Wissenschaftliche Einrichtungen Veterinary Public Health
Institut für Fleischhygiene und – technologie
Fachbereich Veterinärmedizin der Freien Universität Berlin

Molecular Epidemiology and Serodiversity of *Salmonella enterica* in a Pork Chain "From Farm to Fork" in Northern Thailand

Inaugural-Dissertation
zur Erlangung des Grades eines
Doktors der Veterinärmedizin
an der
Freien Universität Berlin

vorgelegt von
Arsooth Sanguankiat
Tierarzt
aus Bangkok / Thailand

Berlin 2013
Journal-Nr.: 3682

Gedruckt mit Genehmigung des Fachbereichs Veterinärmedizin
der Freien Universität Berlin

Dekan:	Univ.-Prof. Dr. Jürgen Zentek
Erster Gutachter:	Univ.-Prof. Dr. Reinhard Fries
Zweiter Gutachter:	Univ.-Prof. Dr. Lothar H. Wieler
Dritter Gutachter:	Univ.-Prof. Dr. Uwe Rösler

Deskriptoren (nach CAB-Thesaurus):
pigs, pork, Thailand, *Salmonella*, serovars, pulsed-field electrophoresis, typing

Tag der Promotion: 27.01.2014

Bibliografische Information der *Deutschen Nationalbibliothek*
Die Deutsche Nationalbibliothek verzeichnet diese Publikation in der Deutschen Nationalbibliografie; detaillierte bibliografische Daten sind im Internet über <http://dnb.ddb.de> abrufbar.

ISBN: 978-3-86387-432-2
Zugl.: Berlin, Freie Univ., Diss., 2013
Dissertation, Freie Universität Berlin
D 188

Choriner Str. 85 - 10119 Berlin
verlag@menschundbuch.de – www.menschundbuch.de

Contents

1. Introduction

Nowadays, the "One Health Concept" is increasingly applied to attain and to maintain health for humans, animals and the environment by collaboration of institutions locally, nationally and globally. Veterinarians work multidisciplinary on carriers of zoonoses and/or food safety (Veterinary Public Health). *Salmonella*, one of the most important zoonotic pathogens, is of concern for the safety of food production. The pattern of *Salmonella* transmission and shedding in swine populations is the result of a variety of factors resulting in a multitude of potential scenarios (Rostagno and Callaway, 2012) and conventional sanitation methods can reduce the microbial load, but cannot eliminate pathogens if present (Olaimat and Holley, 2012). So, control or even elimination of the agent along the production chain is a difficult challenge.

Critical points along the chain have been investigated in various studies to trace back the agent for important sources, e.g.: "*pre-harvest studies"* (Kranker et al., 2003; Lo Fo Wong et al., 2004; Nollet et al., 2004; Dorn-In et al., 2009; Molla et al., 2010; Gotter et al., 2012), studies "*from the farm to the abattoir"* (Rostagno et al., 2003; Botteldoorn et al., 2004; Sorensen et al., 2004; Bahnson et al., 2006; Vieira-Pinto et al., 2006; Dorr et al., 2009; De Busser et al., 2011; Kich et al., 2011; Visscher et al., 2011; Alban et al., 2012; da Silva et al., 2012) *and "post-harvest studies"* (Prendergast et al., 2009; Hansen et al., 2010; Gomes-Neves et al., 2012; van Hoek et al., 2012).

Although several stages and/or risk factors have been reported as important sources of *Salmonella*, the lack of consistency, the methodological limitations, as well as the

complex and dynamic epidemiology of *Salmonella* in swine populations make it difficult to draw definitive conclusions (Rostagno and Callaway, 2012). Moreover, information on the link between living pigs, carcasses and pork pieces is still lacking. So in order to identify possible critical points in the production process, this study was conducted.

In a larger framework, 193 live pigs were sampled individually and followed up into an abattoir and further on to cutting and retail- ready pork. 22 cohorts consisting of 7-10 pigs each were investigated in this study. At the fattening farms, faecal samples were collected just prior to shipment; in addition, samples from the environment were collected in parallel. At slaughter, all pigs in each cohort were sampled (caecal contents and mesenteric lymph nodes). These samples were obtained from 181 pigs. Environmental samples were collected prior, during, and after cleaning and disinfection in addition. 173 freshly cut and transported pork pieces from the same chain were also followed. Finally, pork from retail was sampled. In this study, strains, which have been collected and serotyped, were now genotyped by PFGE to understand the transfer of the agent in the pork chain and in pork products.

2. Literature

2.1 The genus *Salmonella*

The genus *Salmonella* belongs to the family Enterobacteriaceae, which consists of gram-negative, aerobic, facultative anaerobic, a sporogenous rod-shaped bacteria that grow well on artificial media (Edwards and Ewing, 1972). Some genera of the family are atrichous, the genus *Salmonella* is motile by peritrichous flagella. Nonflagellated variants, such as *Salmonella* Pullorum and *S.* Gallinarum, and nonmotile strains resulting from dysfunctional flagella (D'Aoust, 1997). The optimum temperature for growth is 37°C, their limits for growth ranging between 5.2°C and 46.2°C (most serotypes fail to grow at <7°C) (ICMSF, 1996). Glucose and other sugars are fermented, salmonellae usually produce hydrogen sulfide (H_2S) but they do not utilize lactose. They can grow well on MacConkey.

The name of the genus refers to Daniel Elmer Salmon (1850-1914), a veterinary surgeon who studied animal diseases for the United States Department of Agriculture (USDA) and who was a pioneer in public health practice and medical research. Since 1885, more than 2500 serotypes have been identified from different animal species according to their somatic lipopolysaccharide (O-antigen) and flagella antigens (H-antigen) (Grimont and Weill, 2007; Stevens et al., 2009) (Table 2.1). These days, *Salmonella* is one of the most frequently occurring foodborne pathogens, causing gastrointestinal and systemic infection worldwide with 1-5% of gastroenteritis cases in developing countries. The infection usually results from ingestion of contaminated animal products (WHO, 2005).

Table 2.1: Actual number of *Salmonella* species, subspecies and serotypes according to the Kauffmann-White scheme and WHO collaborating Center for Reference and Research on *Salmonella* (WHOCC-Salm)

Group	*Salmonella* species	Subspecies	Number of serotypes with in subspecies according by year		
			1998	2001	2007
1	*Salmonella enterica*	*enterica* (I)	1454	1478	1531
2	*Salmonella enterica*	*salamae* (II)	489	498	505
3	*Salmonella enterica*	*arizonae* (IIIa)	94	94	99
4	*Salmonella enterica*	*diarizonae* (IIIb)	324	327	336
5	*Salmonella enterica*	*houtenae* (IV)	70	71	73
6	*Salmonella enterica*	*indica* (V)	12	12	13
7	*Salmonella bongori*	(VI)	20	21	22
		Total	2463	2501	2579

2.2 Salmonellosis

Salmonellosis is an illness of varying severity, commonly manifested by diarrhea from enterotoxins, abdominal pain, nausea, and sometimes vomiting. Asymptomatic infections may occur. Children less than five years of age, immunocompromised individuals, and members of the older population are in particular at risk. From data of an international outbreak of *Salmonella* Agona, 56 eligible cases showed symptoms including 98% diarrhea, 93% abdominal pain, 73% nausea, 54% fever, 41% vomiting, and 39% blood in the stool (Nicolay et al., 2011).

The pathogenic potential of *Salmonella* serotypes has been classified as (1) serotypes capable of causing a typhoid-like disease in a single-host species [host-restricted serotypes, e.g. *Salmonella* Typhi in humans], (2) host adapted serotypes, which associate to one host species, but which are also able to cause disease in other species as well, [e.g. *Salmonella* Choleraesuis in pigs, *Salmonella* Pullorum in poultry, *Salmonella* Dublin in cattle], (3) ubiquitous serovars occurring without restriction, which are the majority of the serotypes (Boyen et al., 2008). These serovars such as *S.*

Typhimurium and *S.* Enteritidis tend to produce acute but self-limiting enteritis in a wide range of hosts (Stevens et al., 2009).

Peracute septicemia, acute enteritis, chronic enteritis and a subclinical carrier state (Gracey and Collins, 1992) may occur, caused by different serotypes. Even though some animals do not show any clinical sign, infected animals generally carry the agent in their internal organs, e.g. tonsils, intestines or the gut-associated lymphoid tissue (GALT) (Boyen et al., 2008).

2.3 *Salmonella* in the pork chain

Sources of *Salmonella* transmission have been identified in many types of food products of animal origin (Padungtod and Kaneene, 2006; Ben Aissa et al., 2007; Hendriksen et al., 2008; Sanguankiat et al., 2010). Pork products are considered as vehicle for *Salmonella* to humans (Kich et al., 2011). Relationships between *Salmonella* from swine and/or pork and human were reported, too (Padungtod and Kaneene, 2006; Pornruangwong et al., 2008; Hauser et al., 2010).

In the pork chain, *Salmonella* occurrence has been found to be different between and within age groups and within and between herds. Kranker et al. (2003) reported that *Salmonella* was found predominantly in weaners, growers, and finishers, and only occasionally in sows and gilts. These results correspond well with a result from Vigo et al. (2009), who reported a low number of shedder sows and no increase in the shedding rate at farrowing. Results from Dorr et al. (2009) showed significant increase of *Salmonella* prevalence from late nursery to slaughter. In contrast, a report from Denmark indicated that the prevalence declined to undetectable levels prior to

slaughter (Kranker et al., 2003). Weaning stress (such as a change in feed, commingling of litters, and changing of antibodies in sow's milk) might be a reason for rapid increase in *Salmonella* prevalence in nurseries and residual infection in finishers, and also for substantial increase during exposure (e.g. through slurry overflow).

In a farrow to finishing pig farm, a 23 % seroprevalence (ELISA test for detection of IgG-antibodies against the lipopolysaccharide antigen from serovar Typhimurium) in 70-day-old pigs was reported, which increased to 92 % seroprevalence in 150-day-old pigs (Kich et al., 2011). Vigo et al. (2009) reported that out of 35±3 days old piglets, 90 % of faecal samples were positive with *Salmonella*, but the number of positives fell to 10 % at the beginning of nursery (65±3 days old). The authors suggested that surveillance at the beginning of the nursery period should be considered as another important period before early finishing period. Further, shedding was observed during the finishing stage, and the authors suggested that the early finishing period plays a central role in the infection of finisher pigs (Vigo et al., 2009). These findings corroborate findings in a previous study, indicating that infection during finishing may be the major source of *Salmonella* found in market-age hogs, albeit the *Salmonella* status of finishing pigs is dynamic (Davies et al., 1999). The dynamic of the *Salmonella* status is also mentioned by Kranker et al. (2003), who found differences in the prevalence of results in sera and in cultures from Danish farrow to finish swineherds, in the same herds between cohort and between batches (within-herds variation). The authors estimated an average shedding time of 18 or 26 days, depending on the approach used.

Both industrial and developing countries still have *Salmonella* in their farm animals, which included pigs (Swanenburg et al., 2001; Botteldoorn et al., 2003; Kich et al., 2011; van Hoek et al., 2012). In various studies, a higher level of *Salmonella* was observed at the abattoir compared to the farm level (Swanenburg et al., 2001; Hurd et al., 2002; Padungtod and Kaneene, 2006; Kich et al., 2011), easy transfer during transport and/or during lairage at the slaughterhouse may play a role here. An approximately three-fold increase between farm and slaughter was reported (Kranker et al., 2003). However, the slaughter processes might reduce the number of skin-contaminated carcasses: van Hoek et al. (2012) found a *Salmonella* contamination rate on the carcasses of 96.6 % during exsanguination and a reduction to 35.9 % after meat inspection. Hot-water (temperature 80°C/176°F) for 12 to 15 seconds can be used for decontamination the carcasses with a reduction of *Salmonella* (Alban and Sorensen, 2010).

Kich et al. (2011) found the highest pulsotype variability from samples taken from the environment (herd pen floors and lairage). This might be due to the overtime persistence of strains excreted by pigs. The environment is a major source of contamination and infection for the pigs at farm level as well as during transport to the abattoir (Magistrali et al., 2008), while lairage and slaughterline might be a substantial source for contamination by residential *Salmonella* flora in the slaughterhouse (Swanenburg et al., 2001; Mannion et al., 2012). Results suggest that some specific genotypic clusters, which were identified from the caecal contents and/or mesenteric lymph nodes, were not detected on farms, because they originated from the trucks and lairage swabs (Dorr et al., 2009).

In Canada, a longitudinal study in 90 swine-finishing farms was conducted to evaluate the *Salmonella* distribution in the farm environment. 20.1 % of environmental samples were positive, positive samples from the boots accounted to 38.6 %, from dust 5.6 %, in empty pens 11.6 %, the main drain was positive in 31.8 % of cases (Rajić et al. 2005). 66.7 % of farms had at least one *Salmonella*-positive sample and on 14 farms salmonellae were recovered only from the farm environment. The number of *Salmonella*-positive environment samples ranged from 1 to 4 per farm (Rajić et al. 2005).

Salmonella Derby and *Salmonella* Typhimurium were the most prominent serovars from slaughter pigs in Italy, Canada, and the Netherlands (Rajic et al., 2005; Piras et al., 2011; van Hoek et al., 2012). A slaughter pig investigation in Italy showed the highest prevalence in mesenteric lymph nodes (30.5 %), when compared with colon contents (16.4 %), carcasses and livers (14.1 %). *Salmonella* Derby was the most frequent isolate from pigs, followed by *S*. Typhimurium, *S*. Panama, *S*. Livingstone, *S*. Infantis, and *S.* Rissen (Piras et al., 2011). This finding was comparable with data from Rajić et al. (2005), who described the diversity of *Salmonella* serovar on swine finishing farms. Serotypes Derby, Typhimurium and Infantis were the most serovars that were found in Alberta, Canada (Rajic et al., 2005).

Salmonella infections are seldom associated with clinical diseases in pigs (Alban et al., 2012), but contaminated pork, as one of the important sources, is also playing the role as a significant reservoir of non-typhoid human salmonellosis (Boyen et al., 2008). This statement was supported by surveillance data, which showed, that

Salmonella outbreaks were significantly associated to consumption of pork in Germany (Krumkamp et al., 2008).

2.4 Factors associated with the occurrence of *Salmonella*

2.4.1 Fattening pigs

Potential sources of on-farm risk factors in *Salmonella* infection of fattening pigs have been investigated in various studies (Beloeil et al., 2004; Lo Fo Wong et al., 2004; Nollet et al., 2004; Bahnson et al., 2006; Farzan et al., 2006; Dorn-In et al., 2009; Garcia-Feliz et al., 2009; Cardinale et al., 2010; Gotter et al., 2012). These studies investigated farm characteristics, managerial and hygiene practices and pig-health factors (Table 2.2). As presented in the Table, drinkers, housing design and animal flow are likely to effect on *Salmonella* status in fattening pigs. Factors, which are related to managerial and hygiene practices, showed a potential association with *Salmonella,* too, especially feed, sanitation and antibiotic use in the farms.

From farm to abattoir, environment and lairage contamination are of significance in food safety. Dorr et al. (2009) investigated the role of various environment sources and dissemination of *Salmonella* in commercial swine production systems. *Salmonella* isolates were recovered from pigs (faecal, caecal contents, and mesenteric lymph nodes), environmental sources (barn floor, lagoon, barn flush, trucks, and holding pens) (Dorr et al., 2009). Some genotypic clusters were not detected on the farm; they originated from truck and lairage and were then identified from the caecal contents and/or mesenteric lymph nodes. Likewise living vectors such as avian

wildlife may also be considered as a source of infection of domestic animals, including swine and feed plants (Refsum et al., 2002).

Salmonella is a member of water-borne pathogens as well. Manure from animal is a source of infection. Pig slurry is commonly re-used in agriculture as a fertilizer, and it may constitute a risk for the environment when slurry contaminated with *Salmonella* would be spread. Only few studies have investigated the relationships of contamination with slurry (Fablet et al., 2006).

Table 2.2: *Factors associated with Salmonella status in fattening pigs*

Variables	Levels	OR*	Test/Outcomes	References
Farm characteristics				
Drinker design	Some or all bowl drinkers/ Nipple drinker only	8.0/1.0	Isolation/Faecal material	Bahnson et al. (2006)
Lacking of bird proof houses	Yes/No	4.5/1.0	Isolation/Pooled sample	Cardinale et al. (2010)
Pig flow	Continuous/All in-All out	3.9/1.0	Isolation/Faecal material	Farzan et al. (2006)
Proximity to other swine herds	<2km/>2km	3.8/1.0	ELISA/Blood	Hotes et al. (2010)
Herd size	400-800/<400 pigs	2.3/1.0	Isolation/Faecal material	Dorn-In et al. (2009)
Other animals on farm	Yes/No	2.2/1.0	ELISA/Meat juice	Gotter et al.(2012)
Number of fattening barns	1/2/3/4 fattening units	2.2/1.1/2.2/1.0	ELISA/Meat juice	Hotes et al. (2010)
Number of pigs slaughtered per year	≥3500/<3500	1.7/1.0	Isolation/Pooled faeces	García-Feliz et al.(2009)
Snout contact between pens	Yes/No	1.6/1.0	ELISA/Blood	Lo Fo Wong et al. (2004)
Fully slatted floor	Yes/No	0.5/1.0	ELISA/Blood	Hotes et al. (2010)
Lattices pen partition	Yes/No	0.5/1.0	ELISA/Blood	Hotes et al. (2010)
Type of floor in finishing unit	Fully slatted/50-90%slatted/ <50%slatted	0.01/0.01/1.0	Isolation/ Lymph nodes	Nollet et al. (2004)
Management and hygiene practices				
Presence of cockroaches	Yes/No	5.5/1.0	Isolation/Pooled sample	Cardinale et al. (2010)
Application of antibiotics	Yes/No	5.2/1.0	ELISA/Blood	Hotes et al. (2010)
Feed presentation	Dry only/Combined wet/dry	4.9/1.0	Isolation/Faecal material	Bahnson et al. (2006)
Antimicrobial-free system	Yes/No	4.2/1.0	Isolation/Carcass	Gebreyes et al. (2006)
Feed	Dry/Liquid	4.1/1.0	Isolation/Faecal material	Farzan et al. (2006)
Separate transporter for each age group	No/Yes	3.6/1.0	ELISA/Meat juice	Gotter et al.(2012)
Feed structure	Granulate/Pellets/Mix/Flour	3.5/1.6/3.1/1.0	ELISA/Meat juice	Hotes et al. (2010)
Type of feeding during the fattening period	Dry/Wet	3.2/1.0	Isolation/Faecal material	Belœil et al. (2004)
Number of supplier herds	>3/0-3	3.2/1.0	ELISA/Blood	Lo Fo Wong et al. (2004)
Protection clothing worn outside of barn	Yes/No	3.1/1.0	ELISA/Meat juice	Gotter et al.(2012)
Few rodents and no birds in barns	Yes/No	3.0/1.0	ELISA/Blood	Hotes et al. (2010)
Structure of feed	Pellets/Not pellets	3.0/1.0	ELISA/Meat juice	Gotter et al.(2012)
Frequency of sow dung removal during the lactation period in farrowing crate	≥2 /<2	2.9/1.0	Isolation/Faecal material	Belœil et al. (2004)
Functionality of the dosage system for disinfection	No/Yes	2.9/1.0	ELISA/Meat juice	Gotter et al.(2012)
Same protective clothing worn in multiple barns	Yes/No	2.7/1.0	ELISA/Meat juice	Gotter et al.(2012)
Emptying the pit below the slatted floor after remove of the previous batch of sows	No/Yes	2.6/1.0	Isolation/Faecal material	Belœil et al. (2004)
Use of EM	Yes/No	2.6/1.0	ELISA/Meat juice	Dorn-In et al. (2009)
Contact of other animal to pigs	Yes/No	2.2/1.0	ELISA/Meat juice	Gotter et al.(2012)
Disinfection of equipment after use	No/Yes	2.2/1.0	ELISA/Meat juice	Gotter et al.(2012)
Type of ration	Pelleted/Non-pelleted feed	2.2/1.0	Isolation/Pooled faeces	García-Feliz et al.(2009)
Documentation of cleaning and disinfection	No/Yes	2.2/1.0	ELISA/Meat juice	Gotter et al.(2012)
Waste treatment	Slurry/None	2.1/1.0	Isolation/Faecal material	Dorn-In et al. (2009)
Acidification of feed or water	Yes/No	1.8/1.0	ELISA/Meat juice	Hotes et al. (2010)
Certification from national authority	Yes/No	1.8/1.0	Isolation/Faecal material	Dorn-In et al. (2009)
Use of EM	Yes/No	1.5/1.0	Isolation/Faecal material	Dorn-In et al. (2009)
Cleaning ventilation	Regularly/Sometimes/Never	0.9/0.5/1.0	ELISA/Meat juice	Hotes et al. (2010)
Application of antibiotics	Yes/No	0.7/1.0	ELISA/Meat juice	Hotes et al. (2010)
Washing hands consistently	Yes/No	0.6/1.0	ELISA/Blood	Lo Fo Wong et al. (2004)
Protective clothing	Yes/No	0.5/1.0	ELISA/Blood	Hotes et al. (2010)

*OR = Oddsratio

Table 2.2 (continued): Factors associated with *Salmonella* status in fattening pigs

Variables	Level	OR*	Test/Outcome	Reference
Management and hygiene practices (continued)				
Type of feed	Non-pelleted and dry/ Non-pelleted and wet/ Pelleted and dry	0.5/0.4/1.0	ELISA/Blood	Lo Fo Wong et al. (2004)
Possibility of cats being present in the stables	Yes/No	0.4/1.0	Isolation/ Lymph nodes	Nollet et al. (2004)
Cleaning feed tube	Regular/Sometime/Never	0.4/0.35/1.0	ELISA/Blood	Hotes et al. (2010)
Cleaning of transporter	No/Yes	0.4/1.0	ELISA/Meat juice	Gotter et al.(2012)
Use of whey	Yes/No	0.3/1.0	ELISA/Blood	Lo Fo Wong et al. (2004)
Clean boots available	No/Yes	0.3/1.0	ELISA/Meat juice	Gotter et al.(2012)
Greater than one technical visit per month	Yes/No	0.3/1.0	Isolation/Pooled sample	Cardinale et al. (2010)
Feeding system	Liquid/Mash/Mix/Dry	0.3/1.2/2.2/1.0	ELISA/Meat juice	Hotes et al. (2010)
Castration after 1 week of age	Yes/No	0.3/1.0	Isolation/Pooled sample	Cardinale et al. (2010)
How often is the "black side" cleaned	Never/Sometime	0.2/1.0	ELISA/Meat juice	Gotter et al.(2012)
Batch production and hygienic-lock facility	Both/One or neither	0.2/1.0	ELISA/Blood	Lo Fo Wong et al. (2004)
Purchase of gilts	Yes/No	0.3/1.0	Isolation/ Lymph nodes	Nollet et al. (2004)
Decreased with an all-in all-out system	Yes/No	0.1/1.0	Isolation/Pooled sample	Cardinale et al. (2010)
Pig-health status				
No infection at the farrowing stage	Yes/No	5.2/1.0	Isolation/Pooled sample	Cardinale et al. (2010)
Lawsonia intercellularis seroconversion during the second half of the fattening period	Yes/No	3.2/1.0	Isolation/Faecal material	Belœil et al. (2004)
Residual *Salmonella* contamination of the fattening room before loading of the batch follow	Yes/No	3.1/1.0	Isolation/Faecal material	Belœil et al. (2004)
PRRS serological status of the batch followed at the end of the fattening period	Seropositive/Seronegative	3.0/1.0	Isolation/Faecal material	Belœil et al. (2004)

*OR = Odds ratio

2.4.2 Pig carcasses

Several factors showed significant association with *Salmonella* on pig carcasses (Table 2.3). Various authors concluded that carcass contamination at slaughterhouse level were the consequence of *Salmonella*-positive pigs entering the line (Berends et al., 1997; Botteldoorn et al., 2003; Kich et al., 2011; da Silva et al., 2012).

Table 2.3: *Factors associated with Salmonella status on pig carcasses*

Variables	Level	OR*	Test/Outcome	Reference
Carcass swabs after polishing	Positive/Negative	36.7/1.0	Isolation/Carcass swabs after forced chilling	De Busser et al. (2011)
Isolation before chilling	Positive/Negative	14.6/1.0	Isolation/Carcass swabs	Silva et al. (2012)
Isolation from lairage area	Positive/Negative	13.5/1.0	Isolation/Carcass swabs after polishing	De Busser et al. (2011)
Isolation after singeing	Positive/Negative	11.1/1.0	Isolation/Carcass swabs	Silva et al. (2012)
Carcass swabs after polishing	Positive/Negative	10.4/1.0	Isolation/Carcass swabs after splitting	De Busser et al. (2011)
Isolation from swabs oral cavity	Positive/Negative	10.2/1.0	Isolation/Carcass swabs after splitting	De Busser et al. (2011)
Higher frequency of finding *Salmonella*-positive carcass at more than one sampling point	Positive/Negative	9.2/1.0	Isolation/Carcass swabs	Silva et al. (2012)
Finding *Salmonella* isolates in at least one slaughter step	Positive/Negative	7.9/1.0	Isolation/Carcass swabs	Silva et al. (2012)
Aerobic plate count (ACP) on prewash carcass	APC level if increased by 1.0 log CFU/cm^2 /APC level on prewash carcass	7.8/1.0	Isolation/Carcass swabs	Algino et al.(2009)
Isolation after dehairing	Positive/Negative	4.1/1.0	Isolation/Carcass swabs	Silva et al. (2012)
Salmonella in faeces	Yes/No	3.4/1.0	Isolation/Carcass swabs	Berends et al. (1997)
Salmonella from herd serology	Positive/Nogative	1.03/1.0	ELISA/Carcass swabs	Sørensen et al. (2004)
Sampling period	January-March/April-June/July-September/October-December	1.0/2.4/1.1/0.6	Isolation/Carcass swabs	McDowell et al.(2007)
Serology from meat juice	Suspect or positive (imported)/Suspect or positive (homebred)/Negative	0.6/2.1/1.0	ELISA/Carcass swabs	McDowell et al.(2007)
Caecal result	Positive (imported)/ Positive (homebred)/Negative	0.6/2.3/1.0	Isolation/Carcass swabs	McDowell et al.(2007)
Evisceration	Careful/Routine	0.08/1.0	Isolation/Carcass swabs	Berends et al. (1997)
Sampling day	Monday/Tuesday/Wednesday/ Thursday/Friday	1.0/1.5/2.0/1.8/ 2.5	Isolation/Carcass swabs	McDowell et al.(2007)

*OR = Odds ratio

Aerobic plate count (APC) level on prewash carcasses was significantly associated with *Salmonella* prevalence (odd ratio = 7.8 per change of 1.0 log CFU/cm^2) while

leaving the carcass unskinned during chilling was associated with lower mean levels of coliforms, *Enterobacteriaceae,* and APC(Algino et al., 2009).

Also the source of pigs should be considered. In Northern Ireland, the risk of carcass contamination varied with the *Salmonella* status of the individual animal. The occurrence of *Salmonella* in caecal contents or a suspect/positive serological reaction were both independently associated with occurrence of *Salmonella* on carcasses in homebred, but not in imported animals(from different sources) which could suggest possible transport and/or lairage effect nevertheless (McDowell et al., 2007).

Pigs can get infected during resting in the lairage area, when exposed to relatively low amounts of *Salmonella* organisms in the pre-slaughter environment. Shedding of salmonellae under stress condition might occur only 2 h after oral ingestion (Hurd et al., 2001). After pigs were exposed to *Salmonella*, the agent was recovered from the faeces of infected pig within 3 days and from the tonsil and ileum at necropsy (Fedorka-Cray et al., 1994). Also the condition of holding pens and the time of resting affected the *Salmonella* prevalence: solid concrete floors and holding time (more than 45 min before slaughter) were associated with a higher proportion of *Salmonella*-positive samples (Hurd et al., 2005).

The slaughter-line can get contaminated by faeces of carrier pigs (van Hoek et al., 2012). A study in a Dutch pig slaughterline shows a strong correlation between the number of live animals that carry salmonellae in their faeces and the number of contaminated carcasses at the end of the slaughterline (Berends et al., 1997). These researchers showed that live animals that carry salmonellae are 3-4 times more likely

to end up as positive carcass than *Salmonella*-free animals. Hence, separate slaughter of sero-negative pig herds might be a useful practice to lower the prevalence of *Salmonella*-contamination in pork (Swanenburg et al., 2001)

Algino et al. (2009) and da Silva et al.(2012) determined that abattoir procedures as factors being associated with *Salmonella* prevalence and spreading. An effective slaughter process can help to decrease the number of *Salmonella*-positive carcasses in slaughterhouses that receive *Salmonella*-positive pig batches (da Silva et al., 2012). Algino et al. (2009) found that a lower water temperature (<12.8°C) used for washing carcasses was associated with decrease of indicator bacteria when compared to other temperature ranges (≥12.8 to <21.1°C, ≥21.1 to <32.2°C and ≥32.2°C). Meanwhile, singeing was the most important procedure for *Salmonella* reduction and changing the carcass status from *Salmonella* positive to *Salmonella* negative (da Silva et al., 2012).

The equipment of slaughter-lines was also identified as a source of contamination. Carcass splitters were found to harbour *Salmonella* (van Hoek et al., 2012)and were found also as a source of contamination for many carcasses, especially on the cutting side (Smid et al., 2012). Inadequately cleaned polishing machines and inappropriate procedures during evisceration are the most important risk factors (Berends et al., 1997).

2.4.3 Pork cutting and retail-ready pork

Data on the prevalence in pork cutting and retail-ready pork have been reported, too. Here are many opportunities for cross contamination with *Salmonella* spp. Prendergast et al. (2009) recovered *Salmonella* spp. from 2.6 % (13/500) of raw pork

(from butchers' shops and supermarkets) in Ireland between January and November, 2007. Some strains, i.e. *S.* Typhimurium phagetype U310, recovered from a pork abattoir, were being identical (100%) by PFGE analysis to a strain found a year later in a sample from a retail outlet (Prendergast et al., 2009). The authors reported no evidence of an association between the presence of *Salmonella* and the pork type (pieces, chops or minced) or between the presence of *Salmonella* and type of outlet where it was purchased.

Berends et al. (1998) described main risk factors at cutting plants and at retail level with regard to cross contamination: inappropriate cleaning and disinfection (OR = 12.8), manipulation of contaminated materials (OR = 4.7) and (re)contaminated surfaces (OR = 4.4) when contaminated carcasses were being processed. According to the authors, about 90% of the cross contamination occurring in cutting plants is practically unavoidable.

2.5 Methods for *Salmonella* characterization

Phenotypic and/or genotypic techniques may be performed for characterization of strains below species and subspecies level. The ability to identify the source from which human pathogens originated would be of great value in reducing the incidence of foodborne diseases and for intelligence about the spread of diseases due to *Salmonella* outbreaks (Foley et al., 2007).

Efficiency of typing methods would be assessed by typability, reproducibility and discrimination. The first (typability; ability to distinct the strains) and the second (reproducibility; ability of the test to give the same results from the same strain on

repeated tests) assessment are expressed as percentage whereas for the third (discriminatory power; ability to distinguish between unrelated strain), the use of a single numeric index of discrimination (D) or Simpson's index of diversity has been suggested (Hunter and Gaston, 1988).

Foley et al. (2007) and Yan et al. (2003) reviewed *Salmonella* typing methods. Phenotyping and genotyping methods are mainly used to differentiate between *Salmonella* isolates. For phenotypes, biochemical properties reflect the metabolic activity of *Salmonella*, which is based on the phenotypic expression of particular genes. Serotyping, phage typing, antimicrobial susceptibility testing and multilocus enzyme electrophoresis techniques are examples of phenotyping methods, while Pulsed-field gel electrophoresis (PFGE), Restriction fragment length polymorphism (RFLP)/Ribotyping, Amplified fragment length polymorphisms (AFLP), Arbitrary primed-/random amplified polymorphic DNA PCR (AP-RAPD-PCR), Repetitive element PCR (Rep-PCR) and Multilocus sequence typing (MLST) are examples for genotyping methods which identify differences in the nucleotide sequence of the genome (Foley et al., 2007). These techniques have been used to improve the ability of tracing back individual isolates (Yan et al., 2004). Inclusion of molecular-based techniques in monitoring and control programs is recommended to improve the accuracy of dissemination (Michael et al., 2006).

2.5.1 Serotyping

According to antigenic formulae of the *Salmonella* serovars, 2,579 serotypes have been identified from 2 species (*S. enterica* and *S. bongori*). These serotypes have been identified from their somatic lipopolysaccharide [O-antigen] and flagella

antigens [H-antigen] (Grimont and Weill, 2007; Stevens et al., 2009). Serotyping is the most common method to differentiate between *Salmonella* strains (Yan et al., 2004) and widely used for identification. Rajić et al. (2005) reported, that from 81.5 % of samples only one serovar was recovered, whereas 2 serovars and 3 serovars were recovered from 13.0 % and 1.1 % of samples, respectively. However, their discriminatory power is poor (Kerouanton et al., 2007): more than one pattern of PFGE can be found from a serotype (e.g. 46 PFGE patterns of *S.* Typhimurium, 31 patterns of *S.* Senftenberg and 4 patterns of *S.* Bovismorbificans). Nevertheless, conventional antigen-based serotyping continues to be a useful tool for epidemiologic surveillance of *Salmonella*, in combination with molecular subtyping (Tenover et al., 1995; Gaul et al., 2007).

2.5.2 Pulsed-Field Gel Electrophoresis

Salmonella molecular subtyping techniques are shown in Table 2.4. PFGE has been the most widely used method to characterize strains of *Salmonella* serotypes (Gaul et al., 2007). Macrorestriction fragment profile analysis by PFGE (DNA fingerprints or pulsotypes) is used to indicate correlations among strain. This technique has been found to be highly concordant between serotype and PFGE pattern (Kerouanton et al., 2007). Tenover et al. (1995) described criteria for interpreting PFGE patterns when an isolate is considered to be closely related to the outbreak strain as shown in Table 2.5.

Table 2.4: *Salmonella* molecular subtyping techniques

Techniques	Discrimination	Reproducibility	Technical difficulty	Time & cost
Restriction digestion based techniques				
Pulsed-field gel electrophoresis (PFGE)	High	High	High	Medium
Restriction fragment length polymorphism (RFLP)/Ribotyping	Medium	High	Medium	Medium
Amplification based techniques				
Amplified fragment length polymorphisms (AFLP)	High	High	High	Medium
Arbitrary primed-/random amplified polymorphic DNA PCR (AP-RAPD-PCR)	High	Low	Medium	Low
Repetitive element PCR (Rep-PCR)	High	Low	Medium	Low
Nucleotide sequencing based technique				
Multilocus sequence typing (MLST)	Medium/High	High	High	High

Source: Yan et al. (2004)

Table 2.5: Criteria for interpretation of closely related strains

Category	No. of genetic differences compared with outbreak strain	Typical no. of fragment differences compared with outbreak pattern	Epidemiology interpretation
Indistinguishable	0	0	Isolate is part of the outbreak
Closely related	1	2-3	Isolate is probably part of the outbreak
Possibly related	2	4-6	Isolate us possibly part of the outbreak
Different	≥3	≥7	Isolate is not part of the outbreak

Source: Tenover et al. (1995)

Pulsed-field gel electrophoresis (PFGE) has been used in numerous studies both in industrial countries and also in developing countries for veterinary epidemiology studies (Gaul et al., 2007; Pang et al., 2007; Stevens et al., 2008; Vigo et al., 2009; Piras et al., 2011; Chen et al., 2012) and it is a standard typing method for isolates from *Salmonella* outbreaks and for epidemiological investigations (Zou et al., 2010). The main focus of these studies was to indicate the source of contamination and to characterize the genomic DNA of the isolates in order to investigate and to evaluate the distribution of a particular pathogen.

For comparability reasons, standard protocols for PFGE are required. The PulseNet protocol was developed by PulseNet, the national molecular subtyping network for foodborne disease surveillance, which is managed by the CDC (Yan et al., 2004) in order to strengthen the discrimination ability of PFGE among strains and the epidemiological utility of the resulting data (Swaminathan et al., 2001). A most important restriction enzyme, which is commonly used in PFGE for *Salmonella,* is *Xba*I. PulseNet recommends the *Xba*I as primary enzyme for subtyping of *Escherichia coli* O157:H7, *Salmonella* and *Shigella*. Secondary (*Bln*I) and tertiary enzymes (*Spe*I) may be applied, when there is more than one isolate indistinguishable from each isolate and if isolates are likely to be from a same source of contamination (Ribot et al., 2006). In addition, a standard strain of *Salmonella* serotype Braenderup (H9812) restricted with *Xba*I was selected as a universal standard according to the even distribution of bands over the entire range of band sizes (Hunter et al., 2005).

Gaul et al. (2007) determined fragments from DNA, separated by PFGE, within *Salmonella* serotypes to aid in determining the serotype of unknown *Salmonella* isolates from swine. Results indicate that 12 *Salmonella* serotypes from 674 isolates from swine were separated into 66 different *Xba*I PFGE subtypes and the subtypes were separated into groups of identical serotypes based on the PFGE bands. The authors concluded that PFGE characterization would be useful as a preliminary screen for the serovar of an isolate of *Salmonella* based on bands conversed within the serotypes'*Xba*I PFGE subtypes. In additional, Gaul et al. (2007) recommended that, if the isolate had a new PFGE pattern, similarity of more than 72% of the pattern would indicate the same serotype. At the same time, PFGE would be a possible

alternative to serotype based determination or may be used to screen isolates before doing actual serotyping (Gaul et al., 2007).

2.5.3 Computer program-assisted analysis of PFGE data

When PFGE data are more complex, a computer-assisted analysis becomes helpful to analyze the complex database. Comparison of intra- and inter-gel lanes (normalization) as well as comparison of data between different laboratories, also over multi-year time period requires, that every gel contains the same specific PFGE reference pattern, be placed in uniform fashion in several gel lanes. The respective program would then be directed to identify same patterns (Goering, 2010).

To process gels from PFGE with BioNumerics®, 4 steps (1. Strip, 2.Curves, 3.Normalization and 4.Bands) have to be performed.

Step 1 “Strip”: during defining “Strip” patterns in a first step, the program asks researchers to enter approximate number of tracks on the gel in a dialog box, which are:

1) Number of tracks in a gel
2) Thickness, in number of points (pts) of the image strips, that the blue lines enclose in the complete patterns
3) Number of nodes which allow to bend the strip locally
4) Background subtraction and spot removal which allow gel scans with irregular background and spots or artifacts to be cleaned up to a certain extent

Step 2 “Curves” and step 3 “Normalization”: after defining pattern strips, defining densitometric curves (second step) and normalizing gels (third step) are next two steps. The program extracts densitometric curves from the image file, using the information of the strips entered in the previous step (Strip). Reference positions are defined in the normalization step, where the program will be able to determine automatically the molecular weight regression from the sizes entered at this stage.

Step 4 “Bands”: the last step of processing gels is defining bands and quantification (fourth step). In this step, the program defines bands according to band search filters, which involve (Figure 2.1):

1) Percentage of minimum profiling, which is the elevation of the band with respect to the surrounding background
2) Percentage of “Gray zones”, which specifies bands as an “uncertain band”
3) Minimum area, as percentage of the total area of pattern, and shoulder sensitivity allow shoulders without a local maximum as well as doublets of bands with one maximum to be found

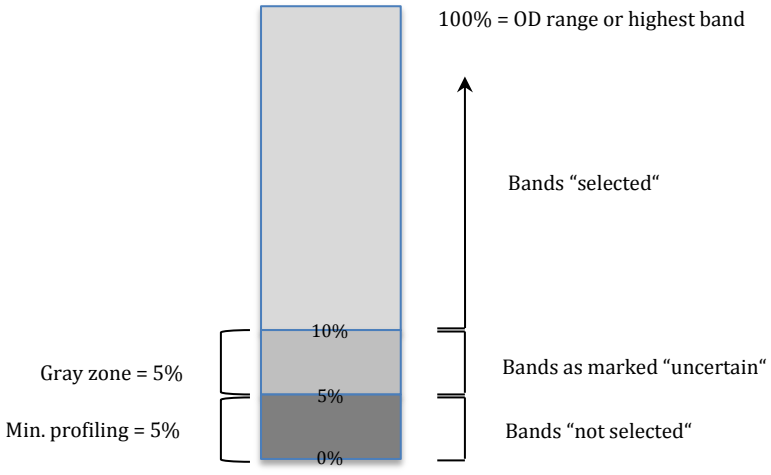


Figure 2.1: Understanding the meaning of processing gels in defining bands(Applied Maths NV, 2011)

Cluster analysis, revealing and visualizing hierarchical structure in complex data sets of fingerprints can be performed by comparisons function in BioNumerics® to identify similarity of samples. To calculate cluster analysis (pairwise similarities), two parameters have to be specified for setting related to similarity coefficient for calculations of similarity matrix and clustering analysis method have to be specified.

For similarity by *Dice* coefficient calculation, two parameters have to be specified:

1) Optimization (%) that researchers allow between any two patterns and within which the program will look for the best possible matching;

2) Band matching position tolerance (%), which is the maximum percentage of shift allowed between two bands to consider them as matching).

For cluster analysis such as UPGMA (Unweighted Pair Group Method with Arithmetic mean) is the result when applying cluster analysis. Various studies set 2 parameters in different values. Examples for setting these parameters were set between 0.5 and 2.0, as show in Table 2.6.

Table 2.6: Studies using cluster analysis with UPGMA technique to generate dendrogramsapplying BioNumerics® or GelCompare II®

Samples	*Salmonella* serotypes	Band matching setting		Reference
		Optimization	Position tolerances	
Lairage area, lymph nodes, content from intestine, carcasses	Typhimurium, Rissen, Derby, Brandenburg, Infantis	ND	1.0	De Busser et al.(2011)
Carcasses, meat and meat handlers	Typhimurium, Derby, Rissen, Mbandaka, London, Give, Enteritidis	1.0	1.0	Gomes-Neves et al.(2012)
Swine finishing herds and slaughter facility	Typhimurium, Panama, Senftenberg, Derby, Mbandaka	ND	1.7	Kich et al.(2011)
Feed and faeces	Serogroup B	1.5	2.0	Molla et al.(2010)
Mesenteric lymph nodes, colon contents, carcasses, livers	Derby, Typhimurium, Panama, Livingstone, Infantis, Rissen	ND	ND	Piras et al.(2011)
Human, swine, pork	Typhimurium, S. 4,[5].12:i:-	ND	ND	Pornruangwong et al.(2008)
Human	Kedougou	1.0	1.5	Pornruangwong et al.(2011)
Pens, faeces, truck caecal contents, mesenteric lymph nodes and carcasses	Typhimurium, Derby, Bovismorbificans, Hardar, Bredeney	1.0	1.0	Magistrali et al.(2008)
Process equipment, faeces, carcasses	Derby Typhimurium Brandenburg Rissen	1.5	1.5	van Hoek et al.(2012)
Retail-ready pork	Typhimurium	0.5	1.5	Prendergast et al.(2009)
Lairage, carcasses after dehairing, after singering, after evisceration, before chilling, caecum	Derby, Typhimurium, Panama	ND	ND	Silva et al.(2012)
Carcasses, colon, mesenteric lymph nodes, slaughterhouse environment	Typhimurium, Derby S. 4,[5].12:i:-	ND	1.0	Botteldorn et al.(2004)
Primary production, pork, human	ND	1.0	1.5	Hauser et al.(2010)

ND = Not determined

2.6 *Salmonella* prevalence data from foods in Thailand

Pigs, chicken, cattle including aquatic animals (fish and shrimps) are widely produced and consumed in Thailand (Padungtod et al., 2008). *Salmonella* prevalence data from various foods in Thailand have been reported from several studies. In chicken meat, *Salmonella* was isolated in a range between 57 % and 72 % (Boonmar et al., 1998; Padungtod and Kaneene, 2006; Vindigni et al., 2007; Minami et al., 2010), while in pork a prevalence of 12 % was observed (Minami et al., 2010), which was lower than results from Padungtod and Kaneena (2006) and Sanguankiat et al. (2010) who reported 29 % and 34.5 % of reatail pork products respectively. In contrast, Vindigni et al. (2007) revealed *Salmonella* prevalences in pork at a higher level: between 74% and 93% from fresh market and supermarket, respectively. For beef, the prevalence was 82% (Vindigni et al., 2007), a lower prevalence was reported (24%) by Miami et al. (2010).

From other food products, *Salmonella* prevalence was reported, too. In 14 % of chicken eggs *Salmonella* was detected (Vindigni et al., 2007), the contamination rate reported from egg farms was 3.2 % (Utrarachkij et al., 2012). Non-detection of *Salmonella* was reported from dairy milk samples (Padungtod and Kaneene, 2006). In a study in Thailand between 2002 and 2007, the 10 most common serotypes were *S*. Enteritidis, Stanley, Weltevreden, Rissen, I [1],4,[5],12:i:-, Choleraesuis, Anatum, Typhimurium, Corvallis, and Panama (Hendriksen et al., 2009).

The aim of this study was to assess the risk and pathways of *Salmonella* contamination in pork from a pig chain and the environment using techniques of molecular biology in Northern Thailand.

3. Materials and Methods

3.1 Materials

3.1.1 Study sites and selection of animal

This study was conducted from December 2004 to May 2005 in Chiang Mai, the largest city, and Lamphun, the area with the highest density of pigs, in Northern Thailand. From a previous study (Patchanee et al., 2002), of 55 % was assumed as a *Salmonella* prevalence in this region, as well as a number of 194 fattening pigs was estimated to determine the overall prevalence of *Salmonella* at individual pig level when using an error level of ± 3.5 % and a 95 % confidence level (Elwood, 2000).

3.1.1.1 The finishing herds

In this longitudinal study, 22 cohorts consisting of 194finishers (about 1-2 days before slaughter, 90-100 kg live weight) were selected conveniently from one large commercial and conventional swine production chain. Each cohort[1]consisted of 7-10 pigs, representing one herd. A total of 22 cohorts reflecting 2 groups of different type of farming was included. The first group represented a type of traditional pig production with open houses (17 herds) and the second one represented an indoor system (5 herds). Spraying on the back identified the individuals. Sterile tags after splitting the carcass were used for identical carcasses along the processing line. Each cohort was followed until the end of the product operations.

[1] Cohort is a group of fattening pigs which shared a particular event together during a particular time span along the process.

3.1.1.2 The abattoir, slaughtering and further procedures

Finishers were transported to a modern abattoir in Chiang Mai, Thailand. After arrival, pigs were again identified at lairage. Then, the animals were showered with potable water and got rest for 1-2 hours. They were then transferred into the slaughter line and were stunned by low-voltage electricity (110-180 Volts) for approximately 15 seconds. Looped around one of the hind ankles by a chain, stunned pigs were hauled to a conveyor rail, the knife was inserted into front of the sternum and pushed forward to sever the anterior vena cava. Scalding and dehairing were done for 1.30 – 3.0 min in a horizontal scalding tank, final dehairing was manually performed with a sharp knife. After dehairing, carcasses were prewashed with potable water, and hauled again to an overhead rail by hooking the hind legs. Evisceration procedures (de-bunging, opening the belly and removing gut) were performed by the same person. Internal organs were removed as part of the plucks and the gutset and were transferred to an offal room for cleaning, where caecal content and mesenteric lymph nodes samples were collected. Then, carcasses were split along the midline; spinal cords were detached from vertebral columns and carcass swabbing after splitting was performed. Afterwards, the split carcasses were washed with potable water (chlorinated 50-100 ppm) before storage in a chilling room (≤4°C) overnight. Tag numbers were attached on the forelegs of each carcass for identification purposes throughout the following procedures. Prior to chilling and after washing, carcasses were swabbed. In the chilling room, tags (denominating the cut piece of meat) were attached to the pork carcasses again.

After chilling overnight, carcasses were delivered to the cutting area between 8 and 9 AM. Cutting was done by person who performed slaughter (staff rotation). Now,

samples were collected from the freshly cut meat. The meat was put into plastic bags and returned to the chilling room, still being fully identified. From these bags, meat was cut into smaller pieces and weighted according to the orders from customers. Packaging was done by wrapping pieces on foam trays. This procedure was done on the day after slaughtering; the packages were kept in the chilling room for 1 or 2 days before delivery.

3.1.2 Collection of samples

For each cohort, 5 sampling occasions were taken place (Figure 3.1): 1st) 1-2 days prior to slaughter (faeces; F) at fattening farm, 2nd) in the morning of the slaughter day, at the abattoir (mesenteric lymph nodes; ML, caecal contents; CC, carcass swabs after splitting; CS, and carcass swabs after washing; CW), 3rd) the day after slaughter, during cutting (pieces of freshly cut pork during the cutting process; FP), 4th) 2 days after slaughter (transport of pieces of FP delivered by a company's truck, "transported pork; TP") and 5th) 2 days after slaughter day, at retail (pork from retail market; R).

3.1.2.1 Faeces

Faecal samples (F) were expected to reflect the shedding of agent in individual pigs at herd stage. Faecal samples (25-30g) from each enrolled pig were collected by hand from the rectum, using disposal gloves, when the herds were visited (1st occasion of sampling). Samples were taken to laboratory and processed immediately after arrival. Times of transportation of sample depended on the distance between herd and laboratory, lasting not more than 2 h. During transport, samples were kept in a cooling box at 4°C. *Salmonella* examination started at the day of sampling.

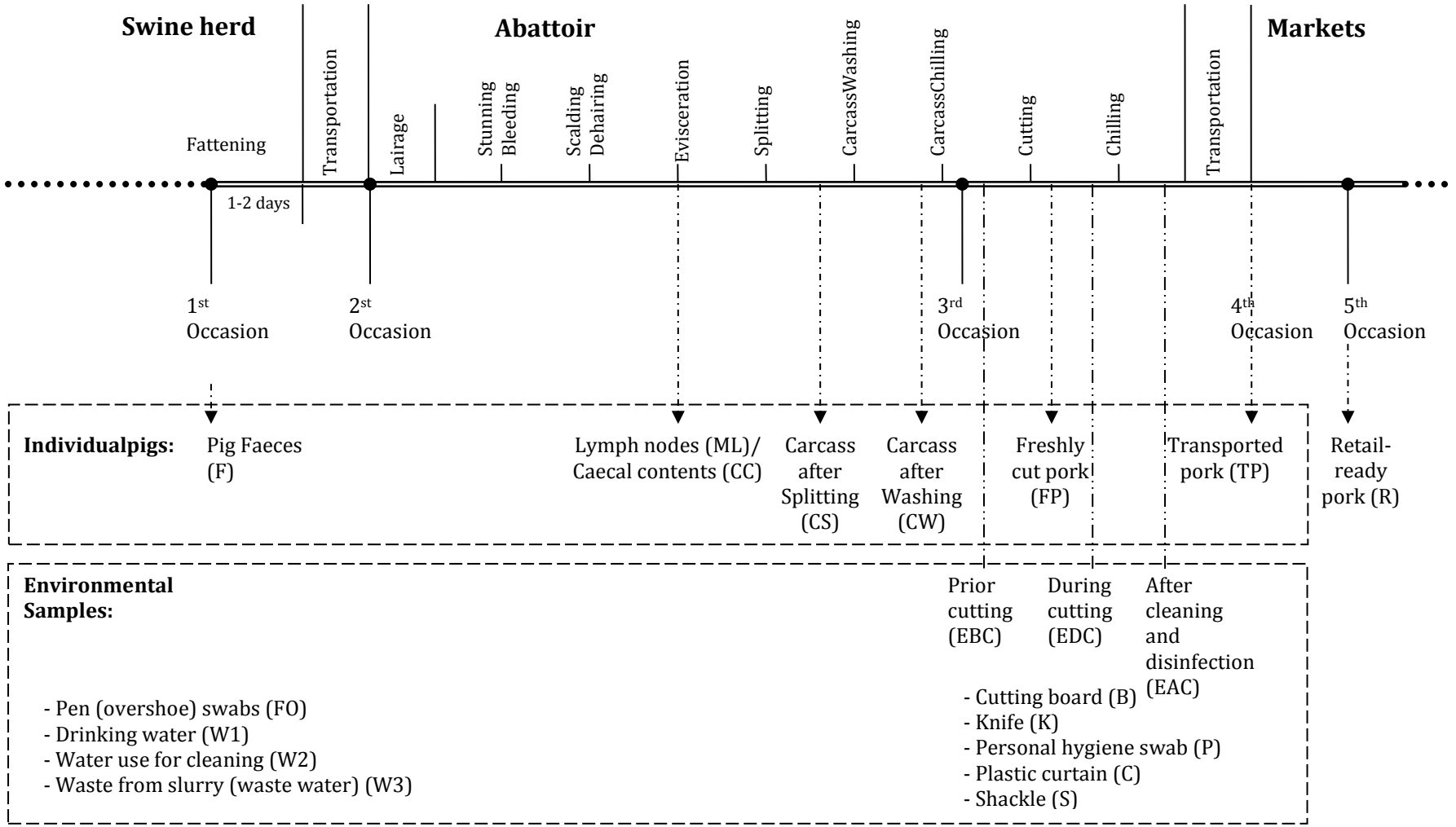


Figure 3.1: Study design and sampling framework of each cohort throughout the chain

3.1.2.2 Mesenteric lymph nodes

Mesenteric lymph nodes (ML) were assumed to represent an infection with *Salmonella*. At the offal room in the abattoir, 25 grams, as a minimum, of mesenteric lymph nodes was collected. They were obtained using sterile gloves, forceps and scalpels. After aseptic dissection, the lymph nodes were placed in a sterile stomacher bag for further microbiological analysis.

3.1.2.3 Caecal contents

Caecal contents (CC) were expected to reflect the agent originating generally from herd, transport and lairage (Sorensen et al., 2004). At the offal room, individual CC samples were collected (25 grams as a minimum) from the caecum of the pig using sterile forceps, scissors and disposal gloves for handling.

3.1.2.4 Carcasses after splitting

Swabbing of carcasses was done to represent the self-contamination and/or cross-contamination during slaughter and dressing (Sorensen et al., 2004). After the removal of internal organs and after splitting, carcasses were sampled prior to washing (Fedorka-Cray et al., 1994). Sampling sites were at neck, belly, back and ham by 100 cm^2 each. Before sampling, sterile cotton layers were moistened with sterile normal saline. Carcass after splitting (CS) sample from each carcass was pooled in a sterile bottle of 50 ml buffered peptone water (BPW). Analysis was conducted at the day of collection.

3.1.2.5 Carcasses after washing

Swabbing of the carcasses after washing (CW) was to reflect the state of sanitation in the slaughter line. Swabs were taken after overnight chilling in the chilling room; the

same sampling site was chosen as the one from the first carcass swabs on the other half of carcasses. Also, sampling sites were identical (neck, belly, back and ham) by 100 cm^2 each. Again, samples from each carcass were pooled in a bottle of 50 ml buffered peptone water (BPW).

3.1.2.6 Pork during cutting ("Freshly cut pork")

Fresh pig meat, in this study called "freshly cut pork" (FP), was taken to reflect the amount of contamination during cutting processes. From each carcass, samples were taken from 5 sample sites (pooling) including belly, tenderloin, jowl, shoulder and loin of individual site, in total of 25 grams for each individual carcass in buffer peptone water.

3.1.2.7 Pork after transport ("Transported pork")

The remaining pork material (including pork tags), was packed, again wrapped on small foam trays and stored in the chilling room for 1 or 2 days. This pork was used for insight into packing, storage and transporting procedures. Products were sent to the laboratory by a factory truck that was also used for the transport of other products to R market. These samples were called "Transported pork" (TP). Microbiological examination was done as soon as possible after arrival.

3.1.2.8 Retail-ready pork

Samples from retail-ready pork (R) were not obtained from the individual cohorts due to the loss of follow up, so, the 4^{th} occasion of sampling took place at the market on the same day of delivery of the TP. Therefore, it was possible that pieces of R pork may have originated from pigs that were included in the study. R was kept in a temperature controlled glass case. Several types of R pork (meat on the bone product,

belly pork, spareribs, jowls, loins, minced pork, pork shoulders, hams, and tenderloins) were collected and used for microbiological analysis.

3.1.3 Collection of environmental samples from herd and abattoir

3.1.3.1 Herd level

3.1.3.1.1 Pen (overshoe) swabs

To investigate the contamination state of the environment at herd level, overshoe swabs (FO) were collected at the 1st occasion of sampling (Figure 3.1). In each herd, seven pens were selected. Swabs were collected using 2 pieces of cotton, each of 15 cm × 20 cm size, wrapped around the boots. Investigators walked around about 30 steps in each pen to be exposed to and to absorb faecal material. After collection, this pair of overshoes was placed in a sterile plastic bag with 225 ml of peptone water. The bag was labeled and kept in an icebox for transport to the laboratory. After arrival, bags were incubated at 37° for 3-5 hours, simultaneously.

3.1.3.1.2 Water

Water was taken from 2 sources (one liter each): the first was from the system for animal drinking (W1) in the pig stalls. The second sample was collected from water pipes (W2), which were used for cleaning the hog stalls. The hog nipple drinker was cleaned with alcohol and flamed prior to water collection. Water was collected in sterile bottles.

3.1.3.1.3 Wastewater from slurry

Waste from the stalls (W3) was drained to slurry pits for processing. This water was collected in sterile bottles, too.

3.1.3.2 Abattoir level

In the cutting room, the cotton swab technique was used for sampling equipment and staff: knives, shackles, plastic curtains and hands of staff (personal hygiene). Times of sampling were prior to cutting (8:00-8:30 AM); EBC, during cutting operations (8:30-11:00 AM); EDC and after cleaning and disinfection of the premises (11:00-12:00 AM); EAC. The technique was described in a previous study (Sanguankiat et al., 2010): A sterile moistened (0.85 % saline solution) cotton swab was held with a sterile forceps; the surface was swabbed 10 times from top to bottom by applying firm pressure to the surface. Swabs were placed into 50 mL sterile (BPW) in media storage bottles with caps and were shaken by hand for 2 min.

3.1.4 Holding and transport of the samples

Having collected the samples, all samples were kept in cool box with ice packs and transported to the laboratory. Upon arrival, they were kept immediately in a refrigerator with controlled temperature (4°C), microbiological examination was done in the same day.

3.2 Methods

All isolates in this study originated from studies described previously (Chantong, 2005; Dorn-In et al., 2009; Sanguankiat et al., 2010). A total of 1982 samples were collected in this study. Isolates were obtained from 22 rounds of sampling from finisher pigs, farm environment and abattoir in Chiang Mai, and kept in half nutrient agar since 2005. Isolates were kept at the Department of Veterinary Public Health, Kasetsart University, some of them were kept at the Institute of Meat Hygiene and Technology, Freie Universität Berlin. For further analysis, strains were recultivated. Details of detection, identification, recovery of the strain and molecular technique for *Salmonella* are described below.

3.2.1 *Salmonella* detection

Laboratory testing for *Salmonella* was conducted following ISO 6579 (2002) with slight modifications: 25 grams (g) of sample or 50 milliliters (Methner et al., 2011) (for swab sample) BPW were used within 2 h after collection. 25 g were suspended and homogenized in 225 mL BPW and incubated at 37±1°C for 18-24 h (pre-enrichment [PE]). As for the swabs, the 50 mL of BPW (cotton swab) was also shaken and incubated at 37±1°C for 18-24 h. An aliquot of 0.1 mL PE was transferred to modified semisolid Rappaport Vassiliadis and incubated at 42±1°C, whereas another 1 mL sample of PE was transferred to 9 mL tetrathionate broth (TTB) and incubated at 37±1°C. After 18-24 h of incubation, a loop of the selective enrichment medium was placed on selective agar, Brilliant-green Phenol-red Lactose Sucrose agar (BPLS) (Merck, Darmstadt, Germany) and Xylose Lactose Tergitol™ 4agar (XLT4) (Oxoid Limited, United Kingdom) and incubated at 37±1°C for 18-24

h. Suspected colonies were confirmed by biochemical tests (triple sugar iron, motility indole, lysine decarboxylase and urease test). Serological testing was done by slide agglutination according to antigenic formulae of the *Salmonella* serovars 2007, 9th edition (Grimont and Weill, 2007). The manufacturer's instructions were followed for agglutination test (Sifin, Berlin, Germany). After testing, 3-5 colonies were collected in 2.0 ml microcentrifuge tube, containing ½ strength nutrient agar and kept in a freezer for storage.

3.2.2 Thawing, recovery, identification and preparation of *Salmonella* isolates for genotyping

To each microcentrifuge tube, 50 µL of brain heart infusion broth (BHIB; Merck, Darmstadt, Germany) was added, and incubated aerobically at 42°C. After 18-24 h of incubation, a loopful of broth was transferred to standard I nutrient agar (Merck, Darmstadt, Germany) and was incubated for 24 h at 37°C. Half of a colony was re-confirmed by streaking on BPLS agar and the other half of the colony was transferred into 5-ml tube of 3.5 ml BHIB for preparing the stock culture. In case of no-growth on standard I nutrient agar (Merck, Darmstadt, Germany), all remaining materials in the microcentrifuge tube were transferred to 3.5 ml of BHIB and incubated at 42°C for cultivation and serological retesting. Isolates were again serotyped using slide agglutination according to antigenic formulae of the *Salmonella* serovars 2007, 9th edition (Grimont and Weill, 2007). Strains were then maintained at -30°C in BHI broth plus 20% glycerol. Prior to use, they were transferred into BHI broth again.

3.2.3 Genotyping by Pulsed-Field Gel Electrophoresis

3.2.3.1 Plug preparation

Isolates were grown overnight at 37°C on Standard I nutrient agar (Merck, Darmstadt, Germany) and in tubes with Standard II Nutrient agar (Merck, Darmstadt, Germany). A colony from Standard I nutrient agar was divided to stab into Standard II tube and to streak on Standard I nutrient agar. After growing on plates at 37°C, genomic DNA was prepared following the method described by CDC (Ribot et al., 2006). Cells were suspended in 2 mL of Cell Suspension Buffer (CSB, 100 mM Tris, 100 mM EDTA [pH 8.0]). This liquid was measured for absorbance values (density) of approximately 0.55-0.60 with a spectrophotometer (Titertek Multiskan®, Labsystems, Finland) at a wavelength of 630 nm. A 200-µL aliquot of each adjusted cell suspension was transferred to a sterile microcentrifuge tube containing 10 µL of Proteinase [(20 mg/mL); Roche Applied Science, Mannheim, Germany]. The agarose used for the plugs consisted of 2% Certified Megabase Agarose (Bio-Rad Laboratories, Hercules, USA), and was gently pipetted up and down for a few times with each cell suspension before dispensing into the well of disposable plug molds (Bio-Rad Laboratories, Hercules, USA). After solidification of the plugs at 4°C for 5 min, they were removed from the molds and immersed in a 20-mL glass tube containing 5 mL of Cell Lysis Buffer (CLB; 50 mM Tris, 50 mM EDTA [pH 8.0]; 1 % Sarcosyl; 0.1 mg/mL Proteinase K). Plugs were incubated in a 54°C water bath shaker for 20 h with gentle agitation (150-175 rpm), after subsequent removal of the plugs form the water bath, the lysis buffer was discarded. The plugs were then quickly washed with 10-15 mL of sterile water (pre-heated to 50°C) in a 50°C water bath shaker for 10-15 min. This procedure was repeated once with double distilled

water and then the plugs were washed four times with 10-15 mL of sterile Tris-EDTA Buffer (TE; 10 mM Tris, 1 mM EDTA [pH 8.0]), pre-heated to 50°C. Plugs were stored in 500 μL TE Buffer at 4°C until use.

3.2.3.2 Digestion with restriction endonuclease *Xba* I

Slices of approximately 2-mm were cut with a single edge razor blade and placed in a sterile microcentrifuge tube that contained 200 μL of a 1× dilution with SuRE/Cut Buffer H (Roche Applied Science, Mannheim, Germany) and were incubated at 37°C for 5-10 min. After incubation, the buffer was discarded and replaced with 200 μL of a restriction endonuclease enzyme (*Xba I*) mixture (50 U/ slice; Roche), subsequently an incubation for 20-24 h at 37°C took place. After incubation, 50 μL of ES solution (0.5 M EDTA [pH 8.0], 1% Sarkosyl) and 100 μL of loading buffer solution were added before loading the plugs into the well of 1.2 % agarose gel (Pulsed-Field Certified Agarose, Bio-Rad Laboratories, Hercules, USA) in 0.5 × Tris-Borate EDTA Buffer (TBE; prepared from 10 × TBE containing 0.89 M Tris borate, 0.02 M EDTA [pH 8.3]).

3.2.3.3 Electrophoresis conditions and casting of the agarose gel

1.2% Pulsed-Field Certified Agarose gel was prepared using a 15-well comb in the wide/long-casting stand (Bio-Rad). Pulse Markers™ 50-1,000 kb (Sigma, Saint Louise, Missouri, USA) were loaded into wells 1,8,15 of 1.2 % Pulsed-Field Certified Agarose gel; test samples were loaded into the remaining wells. Melted 1.2% agarose (equilibrated to 55-60°C) was poured to cover all wells and allowed to solidification. After polymerization, excessive liquid was removed with a tissue paper. The gel was placed into the CHEF-DR II electrophoresis cell (CHEF-DR II, Bio-Rad Laboratories, Richmond, USA). Electrophoresis conditions were as followed: initial switch time of

2.2 seconds and final switch time of 63.8 seconds, electrophoresis running time was 20 hours.

3.2.3.4 Imaging of agarose gel and analysis with bands

The Digital Imaging and Analysis System II (DIAS-II) was used for imaging the agarose gel according to the company's manual. When electrophoresis was over, the machine was turned off. The gel was removed and placed into a covered container. Ethidium bromide [dilute 90µl of ethidium bromide stock solution (10 mg/ml) with 1000 ml of distilled water] was used for staining. The gel was stained for 20 – 30 min in a covered container on a horizontal shaker (Certomat®U) with a speed of 40 min^{-1}. After staining, all ethidium bromide was poured into specific containers. Destaining the gel was done in 500 ml distilled water for 20 min on the horizontal shaker with a speed of 40 min^{-1}. An image was captured with the imaging equipment (DIAS-II). If the background interfered with the resolution, the gel was destained for another 20 min on the horizontal shaker with a speed of 40 min^{-1}. The image was taken and saved as an ".img" or ".1sc" file; for analysis with the BioNumerics®software program those were converted to ".tif[2]" files.

3.2.3.5 Fingerprint image processing with BioNumerics®

BioNumerics® version 6.6 was used for an analysis of the fingerprint images. The images were imported and processed following 4 steps of processing. The process involved the following steps: (i) Strips (defining lanes), (ii) Curves (defining densitometric curves), (iii) Normalization, and (iv) Bands (defining bands and qualification) (Applied Maths NV, 2011)

[2]Tagged Image File Format

In a first step ("Strips"), the program asked researchers to enter the approximate number of tracks on the gel in a dialog box, which were: 1) Number of tracks in a gel (max. 12 samples and 3 markers); 2) Thickness, in the unit of points (pts) of the image strips (the blue lines enclose the complete patterns in a "bounding box"); 3) Number of nodes which allowed researchers to bend the strip locally and 4) Background subtraction and Spot removal which allowed irregular background and spots or artifacts to be cleaned up to a certain extent. After finished the step "Strips", an area of a gel picture was determined by the "bounding box", a green rectangle, which contained 15 lanes of samples and markers.

The blue lines that cover each lane should nearly touch neighboring patterns of each other. "Nodes" in image strip extraction (*Edit > setting*) were also set as 3 nodes (as minimum) to allow bending the strips locally. Three more options, "Background substation", "Spot removal" and "Use bounding box curvature" might be applied in the box of "Raw data" (*Edit > Settings)* which showed "Fingerprint conversion setting" if the gel contained irregular background, spots or artifacts and smiling or sloping band due to distortion in the gel. The gel tone curve was set as linear – a tool to edit the appearance of the image, and adjust weak bands and/or dark bands when bands were not clearly identified.

The second step, defining densitometric curves, shortly called "Curves", was a step where the program automatically defined a densitometric curve using the information of the lane strips which entered in the first step (Applied Maths NV, 2011). The curve was extracted from the image file. Average thickness was set at 10. However, smiling and distortion at the edges of bands should be excluded. Spectral analysis

[3] was done to determine the optimal setting for the least square filtering and background subtraction.

The third step was done according to normalizing the gels, so called "Normalization". Reference patterns were marked and reference positions were defined (reference patterns were identical samples loaded at different positions on the gel for normalization purposes). Fragments of lambda DNA were defined according to the product information of Pulse Marker^{tm} 50-1,000 kb from Sigma®. The first gel was performed as a standard gel. Subsequently the further gels were normalized with the same standard as the first gel, i.e. to calculate the gels for comparison. The gels then were ready to define bands for comparison.

The last step ("Bands and Qualification") is the last step in processing a gel, which involved defining bands and quantifying band area and/or volumes. This step would automatically search for bands processing a 5% minimum profile and 2% gray zone setting (Figure 2.1). Band marking rules were done according to PulseNet USA (Freeman, 2011). More detail of Fingerprint image processing with BioNumerics® has been descried in BioNumerics® Manual Version 6.6.

[3] A function in BioNumerics 6.6 for determining the optimal setting for background and filtering settings.

3.2.3.6 Cluster analysis of fingerprints with BioNumerics®

The cluster analysis [4] was based upon a matrix of similarities between database entries and a subsequent algorithm for calculating bifurcating dendrograms[5] to cluster the entries (Applied Maths NV, 2011). The cluster was performed by selecting *Clustering* > *Calculate* > *Cluster analysis (similarity matrix).* Dice coefficient was selected for measuring the similarity between bands. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) was selected for creating dendrogram types. "Optimization" (a shift that will look for the best possible matching) and "Position tolerance" (the maximum shift between two bands allowing to considering the bands as matching) were both set at 1%. Isolates were regarded as having the same pulsotypes when number and location of the bands were indistinguishable (Kich et al., 2011).

3.2.4 Statistical data analysis

All laboratory results were recorded and managed in MS Excel. For statistical analysis, data were imported into STATA version 11 (StataCorp, 2009). Descriptive statistics was used to determine the prevalence of *Salmonella* in pigs and associated parts of the pork chain environment. Pair-wise comparison of *Salmonella* prevalence between sample prevalence was used to find a correlation[6]. Spearman's correlation technique was used for correlation coefficients (r_s). $r_s > 0.5$ showed high correlation,

[4] Cluster analysis is one of the most popular ways to revealing and visualizing hierarchical structure in complex data set. It is a collective noun for a variety of algorithms that have the common feature of visualizing the hierarchical relatedness between samples by grouping them in a dendrogram or tree (Applied Maths NV 2011).
[5] Dendrogram is a tree diagram frequently used to illustrate the arrangement of the clusters produced by hierarchical clustering.
[6] Correlation is a measure of relationship between two mathematical variables or measured data values.

in the multivariate analysis only one variable with the smallest *P*-value in univariate analysis was used (Garcia-Feliz et al., 2009) to avoid duplication of effects.

Outcomes[7] between sample types of each individual animal (carcass and pork) were combined and displayed by 2-way contingency table to measure associations[8] between exposure (prior status) and outcomes (consequent status). Relative risk [9](RR) was used to measure the strength of an association between an exposure and a *Salmonella* status, which was measured from the contingency table.

Furthermore, McNemar's χ^2 test [10] was used to determine significant differences (probability ≤ 0.05) and the strength of individual pig level agreement between samples. Kappa (κ) statistic [11] was used to assess the level of agreement between these results, following the ranges poor (< 0.00), slight (0.00 to 0.20), fair (0.21 to 0.40), moderate (0.41 to 0.60), substantial (0.61 to 0.80) and almost perfect (0.81 to 1.00) (Landis and Koch, 1977).

The selection process of variables for logistic regression modeling started with an univariate analyses of each variable. The univariable associations were tested between the binary (1 = *Salmonella* positive; 0 = *Salmonella* negative) and outcome variables (1) TP at pig level, (2) FP at pig level, (3) CW at pig level, (4) CS at pig

[7]*Salmonella* status (positive or Negative) from bacteriological test.
[8] Association is used to assess the magnitude of the relationship between an exposure to a disease (*eg* a potential 'cause') and a disease (Dohoo et al. 2003).
[9]Relative risk or risk ratio (RR) is the ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group. RR < 1; exposure is protective, RR = 1; exposure has no effect, RR > 1 exposure is positively associated with disease (Dohoo et al. 2003).
[10]McNemar's test is a normal approximation used on nominal data. It is applied to 2 × 2 contingency tables with a dichotomous trait, with matched pairs of subjects, to determine whether the row and column marginal frequencies are equal ("marginal homogeneity").
[11]Kappa (κ) statistic is a statistical measure of agreement.

level, (5) ML at pig level (6) CC at pig level and all independent variables at farm (faeces, overshoes water). *P*-values from logistic regression were used to examine the significance of the association between dependent and independent variables. All variables with a significance value $P \leq 0.20$ in the univariable analyses were included in the multivariable logistic regression model analysis (Hosmer and Lemeshow, 2000). As a result of this analysis, pigs and their environment, culling area or transport and retail-ready product were found to show close relation. The cohort numbers (farms) was included as a random effect in the analysis. Here, a stepwise backward elimination took place for the multivariable logistic model. A *P*-value for retention (significant impact) of the variable in the models was 0.15 (Hosmer and Lemeshow, 2000) Lastly, from logistic regressions, odd ratios (OR) and 95% confidence intervals (95%CI) were calculated.

4. Results

In a longitudinal study, 193 fattening pigs out of 22 finisher swineherds were investigated. In total, 22 cohorts included 193 faecal samples (F), 181 mesenteric lymph node samples (ML), 181 caecal-content samples (CC), 181 swabs from carcasses after splitting (CS), 180 swabs from carcasses after washing (CW), 173 pieces of freshly cut pork (FP) and 173 TP samples (TP). In addition, 200 R product samples (R) were included in this study.

From the environment at the farms, 22 drinking water samples (W1), 22 cleaning water samples (W2), 22 wastewater samples (W3) and 155 overshoe samples (FO) were collected for analysis. At the slaughterhouse, swabs were taken from cutting boards (B), plastic curtains (C), knives (K), shackles (S) and hands of staff (P) prior to cutting (100 samples), during cutting (100 samples) and after cleaning and disinfection (100 samples).

4.1 Prevalence of *Salmonella* in pigs and associated environments of the pork chain

Overall, *Salmonella* was isolated from 48.9 % (971/1982) of all samples taken (Table 4.1, 4.2 and 4.3). Table 4.1 shows positive findings from environmental samples at the farms and the abattoir. Drinking and cleaning water from farms had a similar prevalence (13.6 %). Results from wastewater were *Salmonella* positive almost every time (21/22) and overshoe samples indicated comparable high positive results (95.5 % and 94.8 %, respectively). At slaughterhouse level, the highest percentage of Salmonella was found during cutting procedures with 23% (95 % CI: 14.8-32.1),

which was much more (about 6 times) than positive samples before cutting (4%). After cleaning and disinfection, positive samples decreased to 14.0% (Table 4.1).

At individual pig level (Table 4.2), the lowest prevalence was obtained from CW (12.9%; 95% CI: 7.0-18.8). With regard to F and ML, no dramatic difference was observed, 61.4% and 63.9%, respectively. The prevalence of CS was about 2.5 times higher than CW. In contrast, CC, which was sampled after transport, yielded the highest percentage of positive samples (83.1%; 95% CI: 74.5-91.6). The number of *Salmonella* positive results was different depending on site and cohorts as shown in Table 4.3.

Table 4.1: *Salmonella* positive samples from the environment

Level	Sample type	Number			Positive (%)	95% Confidence interval
		Samples	Negative samples	Positive samples		
Farms	Drinking water	22	19	3	13.6	-
	Cleaning water	22	19	3	13.6	-
	Waste water	22	1	21	95.5	-
	Overshoes swabs	155	8	147	94.8	90.6-99.0
Abattoir	Environments prior to cutting	100	96	4	4.0	0.2-7.8
	Environment during cutting	100	77	23	23.0	14.8-31.2
	Environment after cleaning and disinfection	100	84	16	14.0	8.7-19.3
Total		521	304	217	41.7	

Table 4.2: *Salmonella* detected at individual level at farms (faeces), slaughterhouse and R

Sample	Number			Prevalence (%)	95% Confidence interval
	Samples	Negative samples	Positive samples		
F	193	74	119	61.4	54.0-68.0
ML	181	65	116	63.9	52.9-74.9
CC	181	30	151	83.1	74.5-91.6
CS	181	123	60	32.0	21.2- 42.9
CW	180	156	24	12.9	7.0-18.8
FP	173	77	96	54.6	40.2-69.0
TP	173	51	122	70.2	57.4-82.9
R	200	134	66	31.1	18.0-44.2
Total	1461	710	754	51.6	46.3-56.1

F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; TP=Transported pork; R=Retail

Table 4.3: Cluster related sampling and detection rate of *Salmonella* (1st-22thcohort) in pigs and environmental samples at different stages of production.

Level	Sample	Number of positive samples (Percentage (%))																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
Farm	F	7 (70)	7 (70)	5 (50)	7 (70)	7 (70)	8 (80)	7 (87.5)	7 (87.5)	5 (62.5)	4 (50)	7 (87.5)	5 (62.5)	6 (60)	4 (50)	5 (50)	3 (30)	5 (62.5)	2 (25)	4 (50)	4 (50)	5 (62.5)	5 (62.5)
	Water	1^{W3} (33.3)	1^{W3} (33.3)	$3^{W1,2,3}$ (100)	1^{W3} (33.3)	$2^{W1,3}$ (66.6)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	$2^{W2,3}$ (66.6)	1^{W3} (33.3)	1^{W3} (33.3)	ND	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	1^{W3} (33.3)	$3^{W1,2,3}$ (100)
Abattoir	Overshoes swabs	8 (100)	6 (85.7)	6 (85.7)	7 (100)	7 (100)	7 (100)	7 (100)	7 (100)	6 (85.7)	7 (100)	7 (100)	7 (100)	7 (100)	6 (85.7)	7 (100)	7 (100)	7 (100)	5 (71.4)	7 (100)	7 (100)	5 (71.4)	7 (100)
	CC	6 (75)	9 (90)	10 (100)	10 (100)	10 (100)	NA	8 (100)	8 (100)	4 (50)	8 (100)	7 (87.5)	7 (100)	7 (70)	7 (87.5)	8 (80)	8 (80)	3 (37.5)	4 (50)	6 (75)	8 (100)	6 (75)	7 (87.5)
	ML	6 (75)	8 (80)	8 (80)	10 (100)	6 (60)	NA	7 (87.5)	8 (100)	3 (37.5)	5 (62.5)	3 (37.5)	2 (28.5)	8 (80)	6 (75)	4 (40)	1 (10)	5 (62.5)	3 (37.5)	6 (75)	5 (62.5)	5 (62.5)	7 (87.5)
	CS	6 (75)	3 (30)	2 (20)	8 (80)	5 (50)	NA	3 (37.5)	2 (25)	3 (37.5)	2 (25)	1 (12.5)	ND	7 (70)	1 (12.5)	1 (10)	5 (50)	1 (12.5)	1 (12.5)	1 (12.5)	1 (12.5)	5 (62.5)	2 (25)
	CW	ND	ND	2 (20)	1 (11.1)	1 (10)	NA	1 (12.5)	ND	ND	2 (25)	ND	ND	3 (30)	ND	2 (20)	3 (30)	ND	1 (12.5)	3 (37.5)	1 (12.5)	3 (37.5)	1 (12.5)
	FP	NA	4 (40)	7 (70)	7 (70)	5 (50)	NA	2 (25)	4 (50)	2 (25)	8 (100)	4 (50)	ND	10 (100)	2 (25)	4 (40)	6 (60)	3 (37.5)	1 (12.5)	8 (100)	4 (50)	7 (87.5)	8 (100)
Environment	Prior to cutting	NA	ND	1^{P} (20)	ND	ND	NA	ND	ND	1^{P} (20)	ND	1^{P} (20)	ND	ND	ND	1^{K} (20)	ND	ND	ND	ND	ND	ND	ND
	During cutting	NA	$2^{B,K}$ (40)	1^{B} (20)	ND	$2^{B,P}$ (40)	NA	$2^{P,K}$ (40)	1^{B} (20)	ND	$2^{B,P}$ (40)	$2^{B,K}$ (40)	1^{K} (20)	1^{P} (20)	1^{B} (20)	1^{P} (20)	1^{B} (20)	1^{P} (20)	ND	$2^{B,K}$ (40)	ND	$3^{B,P,K}$ (60)	ND
	After C&D	NA	1^{B} (20)	1^{B} (20)	1^{B} (20)	1^{B} (20)	NA	ND	1^{B} (20)	ND	ND	ND	1^{B} (20)	ND	ND	$2^{B,P}$ (20)	1^{K} (20)	1^{B} (20)	ND	1^{B} (20)	$2^{B,P}$ (20)	$2^{B,S}$ (40)	1^{B} (20)
Retail	TP	NA	5 (50)	7 (70)	9 (90)	8 (80)	NA	2 (25)	5 (62.5)	3 (37.5)	8 (100)	8 (100)	6 (85.7)	10 (100)	8 (100)	8 (80)	6 (60)	6 (75)	ND	8 (100)	5 (62.5)	5 (62.5)	5 (62.5)
	R	NA	2 (20)	4 (40)	1 (10)	2 (20)	NA	1 (10)	2 (20)	2 (20)	10 (100)	5 (62.5)	2 (20)	4 (40)	2 (20)	ND	1 (10)	3 (30)	ND	10 (100)	4 (40)	4 (40)	2 (20)

ND, not determined; NA, not available;
W1, Drinking water; W2, Cleaning water; W3, water from slurry (waste water);
B, Cutting board; P, Personal hygiene; K, Knife; S, Shackle, C, Plastic curtain.
F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; TP=Transported pork; R=Retail

The fluctuation of *Salmonella* depending on stage and environment is shown in Figure 4.1. Some sample categories included values deviating from other results, shown as bullets (Figure 4.1).

Tracking of *Salmonella* infection/contamination of 22 cohorts is presented in Figure 4.2, visualizing the frequency of positive samples by density. Columns for caecal content and "Transported pork" are dense, whereas columns "Carcass after splitting" and "Carcass after washing" show much lower frequencies of positive results. It is also obvious, that several cohorts had lower *Salmonella* prevalence in mesenteric lymph nodes (9, 11, 12, 15, 16, and 18) than others.

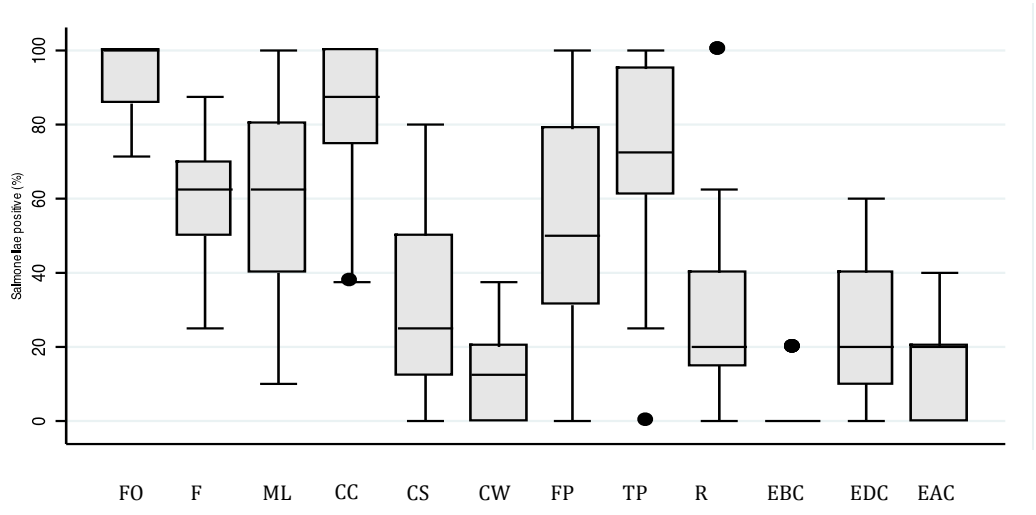


Figure 4.1: Box-and-whisker plots reflecting *Salmonella* findings from the individuals and from related environmental samples; FO=Overshoe swabs; F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; TP=Transported pork; R=Retail; EBC=Environment (cutting boards, hand swabs, knife shackles and plastic curtains) before cutting; EDC=Environment during cutting; EAC=Environment after C&D.

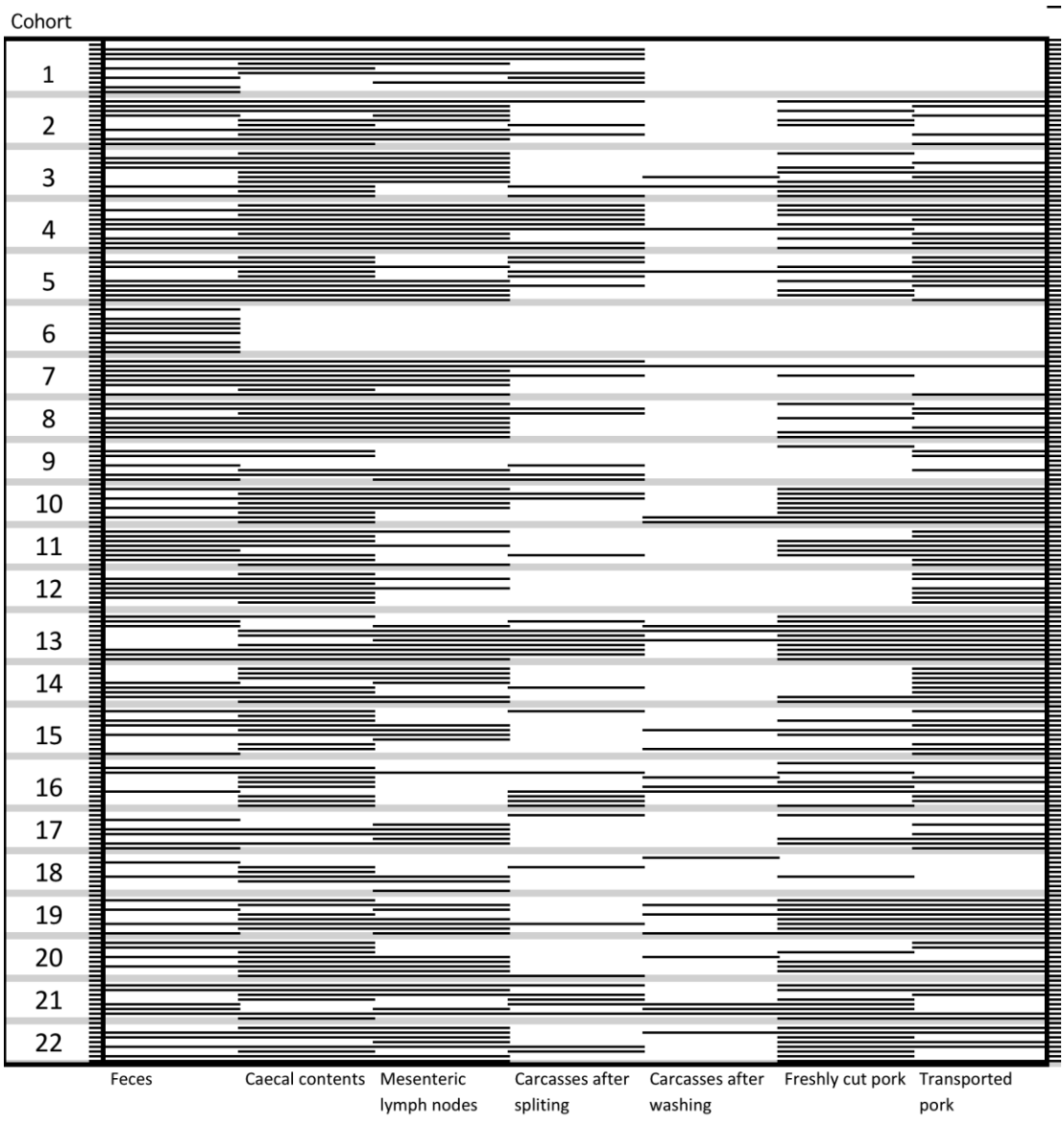


Figure 4.2: Tracking of *Salmonella* infection/contamination of 22 cohorts from Northern Thailand. One bar represented one individual positive sample.

4.2 Correlation of samples prevalence: pairwise comparisons among sample types

Here, each sample type was paired with the others. Correlation coefficients (r_s) and *p*-values are shown in Figure 4.3 and Table 4.4, respectively. 28 pairs were available with r_s indicating the strength of relation. The highest correlation coefficient, i.e., highest relationship, was found between CW and FP (r_s = 0.66; P = 0.0014), which indicated that the carcass quality after splitting related with FP quality.

Other positive associations with a $P \leq 0.05$ in Table 4.4 were

1) FP and R (r_s = 0.5465),

2) TP and R (r_s = 0.5276),

3) CS and FP (r_s= 0.4289).

These results showed correlations between raw material before processing (carcasses and/ or pork cutting) and the R product (Figure 4.3 and Table 4.4).

Another point of interest was the relationship between faeces and other samples: they were positive as related to lymph nodes and caecal samples. However, they were negative as related to samples taken from carcasses being processed, indicating that the processing technology was hygienically acceptable.

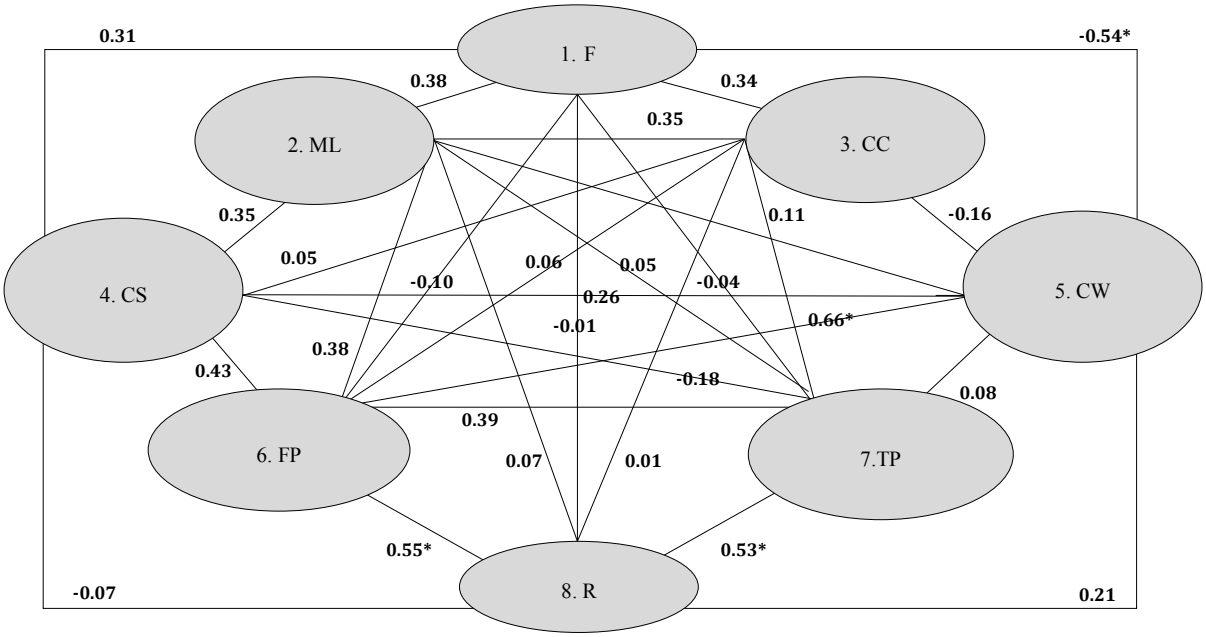


Figure 4.3: Spearman's correlation coefficients between samples at different stages of production (22 sampling cohorts); F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; TP=Transported pork; R= Retail-ready pork

Table 4.4: *P*-values from Spearman's correlation coefficients (r_s) among *Salmonella* prevalence in eight sample types collected from Chiang Mai, Thailand

Samples collected at slaughter and retail	Collection point						
	Retail	Cutting	Slaughter				Farm
	TP	FP	CW	CS	CC	ML	F
R	***0.01***	***0.01***	0.36	0.75	0.95	0.77	0.96
TP		0.08	0.72	0.43	0.64	0.83	0.85
FP			***<0.01***	***0.05***	0.79	0.09	0.67
CW				0.51	0.69	0.97	***<0.01***
CS					0.93	0.10	0.11
CC						0.16	0.20
ML							0.07

Approximately 8-10 pigs were sampled from each herd.
F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; TP=Transported pork; R=Retail-ready pork

4.3 Relative risk (RR) and strength of agreement between samples: Individual pig level

Table 4.5 shows the occurrence of *Salmonella* in a given sample (outcome) as influenced by the presence/absence of the same animal in another sample. All relative risks (RR) are higher than 1.0, which indicates that the *Salmonella* status from a previous step of sampling is positively associated with status of outcome in a consequent sampling. However, only the RR between the *Salmonella* status of CW and FP was statistically significant. The ratio of *Salmonella* in FP in carcasses already contaminated after washing was about 1.64 times (95 % CI: 1.294-2.089) higher than the that in FP in non-exposed (*Salmonella*-free [12]) CW. From 77 *Salmonella*-free FP samples, 73 samples of *Salmonella*-free FP (94.8 % of total) were from *Salmonella*-free carcasses. On the other hand, if CW were still contaminated (24), a high change to get contaminated FP was remarked (20).

Based on the McNemar test (lower right-angled triangle of Table 4.6) between 21 pairs of sample types were tested for the two marginal probabilities for each sample are the same. Among them, 17 sample pairs were significantly different ($p < 0.05$) from each other and 4 sample pairs showed no significant difference (the marginal proportions were not significantly different from each other) and it indicated that no change of results was found after various processing steps during sample collection.

[12] *Salmonella* not found by bacteriological testing

Table 4.5:2-way contingency table and measure of associations (relative risk) between *Salmonella* exposure status and outcome of *Salmonella* detection

Outcome	Status	F– Exposure status Negative	Positive	Sum	*Relative risk* (95% CI)	P-value
Salmonella in CC	Absent	12	17	29	1.016 (0.890-1.159)	0.817
	Present	59	92	151		
		71	109	180		

Outcome	Status	F- Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella in ML	Absent	31	33	64	1.238 (0.974-1.572)	0.081
	Present	40	76	116		
		71	109	180		

Outcome	Status	CC- Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella in ML	Absent	16	49	65	1.447 (0.972-2.156)	0.069
	Present	14	102	116		
		30	151	181		

Outcome	Status	ML -Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella on CS	Absent	44	77	121	1.041 (0.674-1.608)	0.858
	Present	21	39	60		
		65	116	181		

Outcome	Status	CC-Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella on CS	Absent	21	100	121	1.492 (0.844-2.638)	0.169
	Present	9	51	60		
		30	151	181		

Outcome	Status	CS -Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella on CW	Absent	106	50	156	1.231 (0.572-2.646)	0.595
	Present	15	9	24		
		121	59	180		

Outcome	Status	CW -Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella in FP	Absent	73	4	77	1.644 (1.294-2.089)	***<0.0005***
	Present	75	20	95		
		148	24	172		

Outcome	Status	FP -Exposure status Negative	Positive	Sum	Relative risk (95% CI)	*P-value*
Salmonella in TP	Absent	27	24	51	1.155 (0.945-1.412)	0.159
	Present	50	72	122		
		77	96	173		

Table 4.6: A crosstab of Kappa index and *P*-value for McNemar test from *Salmonella* positive test results of slaughtered pigs, carcasses and pork at individual animal basis

		Kappa index						
		F	ML	CC	CS	CW	FP	TP
P-value for	F		0.15	0.00	<0.00	<0.00	<0.00	<0.00
McNemar test	ML	0.42		0.13	<0.00	<0.00	0.16	0.03
	CC	<0.00	<0.00		0.03	<0.00	0.04	0.12
	CS	<0.00	<0.00	<0.00		0.05	0.11	0.03
	CW	<0.00	<0.00	<0.00	<0.00		0.15	0.02
	FP	0.45	0.12	<0.00	<0.00	<0.00		0.10
	TP	0.05	0.20	<0.00	<0.00	<0.00	<0.00	

F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP= Freshly cut pork; TP=Transported pork.

The strength of agreement by Kappa (κ) statistic (Table 4.6, the right-angled triangle) in the relationship between 21 pairs of variables determined the consistency of the *Salmonella* results (positive/negative) in each individual animal. In most cases, only a weak concordance was seen between in this study (poor agreement: $\kappa \leq 0.0$; slight agreement: $\kappa = 0.00\text{-}0.20$). The highest agreement ($\kappa = 0.16$) was obtained between *Salmonella* status in ML and *Salmonella* status in FP.

4.4 Univariable and Multivariable analyses

In a univariable analysis (by logistic regression), some available variables showed an effect on outcomes of variables as followed: overshoes at herd level and cutting board during cutting were associated with *Salmonella* status in TP; ML, CW and cutting board during cutting associated with *Salmonella* status in FP; F and CC status associated with *Salmonella* status in ML; drinking water and ML status associated with *Salmonella* status in CC, as shown in Table 4.7.

- Detection of *Salmonella* in CC was influenced by the presence of *Salmonella* in drinking water at herd level (OR = 6.2; 95% CI: 0.8 – 47.9; *P* = 0.022) and in ML (OR = 2.3; 95% CI: 1.0 – 5.2; *P* = 0.045).
- Detection of *Salmonella* in ML was influenced by the presence of *Salmonella* in F at pig farm level (OR = 2.1; 95% CI: 1.1 – 3.8; *P* = 0.030) and in ML (OR = 2.3; 95% CI: 1.0 – 5.2; *P* = 0.045).
- Detection of *Salmonella* in FP was influenced by 4 variables: cleaning water at heard level (OR = 2.5; 95% CI: 0.9 – 6.3;*P* = 0.042), ML at pig level (OR = 2.0; 95% CI: 1.1 – 3.7;*P* = 0.030), CW at pig level (OR = 4.9; 95% CI: 1.9 – 14.9;*P* = 0.001), and cutting board during cutting (OR = 1.8; 95% CI: 1.0 – 3.4;*P* = 0.049).
- Detection of *Salmonella* in overshoe swabs and in cutting board during cutting increased odds ratios of *Salmonella* detection in TP (OR = 3.0; 95% CI: 1.5 – 5.9;*P* = 0.002 and OR = 2.0; 95% CI: 1.0 – 3.9;*P* = 0.041, respectively).

Multivariable analyses using logistic regression equations with herd as random effect are shown in Table 4.7, too. Only some factors, which showed a significant effect with the univariable analysis, were accounted into the multivariable analyses. Detection of *Salmonella* on CW increased the odds (OR = 3.9) of FP. *Salmonella* from cutting boards during cutting increased odds of TP (OR = 3.9) significantly. Additionally, *Salmonella* on overshoes at fattening farms positively increased odds of *Salmonella* in TP (OR = 5.5; *P* = 0.01), as well as detection of *Salmonella* in ML increased the odds of *Salmonella* findings in CC (OR = 2.3) and that of contaminated FP (OR = 2.0) (*P* = 0.045 and 0.030, respectively).

Table 4.7: Results of the univariable and multivariable analyses on the 172 fattening pigs (*Salmonella* status of caecal content, mesenteric lymph nodes, carcass after spitting, carcass after washing, freshly cut pork and transported pork as outcome variables)

	Univariable			Multivariable		
	Odds ratio	95 % Confidence interval	*P*-value	Odds ratio	95% Confidence interval	*P*-value
Caecal contents						
Faeces at pig level	1.14	0.50-2.60	0.747			
Overshoes at herd level	1.95	0.85-4.48	0.120			
Drinking water at herd level	6.23	0.81-47.88	0.022			
Cleaning water at herd level	2.60	0.58-11.69	0.164			
Waste water at herd level	0.72	0.09-6.13	0.759			
Mesenteric lymph nodes at pig level	2.31	1.02-5.24	0.045			
Mesenteric lymph nodes						
Faeces at pig level	2.01	1.07-3.78	0.030			
Overshoes at herd level	0.99	0.51-1.95	0.987			
Drinking water at herd level	1.91	0.76-4.78	0.152			
Cleaning water at herd level	1.36	0.55-3.34	0.496			
Waste water at herd level	0.56	0.11-2.88	0.472			
Caecal content at pig level	2.31	1.02-5.24	0.045			
Carcass after splitting						
Faeces at pig level	0.92	0.48-1.78	0.803			
Overshoes at herd level	1.14	0.56-2.23	0.711			
Drinking water at herd level	1.08	0.45-2.57	0.868			
Cleaning water at herd level	0.49	0.17-1.37	0.149			
Waste water at herd level	3.25	0.39-27.10	0.214			
Mesenteric lymph nodes at pig level	0.93	0.48-1.82	0.840			
Caecal content at pig level	1.41	0.55-3.55	0.459			
Carcass after washing						
Faeces at pig level	0.63	0.26-1.49	0.290			
Overshoes at herd level at pig level	1.35	0.50-3.63	0.541			
Drinking water at herd level	1.03	0.32-3.30	0.955			
Cleaning water at herd level	0.78	0.21-2.82	0.693			
Waste water at herd level	Nc	Nc	Nc			
Mesenteric lymph nodes at pig level	0.64	0.27-1.53	0.318			
Caecal content at pig level	0.40	0.15-1.09	0.084			
Carcass after splitting at pig level	1.42	0.58-3.48	0.451			
Freshly cut pork						
Faeces at pig level	0.92	0.50-1.69	0.780			
Overshoes at herd level	1.89	0.98-3.65	0.056			
Drinking water at herd level	2.30	0.95-5.56	0.055			
Cleaning water at herd level	2.50	0.99-6.31	0.042			
Waste water at herd level	3.93	0.77-20.05	0.075			
Mesenteric lymph nodes at pig level	1.99	1.06-3.74	0.030	2.01	0.88-4.59	0.097
Caecal content at pig level	1.29	0.57-2.89	0.543			
Carcass after splitting at pig level	1.70	0.87-3.31	0.114			
Carcass after washing at pig level	4.87	1.59-14.93	0.001	3.94	1.07-14.57	0.039
Personal hygiene before cutting	0.78	0.34-1.80	0.561			
Knife before cutting	0.52	0.14-1.91	0.319			
Personal hygiene during cutting	1.64	0.87-3.09	0.127			
Cutting board during cutting	1.83	1.00-3.38	0.049			
Knife during cutting	0.79	0.41-1.53	0.483			
Transported pork						
Faeces at pig level	0.95	0.49-1.85	0.875			
Overshoes at herd level	2.95	1.48-5.91	0.002	5.54	1.39-22.10	0.015
Drinking water at herd level	1.06	0.44-2.60	0.890			
Cleaning water at herd level	1.49	0.56-3.95	0.416			
Waste water at herd level	Nc	Nc	Nc			
Mesenteric lymph nodes at pig level	1.17	0.60-2.30	0.648			
Caecal content at pig level	2.02	0.87-4.65	0.103			
Carcass after splitting at pig level	1.25	0.61-2.59	0.532			
Carcass after washing at pig level	1.31	0.49-3.52	0.585			
Personal hygiene before cutting	0.94	0.38-2.32	0.892			
Knife before cutting	1.73	0.35-8.47	0.475			
Personal hygiene during cutting	1.52	0.75-3.08	0.235			
Cutting board during cutting	1.99	1.02-3.87	0.041	3.86	1.05-14.16	0.042
Knife during cutting	0.94	0.46-1.93	0.862			
Freshly cut pork at pig level	1.56	0.83-3.09	0.162			
Personal hygiene after C&D	1.11	0.37-3.29	0.853			
Cutting board after C&D	1.39	0.71-2.70	0.334			
Knife after C&D	0.61	0.17-2.27	0.472			

Nc: Model not able to convert.

4.5 *Salmonella* serotypes

From 971 positive samples, 1,000 *Salmonella* isolates were available for serotyping (one to a maximum of five isolates from each sample) in order to acquire serovars. The distribution of *Salmonella* serovars among the 22 cohorts is shown in Table 4.9. Among them, *Salmonella* serovars in each individual pig are shown in Table 4.8. Overall, 26 serovars were identified in this study. *Salmonella* Rissen was the predominant serovar (45.9 %), and was found in all cohorts (Table 4.9). With 44 isolates (4.4 %), the highest number of *S.* Rissen isolates was found in cohort 8 and 19, whereas the lowest amount of *S.* Rissen occurred in the cohort number 16 with 4 (0.4%) samples.

The highest number of *S.* Rissen was detected from mesenteric lymph nodes with 68 samples (Table 4.10) accounting to 14.6%. Simultaneously, from the caecal contents and from faecal samples no different numbers were obtained (67 of each sample type). *Salmonella* Typhimurium (17.4%) and *Salmonella* Stanley (9.2%) were common serotypes, too. These two serotypes were detected in 20th and 19th cohorts, respectively, from total 22 cohorts enrolled in this study.

7 serovars (*Salmonella* Anatum, Krefeld, Panama, Rissen, Stanley, Typhimurium and Weltevreden) and the 3 most common serotypes (Rissen, Typhimurium and Stanley) were identified throughout the complete chain (farms, slaughterhouse and R samples). 11 serovars (*Salmonella* Afula, Agona, Alfort, Bovismorbificans, Chittagong, Corvallis, Derby, Hato, Israel, Langensalza, Regent and Rideau) were detected only in farm samples including faeces from animal entering slaughterhouse, whereas 3 serovars (*Salmonella* Eppendorf, Livingstone and Tsevie) were detected only from

samples taken from the slaughterhouse. One serovar (Enteritidis) was isolated only from 1 R sample and could not be detected from any other place (Table 4.8 and 4.9).

Table 4.8: Distribution of *Salmonella* serotypes along the pork chain of the same animal in different samples

Cohort No.	Pig ID	F	ML	CC	CS	CW	FP	TP	R
2	11	Rissen	Rissen	Rissen	Panama		Panama	Rissen/Panama	Rissen
	12	Rissen	Weltevreden	Rissen				Rissen	Panama
	13	Stanley	Rissen	Weltevreden			Panama/Typhimurium		Panama
	14	Stanley	Gloucester					Panama	
	15		Lagos	Typhimurium			Panama/Typhimurium		
	16			Rissen	Rissen		Rissen		
	17	Rissen	Rissen	Rissen					
	18		Gloucester	Stanley	Rissen			Panama	
	19	Rissen	Rissen	Rissen					
	20	Typhimurium		Rissen				Rissen	
3	21		Gloucester	Typhimurium			Rissen		Rissen
	22	Anatum	Typhimurium	Rissen					Rissen
	23	Panama	Typhimurium	Typhimurium				Rissen	Rissen
	24	Rissen	Rissen	Rissen			Anatum		Anatum
	25		Rissen	Stanley			Krefeld	Krefeld	
	26		Rissen	Panama		Rissen		Rissen	
	27		Rissen	Rissen			Rissen	Krefeld	
	28	Panama		Typhimurium	Krefeld	Rissen	Rissen	Rissen	
	29			Panama			Rissen	Rissen	
	30	Rissen	Panama	Anatum	Stanley		Rissen	Rissen	
4	31		Typhimurium	(F-67)	Rissen		(F-67)	(F-67)	Lagos
	32	Typhimurium	(F-67)	Lagos	(F-67)		(F-67)	(F-67)	
	33		(F-67)	(F-67)	(F-67)		(F-67)	(F-67)	
	34	(F-67)	Gloucester	(F-67)	(F-67)			(F-67)	
	35	(F-67)	(F-67)	(F-67)	(F-67)		(F-67)	(F-67)	
	36	(F-67)	Rissen	(F-67)	Rissen	Rissen	Rissen		
	37		Rissen	Rissen				Rissen	
	38	Rissen	Rissen	(F-67)			Rissen	(F-67)	
	39	Rissen	Rissen	Rissen	Rissen			Rissen	
	40	(F-67)	Rissen	Rissen	(F-67)		(F-67)	Rissen	
5	41			Rissen	Rissen			Typhimurium	Krefeld
	42	Rissen		Rissen	Tsevie			Rissen	Tumodi
	43	Rissen	Rissen	Lagos			Rissen	Lagos	
	44			Rissen	Typhimurium	Rissen	Rissen	Rissen	
	45			Typhimurium	Panama			Typhimurium/Tumodi	
	46	Rissen	Panama	Panama			Panama	Panama/Typhimurium	
	47	Panama	Panama	Stanley	Rissen			Typhimurium	

Table 4.8: Distribution of *Salmonella* serotypes along the pork chain of the same animal in different samples

Cohort No.	Pig ID	F	ML	CC	CS	CW	FP	TP	R
	48	Stanley	Panama	Rissen			Typhimurium		
	49	Rissen	Panama	Stanley			Typhimurium/Tumodi		
	50	Rissen	Panama	Rissen				Typhimurium	
7	61	Rissen	Rissen	Stanley	Typhimurium				Rissen/Typhimurium
	62	Agona	Rissen	Rissen	Typhimurium		Typhimurium/Lagos	Typhimurium	Rissen/Typhimurium
	63	Rissen	Rissen	Rissen					Rissen
	64	Rissen	Lagos	Rissen	Typhimurium		Rissen		
	65	Rissen	Weltevreden	Rissen					
	66	Rissen	Stanley	Rissen					
	67			Stanley					
	68	Rissen	Rissen	Stanley				Rissen	
8	69	Rissen	Rissen	Rissen			Rissen		Rissen
	70	Rissen	Rissen	Rissen	Rissen			Rissen/Typhimurium	Rissen/Typhimurium /Enteritidis
	71		Rissen	Rissen	Rissen			Rissen	Rissen/Typhimurium
	72	Rissen	Rissen	Rissen			Rissen		
	73	Stanley	Rissen	Rissen					
	74	Rissen	Rissen	Rissen				Rissen	
	75	Rissen	Rissen	Rissen			Rissen	Rissen/Typhimurium	
	76	Rissen	Rissen	Rissen			Rissen	Rissen/Typhimurium	
9	77						Agama		Typhimurium
	78	Rissen		Anatum				Rissen	Rissen
	79	Typhimurium		Rissen				Rissen	
	80								
	81	Regent			Rissen				
	82		Rissen	Stanley				Rissen	
	83	Typhimurium	Rissen	Typhimurium	Rissen		Unidentified		
	84	Rissen	Rissen		Rissen				
10	85	Rissen	Gloucester	Krefeld			Krefeld	Krefeld	Krefeld
	86		Krefeld	Krefeld	Rissen		Krefeld	Rissen	Krefeld
	87	Stanley	Rissen	Rissen	Krefeld		Rissen	Rissen	Krefeld
	88		Rissen	Rissen			Krefeld	Krefeld	Krefeld
	89	Rissen	Rissen	Eppendorf			Rissen	Krefeld	Krefeld
	90			Unidentified			Krefeld	Rissen	Krefeld
	91	Stanley		Gloucester		Stanley	Rissen	Krefeld	Rissen
	92			Krefeld		Unidentified	Lagos	Krefeld	Rissen
11	93	Rissen	Rissen	Rissen				Krefeld	Krefeld
	94	Rissen		Typhimurium				Typhimurium	Lagos
	95	Rissen		Rissen			Rissen	Rissen	Lagos

Table 4.8: Distribution of *Salmonella* serotypes along the pork chain of the same animal in different samples

Cohort No.	Pig ID	F	ML	CC	CS	CW	FP	TP	R
	96	Rissen	Typhimurium	Rissen			Lagos	Lagos	Lagos
	97	Rissen					Lagos	Krefeld	
	98	Rissen		Rissen	Rissen		Rissen	Krefeld	
	99	Rissen		Rissen				Krefeld	
	100		Rissen	Typhimurium				Krefeld	
12	101			Rissen				Rissen	Rissen
	102	Rissen	Rissen	Rissen				Rissen	Rissen
	103	Stanley		Rissen					Rissen
	104	Stanley	Rissen	Rissen				Rissen	Rissen
	105	Stanley		Stanley				Rissen	Rissen
	106	Rissen		Rissen				Rissen	Krefeld
	107			Rissen				Krefeld	
	108								
13	109	Rissen		Typhimurium			Typhimurium	Typhimurium	Rissen
	110	Rissen			Rissen		Typhimurium	Typhimurium	Rissen
	111	Rissen	Typhimurium			Typhimurium	Typhimurium	Typhimurium	Typhimurium
	112		Typhimurium	Typhimurium	Typhimurium	Typhimurium	Typhimurium	Typhimurium	
	113		Typhimurium	Rissen	Typhimurium		Typhimurium	Typhimurium	
	114		Rissen		Typhimurium	Typhimurium	Typhimurium	Typhimurium	
	115		Rissen	Typhimurium	Typhimurium		Typhimurium	Typhimurium	
	116	Typhimurium	Typhimurium	Typhimurium	Typhimurium		Typhimurium	Typhimurium	
	117	Typhimurium	Typhimurium	Stanley	Typhimurium		Typhimurium	Typhimurium	
	118	Rissen	Typhimurium	Stanley			Typhimurium	Typhimurium	
14	119		Typhimurium	Stanley				Rissen	Rissen
	120		Stanley	Stanley				Weltevreden	Rissen
	121		Stanley	Stanley				Weltevreden	Weltevreden
	122	Bovismorbificans	Stanley					Weltevreden	
	123	Bovismorbificans		Stanley	Stanley			Rissen	
	124	Stanley		Rissen				Rissen	
	125	Stanley	Anatum	Stanley			Rissen	Rissen	
	126		Stanley	Weltevreden			Unidentified	Weltevreden	
15	127	Chittagong		Panama	Gloucester			Rissen	
	128			(F-67)					
	129	Stanley		Anatum			Rissen	Krefeld	
	130	Rissen	Stanley	Weltevreden				Krefeld	
	131		Stanley	Weltevreden		Stanley	Typhimurium	Typhimurium	
	132	Bovismorbificans	Anatum	Rissen			Krefeld	Krefeld	
	133		Rissen						
	134			Panama				Rissen	

Table 4.8: Distribution of *Salmonella* serotypes along the pork chain of the same animal in different samples

Cohort No.	Pig ID	F	ML	CC	CS	CW	FP	TP	R
	135			Panama		Typhimurium	Typhimurium	Typhimurium	
	136	Corvallis						Typhimurium	
16	137						Lagos	Lagos	Agama
	138	Typhimurium		Tsevie					
	139	Typhimurium	Rissen	Typhimurium	Gloucester		Lagos		
	140			Gloucester		Typhimurium		Gloucester	
	141			Weltevreden			Gloucester	Typhimurium/Agama	
	142			Stanley		Gloucester	Typhimurium		
	143	Rissen			Gloucester	Lagos	Agama	Typhimurium	
	144			Typhimurium	Lagos			Gloucester	
	145			Gloucester	Lagos			Typhimurium	
	146			Gloucester	Gloucester		Typhimurium		
17	147				Gloucester		Typhimurium	Typhimurium	Typhimurium/Stanley
	148	Typhimurium							Stanley
	149		Rissen					Gloucester	Agama
	150	Rissen	Rissen	Rissen					
	151	Rissen	Rissen	Rissen				Typhimurium	
	152		Gloucester				Lagos	Lagos	
	153	Typhimurium	Rissen	Rissen			Typhimurium	Typhimurium	
	154	Rissen						Agama	
18	155								
	156	Rissen							
	157			Unidentified	Rissen				
	158			Rissen					
	159	Stanley	Rissen	Rissen	Rissen		Stanley		
	160		Rissen	Rissen					
	161								
	162		Unidentified						
19	163	Stanley		Rissen			Rissen	Rissen	Rissen
	164		Rissen	Rissen		Rissen	Rissen	Rissen	Rissen
	165	Stanley	Rissen				Rissen	Rissen	Rissen
	166			Rissen		Rissen	Rissen	Rissen	Rissen
	167		Unidentified	Stanley			Rissen	Rissen	Rissen
	168	Rissen	Stanley	Stanley	Stanley		Rissen	Rissen	Rissen
	169		Rissen	Typhimurium			Rissen	Rissen	Rissen
	170	Stanley	Stanley			Rissen	Rissen	Rissen	Typhimurium
20	171	Rissen		Gloucester				Rissen	Typhimurium
	172	Rissen		Anatum				Stanley	Rissen
	173			Anatum			Rissen		Rissen

Table 4.8: Distribution of *Salmonella* serotypes along the pork chain of the same animal in different samples

Cohort No.	Pig ID	F	ML	CC	CS	CW	FP	TP	R
	174	Rissen	Rissen	Anatum		Unidentified			Rissen
	175		Anatum	Anatum			Rissen	Rissen	
	176	Anatum	Rissen	Lagos			Rissen	Stanley	
	177		Anatum	Stanley			Rissen	Stanley	
	178		Rissen	Stanley	Rissen				
21	179	Rissen	Rissen	Stanley	Rissen		Stanley	Stanley	Stanley
	180	Rissen	Rissen	Rissen			Rissen	Stanley	Stanley
	181		Rissen	Rissen	Rissen			Stanley	Typhimurium
	182			Stanley	Rissen		Rissen		Rissen
	183	Typhimurium			Rissen	Rissen	Rissen		
	184	Typhimurium	Rissen			Rissen	Rissen		
	185	Agona	Rissen	Stanley	Rissen	Rissen	Rissen	Rissen	
	186			Tsevie			Rissen	Stanley	
22	187		Rissen	Rissen			Rissen	Stanley	Stanley
	188	Weltevreden	Weltevreden	Unidentified		Rissen	Stanley	Weltevreden	Stanley
	189	Rissen	Stanley	Rissen			Rissen		
	190		Rissen				Rissen	Anatum	
	191	Weltevreden	Rissen	Rissen	Anatum		Rissen	Stanley	
	192			Rissen	Rissen		Rissen		
	193	Rissen	Rissen	Weltevreden			Rissen		
	194	Weltevreden	Rissen	Rissen			Typhimurium	Typhimurium	

F=Faecal; ML=Mesenteric lymph nodes; CC=Caecal content; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; TP=Transported pork; R=Retail

Table 4.9: Number of *Salmonella* serovar isolates from 22 cohorts, Northern Thailand, 2004/2005

Serotypes	Number of isolates among batch per serotype																						Total	%
	Cohort																							
	1*	2	3	4	5	6**	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	(n)	
Afula			1									1											2	0.2
Agama									1							3	2						6	0.6
Agona							1		1										1		1		4	0.4
Alfort			1													1							2	0.2
Anatum			4						1					1	2					9		2	19	1.9
Bovismorbificans														3	2								5	0.5
Chittagong															1								1	0.1
Corvallis													1		4	1							6	0.6
Derby								1															1	0.1
Enteritidis								1															1	0.1
Eppendorf										1													1	0.1
Gloucester		2	1	1						2					1	10	3			1			21	2.1
Hato															1								1	0.1
Israel					1																		1	0.1
Krefeld	10		4		1					22	7	1			5								50	5.0
Lagos		1		2	2		3			1	7			1	1	7	2			1			28	2.8
Langensalza					1																		1	0.1
Livingstone		1																					1	0.1
Panama		12	5		12										3								32	3.2
Regent			1		1				1														3	0.3
Rideau	1																						1	0.1
Rissen	20	29	29	20	20	16	28	44	15	21	20	24	13	9	8	4	12	8	44	23	30	21	458	45.9
Stanley	3	3	2		5		4	1	3	4		4	2	12	8	1	2	4	8	5	13	6	87	8.7
Tsevie					1											2					1		4	0.4
Tumodi					4																		4	0.4
Typhimurium		6	8	4	13		10	5	4	4	13	2	48	1	6	15	16	3	3	3	6	2	172	17.2
Weltevreden		3			1		1		2					7	4	1						10	29	2.9
S. O:3,10 H:e,h			3																				3	0.3
S. O:3,10 H:z_6																						6	6	0.6
S. O:3,15 H:f,g,r														3									3	0.3
Unidentified (A-E)			1	1					2	2				1				2	1	1		1	12	1.2
Unidentified (F-67)				34											1								35	3.5
Total (n)	34	56	60	62	62	16	47	52	30	57	47	32	64	38	44	45	37	17	57	43	51	48	1000	
In percent (%)	3.4	5.6	6.0	6.2	6.2	1.6	4.7	5.2	3.0	5.7	4.7	3.2	6.4	3.8	4.4	4.5	3.7	1.7	5.7	4.3	5.1	4.8		100.0
Ratio isolates/serotype	8.5	7.0	5.6	6.7	5.1	16.0	7.8	10.4	3.5	7.8	11.7	8.0	16.0	4.8	3.3	4.5	6.1	5.0	14.0	7.0	10.2	8.2	34.8	

*)Samples were obtained from farm to carcass after washing;**)Samples were obtained from farm only.

Table 4.10: Distribution of *Salmonella*, sample type and place related, Northern Thailand, 2004/2005

Serotypes	Number of isolates among type of sample type per serotype																						
	Farm				Slaughterhouse and cutting room															R		Total	%
	FD	FC	FW	FO	F	CC	ML	CS	CW	FP	P1	P2	P3	B2	B3	K1	K2	K3	S3	TP	R		
Afula			1	1																		2	0.2
Agama										2										2	2	6	0.6
Agona			1	1	2																	4	0.4
Alfort		1		1																		2	0.2
Anatum				2	2	7	4	1		1										1	1	19	1.9
Bovismorbificans			2		3																	5	0.5
Chittagong					1																	1	0.1
Corvallis			2	3	1																	6	0.6
Derby				1																		1	0.1
Enteritidis																					1	1	0.1
Eppendorf						1																1	0.1
Gloucester						5	6	5	1	1										3		21	2.1
Hato				1																		1	0.1
Israel	1																					1	0.1
Krefeld				5	2	4	1	4		6			1							16	11	50	5.0
Lagos						3	2	2	1	7	1	1		2		1				4	4	28	2.8
Langensalza				1																		1	0.1
Livingstone														1								1	0.1
Panama				2	3	6	6	2		4				1	1		1			4	2	32	3.2
Regent			1	1	1																	3	0.3
Rideau					1																	1	0.1
Rissen			7	60	67	67	68	27	11	46	2	1	1	6	9		4		1	48	33	458	45.9
Stanley				8	18	25	10	3	2	3		1		1			1			9	6	87	8.7
Tsevie						2		1	1													4	0.4
Tumodi										1										2	1	4	0.4
Typhimurium		1	6	35	12	15	11	10	4	22	1	4		3	4		1	1		30	12	172	17.2
Weltevreden		1	1	7	3	6	3					1		1						5	1	29	2.9
S. O:3,10 H:e,h				3																		3	0.3
S. O:3,10 H:z_6	1		1	4																		6	0.6
S. O:3,15 H:f,g,r				3																		3	0.3
Unidentified (A-E)	1		1	1		3	2		2	2												12	1.2
Unidentified (F-67)				4	4	7	3	5		5					1					6		35	3.5
Total (n)	3	3	21	147	119	151	116	60	22	100	4	8	2	15	15	1	7	1	1	130	74	1000	
%	0.3	0.3	2.1	14.7	11.9	15.1	11.6	6.0	2.2	10.0	0.4	0.8	0.2	1.5	1.5	0.1	0.7	0.1	0.1	13.0	7.4		100
Ratio isolates/serotype	1.0	1.0	2.3	8.8	8.8	12.8	12.3	6.1	3.3	9.3	1.3	1.6	1.0	2.1	4.6	1.0	1.7	1.0	1.0	11.2	6.7	34.8	

FD=Drinking water; FC=Cleaning water; FW=Waste water; FO=Overshoes swab; F=Faecal, CC=Caecal content; ML=Mesenteric lymph nodes; CS=Carcass swabs after splitting; CW=Carcass swabs after washing; FP=Freshly cut pork; P=Personal hygiene; B=Cutting board; K=Knives; S=Shackle (1=Prior cutting; 2=During cutting; 3=After C&D); TP=Transported pork; R=R pork.

4.6 Pulsed-field gel electrophoresis

Pattern of variability among serotypes and sample origin are shown in Table 4.11 and 4.13, respectively. Available strains were analyzed and processed by BioNumerics®6.6. 83 pulsotypes[13] from3 27 strains were observed among twelve serotypes (Anatum, Chittagong, Stanley, Typhimurium, Rissen, Krefeld, Enteritidis, Panama, Livingstone, Bovismorbificans, Corvallis and Weltevreden), together with 12 unidentified strains (Table 4.11). 12-20 DNA fragments (bands) were obtained ranging from 33 to 1022 kb[14]. The origin of the 327 strains of *Salmonella* from the investigated pork chain is shown in Table 4.12.

Table 4.11: Number of studied isolates, PFGE patterns obtained for each serotype

Serotypes	Isolates	Pulsotypes	Ratio (isolate/pulsotype)
Rissen	169	31	5.5
Stanley	36	15	2.4
Typhimurium	53	10	5.6
Corvallis	6	5	1.2
Panama	23	4	5.8
Anatum	9	3	3.0
Krefeld	8	3	2.7
Weltevreden	3	2	1.5
Bovismorbificans	5	1	5.0
Chittagong	1	1	1.0
Enteritidis	1	1	1.0
Livingstone	1	1	1.0
Unidentified serotypes	12	6	2.0
Total	327	83	3.9

Dendrograms of PFGE patterns (pulsotypes) of *S.* Krefeld, *S.* Panama and *S.* Bovismorbificans were highly similar, while those of *S.* Stanley, *S*. Typhimurium, *S.* Rissen and *S.* Corvallis were highly diverse (Figure 4.4, 4.5, 4.6, 4.7, 4.8 and 4.9).

[13]Pulsotype is a pattern (PFGE-pattern) which shows distinguishability from other pulsotypes
[14] kb = kbp = kilo base pairs = 1,000 bp

Table 4.12: Pulsotype distribution of the 327 strains of *Salmonella* along the pork chain obtained from different samples

Cohort	Environment at farms[a]	Pig[c]	F	CC	ML	CS	CW	Environment prior cutting[b]	FP	TP	Environment during cutting[b]	R	Environment after C&D[b]
1	SKRX01(0)	1				SRX15							
	SKRX03(0)	2	SSTX11										
	SSTX11(0)	3		SRX09, 13		SRX09							
		7	SKRX02										
		8			SRX02								
		9	SKRX01										
2	SWEX02(3)	11		SRX19					SPAX01	SPAX01	SLIX01(B)	SRX02, 06	STYX09(B)
										SRX06, 11			SPAX01(B)
		12								SRX01, 5	SRX13(B),R15(B)	SPAX01, 02, 03	
		13	SSTX10						STYX09		STYX09(B)		
									SPAX01				
		14	SRX20							SPAX01	SPAX01(K)		
		15							STYX09				
									SPAX01				
		16							SRX011, 18				
		18								SPAX01, 04			
3	SRX13(0,3)	23						SRX23(P), 25(P)		SRX18	SRX18(B)	SRX18	SRX17(B)
	SANX03(0,2)	24			SRX18			STYX09(P)			STYX09(B)	SRX19	STYX09(B)
		27		SRX16	SRX18								
		28							SRX18, 22, 24				
		30							SRX18				
4		31						STYX07(0)	S. (F-67) U1				
		32							S. (F-67) U1,U2				
		36							SRX18				
		37								SRX18			
		38							SRX18				
		40								SRX18			
5	SRX13(0)	41								STYX09	STYX09(P)	STYX09	STYX08
	SSTX01(0),10(0)	42								SRX16			
		43			SRXR18				SRX18				
		44							SRX18	SRX12			
		45								STYX09			
		46								STYX09			
		48	SSTX08						STYX09				
		49							STYX09				
		50								STYX09			

Table 4.12: Pulsotype distribution of the 327 strains of *Salmonella* along the pork chain obtained from different samples

Cohort	Environment at farms[a]	Pig[c]	F	CC	ML	CS	CW	Environment prior cutting[b]	FP	TP	Environment during cutting[b]	R	Environment after C&D[b]
7		62					SSTX05		STYX01,09		STYX09(K,P)	STYX09	
		64		SRX18		STYX05			SRX18		SRX18(K)	SRX13, 18	
		66		SSTX05									
8		70								STYX09		STYX09	
		72							SRX18			SENX01	
		73	SSTX10										
		75							SRX18	STYX09			
		76								STYX09			
9	SSTX04(0),08(0) SRX13(0)	78	SRX13	SSTX05									
		79											
		84			SRX18								
10	STYX05(3) SSTX12(0)	87	SSTX07		SRX18							SRX27	
		88			SRX18								
		91	SSTX01						SRX18				
11	SSTX07(0), 08(0)	93		SRX02	SRX02						SRX02(B)		
		95		SRX02					SRX18	SRX02			
		96		SRX01									
		98		SRX02		SRX02							
		99		SRX31									
		100			SRX02								
12	SRX27(0) SSTX08(0)	101		SRX26	SRX26					SRX27		SRX28	
		102											
		103	SSTX08	SRX19									
		104	SSTX08		SRX26					SRX28			
		105	SSTX08							SRX27			
		106		SRX26									
13	SCOX01(0)	110				SRX19						SRX7	
14	SBOX01(0) SRX21(0)	119								SRX13		SRX12 SRX13	
		122	SBOX01										
		123	SBOX01										
		124	SSTX06	SRX29						SRX13			
		125	SSTX06		S. 3,10:z_6:- U1								

Table 4.12: Pulsotype distribution of the 327 strains of *Salmonella* along the pork chain obtained from different samples

Cohort	Environment at farms[a]	Pig[c]	F	CC	ML	CS	CW	Environment prior cutting[b]	FP	TP	Environment during cutting[b]	R	Environment after C&D[b]
15	SSTX13(0)	127	SCHX01							SRX12			
	SSTX15(0)	129	SSTX14										
	SBOX01(0)	130		SWEX01									
	SWEX01(0)	131					STYX02						
	SCOX02(0)	132	SBOX01	SRX0R8									
	SCOX03(3)	134								SRX12			
	SCOX04(0)	136	SCOX05										
16	STYX04(0)	139			SRX12						STYX03(B)		
	SKRX01(0)	140		STYX06									
	SCOX03(3)	141								STYX03			
		143								STYX03			
17	STYX05(0)	150		SRX12	SRX12							STYX03	
		151		SRX12	SRX12								
18	STYX03(0)	156	STYX03										
	SSTX01(0)	159	SSTX01	SRX14	SRX12								
	SSTX06(0)	160		SRX14									
19	SRX16(0,3)	163	SSTX02	SRX16								STYX03	
		164				SRX19	SRX27		SRX13			SRX16	
		165	SSTX01							SRX16		SRX09	
		166								SRX12		SRX10	
		168	SSTX09		SSTX03					SRX16			
		170	SSTX01				SRX16			SRX16			
20	SRX15(0)	171	SRX13							SRX09		SRX02	SRX13(B)
	SRX02(0)	172	SRX13									SRX17	
	SANX01(0)	173		SANX01								SRX19	
		174	SRX13		SRX14		SRX13					STYX09	
		176	SANX02		SRX07								
21	SRX02(0,3)	179	SRX02	SRX02								SRX02	
	SRX30(0)	180	SRX02		SRX02				SRX02				
		181				SRX02							
		182				SRX03							
		183				SRX04	SRX02		SRX02				
		184			SRX13		SRX02						
		185				SRX04	SRX02			SRX03			
		186							SRX02				

Table 4.12: Pulsotype distribution of the 327 strains of *Salmonella* along the pork chain obtained from different samples

Cohort	Environment at farms[a]	Pig[c]	F	CC	ML	CS	CW	Environment prior cutting[b]	FP	TP	Environment during cutting[b]	R	Environment after C&D[b]
22	S. 3,10:z_6:- U2$^{(0,3)}$	187			SRX12				SRX01				
	S. 3,10:z_6:- U3$^{(0)}$	188					SRX13						
	S. 3,10:z_6:- U2$^{(1)}$	189	SRX12	SRX12									
		191	S. 3,10:z_6:- U2	SRX12	SRX12				SRX02				
		192		SRX12		SRX02							
		193	S. 3,10:z_6:- U2		SRX12								
		194		SRX13	SRX12								

[a] Superscript number $^{(0)}$=Overshoes swab; $^{(1)}$=Drinking water; $^{(2)}$= Cleaning water; $^{(3)}$ = Waste water.
[b]Superscript alphabets $^{(B)}$=Cutting board; $^{(P)}$=Personal hygiene (hands) ; $^{(K)}$=Knife.
[c]Indicated individual animals, each cohort consisted of 7-10 pigs, representing each herd.
Serotypes in brackets present in one cohort, which could not refer to individual animal.

SRX = S. Rissen; STYX = S. Typhimurium; SSTX = S. Stanley; SPAX = S. Panama; SKRX = S. Krefeld; SANX = S. Anatum; SCOX = S. Corvallis; SWEX = S. Weltevreden; SENX = S. Enteritidis; SBOX = S. Bovismorbificans; SLIX = S. Livingstone; SCHX = S. Chittagong

The highest number (58) of isolates was obtained from overshoes (Table 4.13); however, the largest variability among pulsotypes (excluding samples with only one isolate) was observed in isolates from waste water, cutting boards during cutting and personal hygiene prior cutting (hands swab) with isolate/pulsotype ratios of 1.1, 1.2 and 1.3, respectively (Table 4.13). Meanwhile, considering a number of more than 20 isolates, the largest variability (the lower the ratio between the numbers of isolate per pulsotype, the higher the variability was noticed) among pulsotypes was observed in isolates from overshoes, faeces and CC with 1.8 isolates per pulsotype. In this study, the ratio of 3.1 isolates per pulsotype indicated the smallest variability, which was observed in those from FP.

Table 4.13: Distribution of 327*Salmonella* isolates among serotypes and *XbaI* pulsotypes according to the original of samples taken from 22 cohorts

Sample origins	Isolates	Serotypes	Pulsotypes	Ratio	
				isolates/serotype	isolates/pulsotype
Overshoes	58	9	32	6.4	1.8
TP	42	3	17	14.0	2.5
R	40	4	19	10.0	2.1
FP	37	4	12	9.3	3.1
F	36	9	20	4.0	1.8
CC	30	5	17	6.0	1.8
ML	26	3	10	8.7	2.6
CS	10	2	7	5.0	1.4
CW	9	3	6	3.0	1.5
Cutting boards after cleaning	8	3	5	2.7	1.6
Waste water	8	6	7	1.3	1.1
Cutting boards during cutting	7	3	6	2.3	1.2
Knives during cutting	7	3	3	2.3	2.3
Personal hygiene prior cutting	4	2	3	2.0	1.3
Personal hygiene during cutting	3	1	1	3.0	3.0
Cleaning water	1	1	1	1.0	1.0
Drinking water	1	1	1	1.0	1.0
Total	327				

4.6.1 *Salmonella* Rissen

In the present study, 31 isolates were obtained among 169 isolates, percentage of similarity between 84.2 and 100 % among pulsotypes were found. Between 16 and 18 bands were observed in this serovar (Figure 4.4). 27 isolates were undistinguishable and were grouped in the same pulsotype, which was classified in pulsotype "SRX02[15]" in this study (Figure 4.4). These isolates were recovered from cohorts1 (Mae Tha), 2 (Ban Thi), 11 (Mae Tang), 20 (Mae Tha), 21 (Mae Tha) and 22 (Mae Tha). Mae Tha and Mae Tang were two districts, where SRX02 was mainly found.

Transfer/ trace back to the farm: the pulsotype SRX02 strain could be traced back to the farm of origin, for example in the 21st cohort, it was obtained from wastewater, overshoes, F, CC, ML, CS, CW, FP and R samples (from the same Pig ID 180 with faecal, mesenteric lymph nodes, and FP samples) (Table 4.12). The strain indicated that *Salmonella* from farm and from pork was related to each other and the fattening pigs could be carriers, transferring *Salmonella* to pork.

Moreover, evidence for contamination during slaughter could be observed. Carcasses after spitting produced the same pulsotype, which was taken from CC. Pulsotypes SRX09 and SRX02 could be found in CC and carcasses after spitting from the same pigs [Pig number 3 in cohort 1 and pig number 98 in cohort 11 (Table 4.12), respectively] showing indistinguishable pulsotypes (Figure 4.4).

[15] SRX02; "S"= *Salmonella*, "R" = Serotype Rissen, "X" = Endonuclease*Xba*I, "02" = number of classification among the same serotype.

R: frequently, *Salmonella* Rissen isolates from R pork were more closely related to those from mesenteric lymph nodes and/or samples from environment during cutting and/or FP and/or TP (cohort 2: SRX06; cohort 3: SRX08; cohort 7: SRX18; cohort 12: SRX28, cohort 14: SRX13, cohort 19: SRX16, cohort 20: SRX02 and cohort 1: SRX02) (Table 4.12). Nonetheless, some isolates (from R pork) were slightly different within the same cohort (SRX17 and SRX 19 from cohort 20). In cohort 19, *Salmonella* Rissen pulsotype "SRX16" was mainly observed (Figure 4.4); it, besides, transferred the same pulsotype from wastewater and overshoe samples at farm through CC by animals to carcasses after washing and also to TP. SRX16 was detected in R pork with 100% similarity with the pre-harvest isolates, too. Closely related strain which presented in a slightly different shape from pulsotype "SRX16" was also observed from FP in cohort 19 but it was grouped in pulsotype "SRX13"

Transfer and/or persistence of *Salmonella* from farm and environments of slaughterhouse could be found in this study too. *Salmonella* pulsotype "SRX13" that presented in number 171, 172 and 174 (cohort 20) was also found in carcass samples after washing of pig number 174, and cutting boards after cleaning and disinfection (Table 4.12).

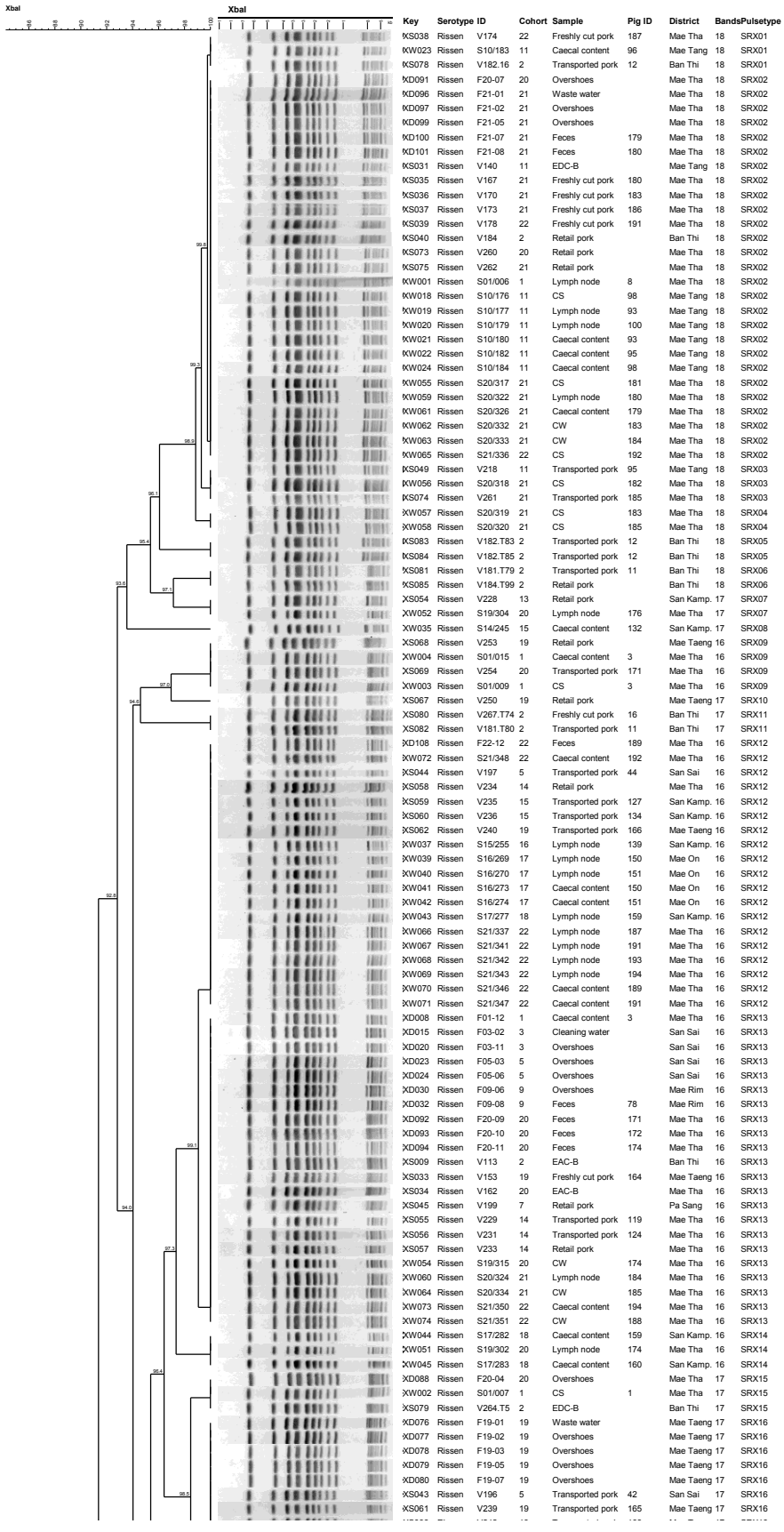


Figure 4.4: Dendrogram of S. Rissen isolates from a pork chain at different stages of production and environment from Northern Thailand. CS: Carcass swabs after splitting; CW: Carcass swabs after washing; EBC: Environments before cutting; EDC: Environments during cutting; EAC: Environments after C&D; B: Cutting board; K: Knife; P: Personal hygiene swab.

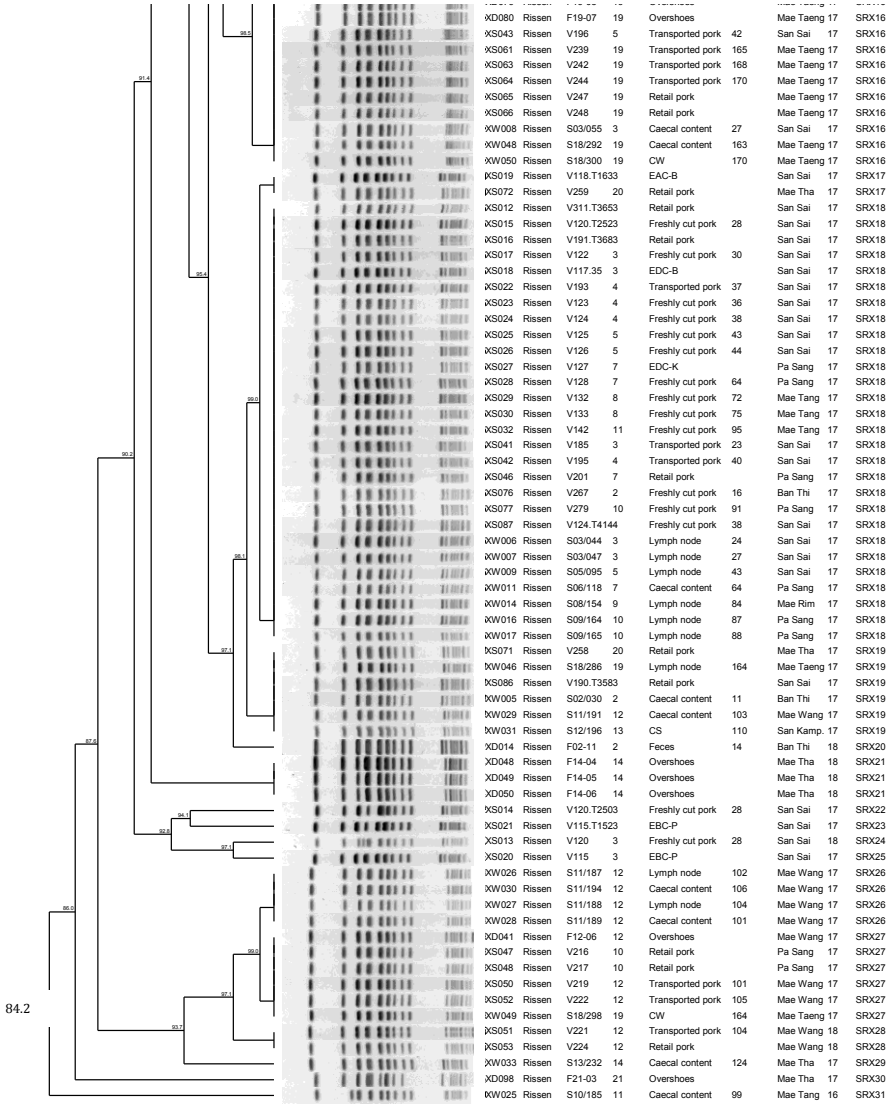


Figure 4.4 (continued): Dendrogram of S. Rissen isolates from a pork chain at different stages of production and environment from Northern Thailand. CS: Carcass swabs after splitting; CW: Carcass swabs after washing; EBC: Environments before cutting; EDC: Environments during cutting; EAC: Environments after C&D; B: Cutting board; K: Knife; P: Personal hygiene swab.

4.6.2 *Salmonella* Typhimurium

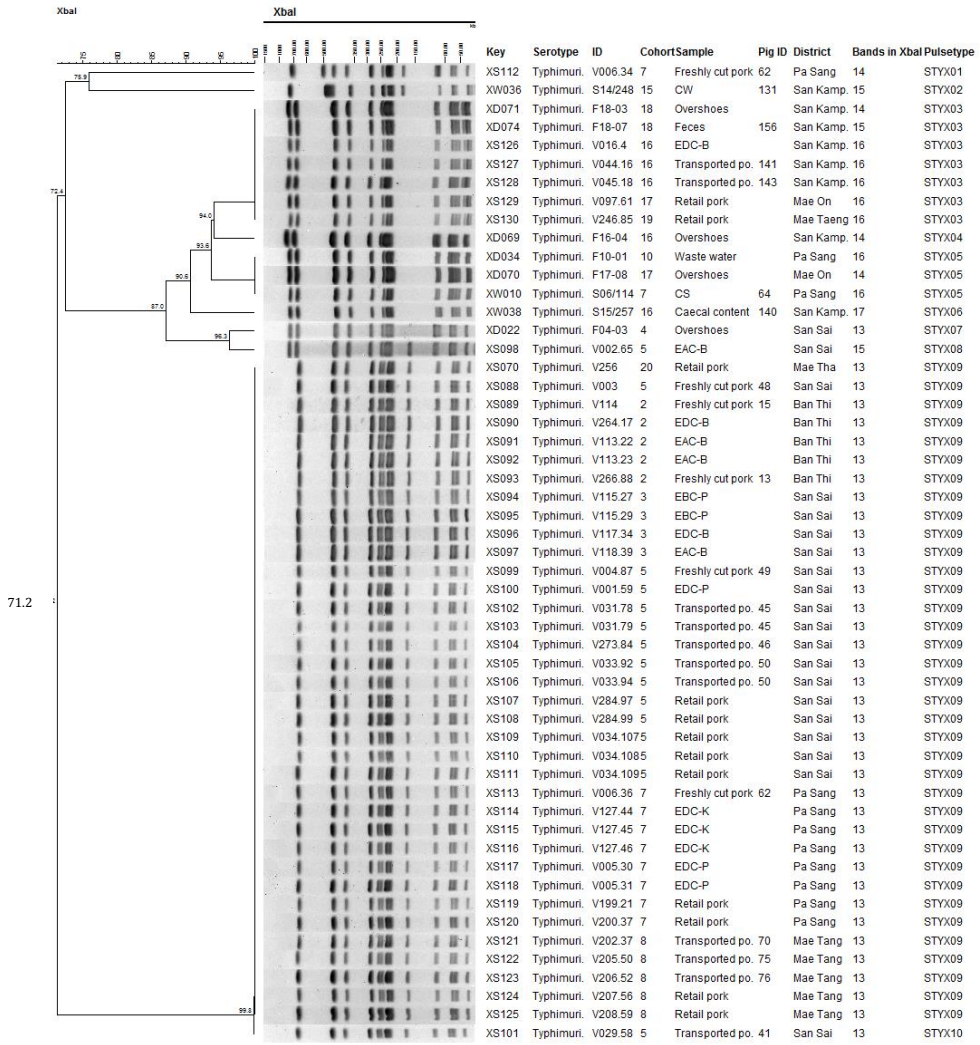
Among 53 *S.* Typhimurium isolates, 10 pulsotypes (5.6 isolates/pulsotype) (Table 4.11 and Figure 4.5) with similarities between 72.4 % – 100.0 % were observed. Number of bands ranged between 13-17 bands in each pulsotype (16-18 bands in *Salmonella* Rissen). Persistence and/or transfer of *Salmonella* could be seen too (pulsotype STYX09).

Starting with cohort 2, this pulsotype was first found in FP, cutting board during cutting and again cutting board after cleaning and disinfection.

Cohort 3: This pulsotype was again identified in samples from hand swabs before cutting, cutting board during cutting and remained still after cleaning and disinfection on cutting board.

Furthermore, cohort 5 (14 isolates: FP, hand swabs during cutting, TP and R pork), cohort 7 (8 isolates: FP, knife swabs during cutting, hand swabs during cutting and R pork), cohort 8 (5 isolates: TP and R pork) and cohort 20 (1 isolate from R pork). Transfer and/or persistence of *Salmonella* could be seen in this serovars and the results indicated the similarity of pulsotypes between environment samples and pork.

Pulsotype STYX03 (second frequent pulsotype within serovar Typhimurium), was identified in 7 isolates from cohort 15-19, obtained from overshoe swabs (cohort 18), faecal samples (cohort 18), cutting board during cutting (cohort 16), TP (cohort 16) and R pork (cohort 17 and 19) samples. This strain was only obtained from district in Chiang Mai province (Appendix 9.1).



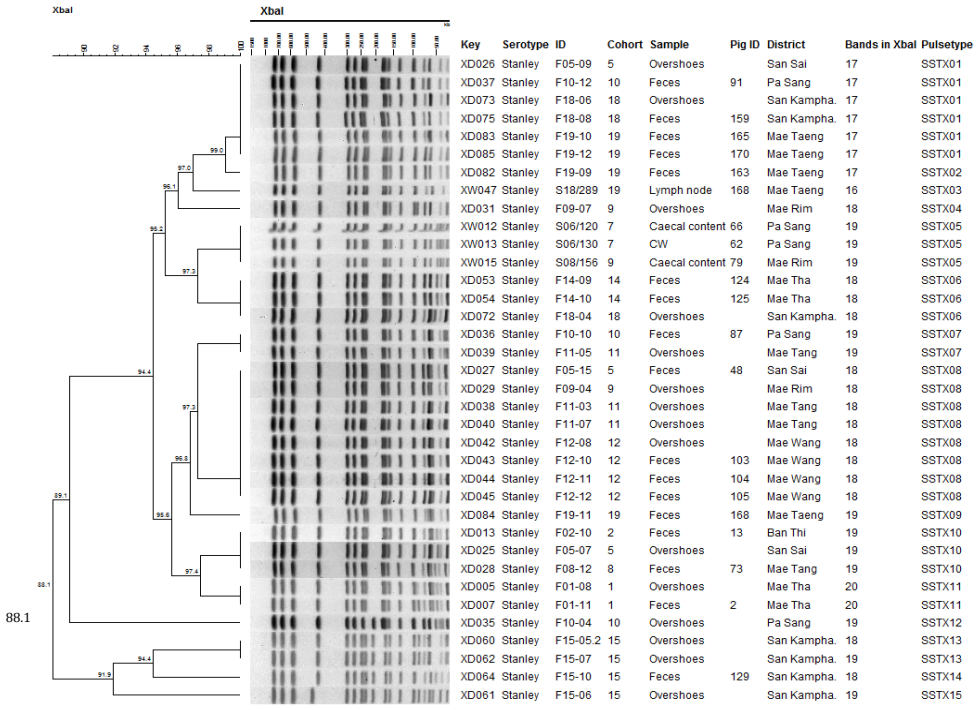
Key	Serotype	ID	Cohort	Sample	Pig ID	District	Bands in XbaI	Pulsetype
XS112	Typhimuri.	V006.34	7	Freshly cut pork	62	Pa Sang	14	STYX01
XW036	Typhimuri.	S14/248	15	CW	131	San Kamp.	15	STYX02
XD071	Typhimuri.	F18-03	18	Overshoes		San Kamp.	14	STYX03
XD074	Typhimuri.	F18-07	18	Feces	156	San Kamp.	15	STYX03
XS126	Typhimuri.	V016.4	16	EDC-B		San Kamp.	16	STYX03
XS127	Typhimuri.	V044.16	16	Transported po.	141	San Kamp.	16	STYX03
XS128	Typhimuri.	V045.18	16	Transported po.	143	San Kamp.	16	STYX03
XS129	Typhimuri.	V097.61	17	Retail pork		Mae On	16	STYX03
XS130	Typhimuri.	V246.85	19	Retail pork		Mae Taeng	16	STYX03
XD069	Typhimuri.	F16-04	16	Overshoes		San Kamp.	14	STYX04
XD034	Typhimuri.	F10-01	10	Waste water		Pa Sang	16	STYX05
XD070	Typhimuri.	F17-08	17	Overshoes		Mae On	14	STYX05
XW010	Typhimuri.	S06/114	7	CS	64	Pa Sang	16	STYX05
XW038	Typhimuri.	S15/257	16	Caecal content	140	San Kamp.	17	STYX06
XD022	Typhimuri.	F04-03	4	Overshoes		San Sai	13	STYX07
XS098	Typhimuri.	V002.65	5	EAC-B		San Sai	15	STYX08
XS070	Typhimuri.	V256	20	Retail pork		Mae Tha	13	STYX09
XS088	Typhimuri.	V003	5	Freshly cut pork	48	San Sai	13	STYX09
XS089	Typhimuri.	V114	2	Freshly cut pork	15	Ban Thi	13	STYX09
XS090	Typhimuri.	V264.17	2	EDC-B		Ban Thi	13	STYX09
XS091	Typhimuri.	V113.22	2	EAC-B		Ban Thi	13	STYX09
XS092	Typhimuri.	V113.23	2	EAC-B		Ban Thi	13	STYX09
XS093	Typhimuri.	V266.88	2	Freshly cut pork	13	Ban Thi	13	STYX09
XS094	Typhimuri.	V115.27	3	EBC-P		San Sai	13	STYX09
XS095	Typhimuri.	V115.29	3	EBC-P		San Sai	13	STYX09
XS096	Typhimuri.	V117.34	3	EDC-B		San Sai	13	STYX09
XS097	Typhimuri.	V118.39	3	EAC-B		San Sai	13	STYX09
XS099	Typhimuri.	V004.87	5	Freshly cut pork	49	San Sai	13	STYX09
XS100	Typhimuri.	V001.59	5	EDC-P		San Sai	13	STYX09
XS102	Typhimuri.	V031.78	5	Transported po.	45	San Sai	13	STYX09
XS103	Typhimuri.	V031.79	5	Transported po.	45	San Sai	13	STYX09
XS104	Typhimuri.	V273.84	5	Transported po.	46	San Sai	13	STYX09
XS105	Typhimuri.	V033.92	5	Transported po.	50	San Sai	13	STYX09
XS106	Typhimuri.	V033.94	5	Transported po.	50	San Sai	13	STYX09
XS107	Typhimuri.	V284.97	5	Retail pork		San Sai	13	STYX09
XS108	Typhimuri.	V284.99	5	Retail pork		San Sai	13	STYX09
XS109	Typhimuri.	V034.107	5	Retail pork		San Sai	13	STYX09
XS110	Typhimuri.	V034.108	5	Retail pork		San Sai	13	STYX09
XS111	Typhimuri.	V034.109	5	Retail pork		San Sai	13	STYX09
XS113	Typhimuri.	V006.36	7	Freshly cut pork	62	Pa Sang	13	STYX09
XS114	Typhimuri.	V127.44	7	EDC-K		Pa Sang	13	STYX09
XS115	Typhimuri.	V127.45	7	EDC-K		Pa Sang	13	STYX09
XS116	Typhimuri.	V127.46	7	EDC-K		Pa Sang	13	STYX09
XS117	Typhimuri.	V005.30	7	EDC-P		Pa Sang	13	STYX09
XS118	Typhimuri.	V005.31	7	EDC-P		Pa Sang	13	STYX09
XS119	Typhimuri.	V199.21	7	Retail pork		Pa Sang	13	STYX09
XS120	Typhimuri.	V200.37	7	Retail pork		Pa Sang	13	STYX09
XS121	Typhimuri.	V202.37	8	Transported po.	70	Mae Tang	13	STYX09
XS122	Typhimuri.	V205.50	8	Transported po.	75	Mae Tang	13	STYX09
XS123	Typhimuri.	V206.52	8	Transported po.	76	Mae Tang	13	STYX09
XS124	Typhimuri.	V207.56	8	Retail pork		Mae Tang	13	STYX09
XS125	Typhimuri.	V208.59	8	Retail pork		Mae Tang	13	STYX09
XS101	Typhimuri.	V029.58	5	Transported po.	41	San Sai	13	STYX10

Figure 4.5: Dendrogram of *Salmonella* Typhimurium isolates from a pork chain at different stages of production and environment from Northern Thailand. CS: Carcass swabs after splitting; CW: Carcass swabs after washing; EBC: Environments before cutting; EDC: Environments during cutting; EAC: Environments after C&D; B: Cutting board; K: Knife; P: Personal hygiene swab.

4.6.3 *Salmonella* Stanley

15 pulsotypes were found among 36 isolates of *Salmonella* Stanley. This serovar showed higher variability (smaller number of ratio between isolates and pulsotype) (isolates/pulsotype = 2.4) than serovar Rissen (5.5) and Typhimurium (5.6) (Table 4.11), respectively. The lowest similarity value, which was found among this serovar, was 88.1 % (Figure 4.6). In this serovar, pulsotypes “SSTX08” and “SSTX01” were

the most two frequent pulsotypes. SSTX08 could be found from cohort 5, 9, 11 and 12; SSTX01 could be found from cohort 5, 10, 18 and 19. These available isolates for PFGE showed indistinguishable pulsotype between farm sample and animal sample isolates. Contamination during slaughtering could be found from this study, pulsotype "SSTX05" could be identified from CW and CC from animal number 62 and 66 respectively, with the highest number of bands (20 bands in XbaI) (Figure 4.6).



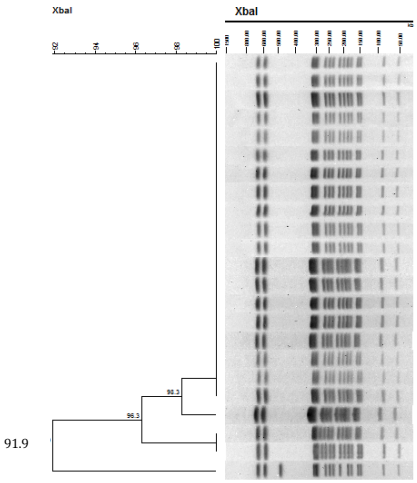
Key	Serotype	ID	Cohort	Sample	Pig ID	District	Bands in XbaI	Pulsetype
XD026	Stanley	F05-09	5	Overshoes		San Sai	17	SSTX01
XD037	Stanley	F10-12	10	Feces	91	Pa Sang	17	SSTX01
XD073	Stanley	F18-06	18	Overshoes		San Kampha.	17	SSTX01
XD075	Stanley	F18-08	18	Feces	159	San Kampha.	17	SSTX01
XD083	Stanley	F19-10	19	Feces	165	Mae Taeng	17	SSTX01
XD085	Stanley	F19-12	19	Feces	170	Mae Taeng	17	SSTX01
XD082	Stanley	F19-09	19	Feces	163	Mae Taeng	17	SSTX02
XW047	Stanley	S18/289	19	Lymph node	168	Mae Taeng	16	SSTX03
XD031	Stanley	F09-07	9	Overshoes		Mae Rim	18	SSTX04
XW012	Stanley	S06/120	7	Caecal content	66	Pa Sang	19	SSTX05
XW013	Stanley	S06/130	7	CW	62	Pa Sang	19	SSTX05
XW015	Stanley	S08/156	9	Caecal content	79	Mae Rim	19	SSTX05
XD053	Stanley	F14-09	14	Feces	124	Mae Tha	18	SSTX06
XD054	Stanley	F14-10	14	Feces	125	Mae Tha	18	SSTX06
XD072	Stanley	F18-04	18	Overshoes		San Kampha.	18	SSTX06
XD036	Stanley	F10-10	10	Feces	87	Pa Sang	19	SSTX07
XD039	Stanley	F11-05	11	Overshoes		Mae Tang	19	SSTX07
XD027	Stanley	F05-15	5	Feces	48	San Sai	18	SSTX08
XD029	Stanley	F09-04	9	Overshoes		Mae Rim	18	SSTX08
XD038	Stanley	F11-03	11	Overshoes		Mae Tang	18	SSTX08
XD040	Stanley	F11-07	11	Overshoes		Mae Tang	18	SSTX08
XD042	Stanley	F12-08	12	Overshoes		Mae Wang	18	SSTX08
XD043	Stanley	F12-10	12	Feces	103	Mae Wang	18	SSTX08
XD044	Stanley	F12-11	12	Feces	104	Mae Wang	18	SSTX08
XD045	Stanley	F12-12	12	Feces	105	Mae Wang	18	SSTX08
XD084	Stanley	F19-11	19	Feces	168	Mae Taeng	19	SSTX09
XD013	Stanley	F02-10	2	Feces	13	Ban Thi	19	SSTX10
XD025	Stanley	F05-07	5	Overshoes		San Sai	19	SSTX10
XD028	Stanley	F08-12	8	Feces	73	Mae Tang	19	SSTX10
XD005	Stanley	F01-08	1	Overshoes		Mae Tha	20	SSTX11
XD007	Stanley	F01-11	1	Feces	2	Mae Tha	20	SSTX11
XD035	Stanley	F10-04	10	Overshoes		Pa Sang	19	SSTX12
XD060	Stanley	F15-05.2	15	Overshoes		San Kampha.	18	SSTX13
XD062	Stanley	F15-07	15	Overshoes		San Kampha.	19	SSTX13
XD064	Stanley	F15-10	15	Feces	129	San Kampha.	18	SSTX14
XD061	Stanley	F15-06	15	Overshoes		San Kampha.	19	SSTX15

Figure 4.6: Dendrogram of *Salmonella* Stanley isolates from pork chain at different stages of production and environment from Northern Thailand. CS: Carcass swabs after splitting; CW: Carcass swabs after washing.

4.6.4 *Salmonella* Panama

Salmonella Panama was obtained from cohort 2, 3, 5 and 15 (Table 4.9). Among them, 23 isolates from cohort 2 are shown in Figure 4.7, which were subtyped into 4 pulsotypes. 91.9 % was the lowest similarity among the isolates. The most frequent

pulsotype found here was "SPAX01". This pattern was found in environment samples (knife during cutting and cutting board after cleaning and disinfection), and pork (FP, TP and R pork). This result indicated a close relationship between environment in cutting unit and pork at R. Mostly, isolates from the same samples were grouped in the same pulsotype, but an isolate from TP of pig number 18 was closely related to those were isolated from the same sample, (SPAX04 and SPAX01) (Figure 4.7). It indicated that more than one pulsotype could be found from one sample.



Key	Serotype	ID	Cohort	Sample	Pig ID	District	Bands in XbaI	Pulsetype
XS133	Panama	V113.T10	2	EAC-B		Ban Thi	15	SPAX01
XS134	Panama	V265.T11	2	EDC-K		Ban Thi	15	SPAX01
XS135	Panama	V265.T12	2	EDC-K		Ban Thi	15	SPAX01
XS136	Panama	V265.T14	2	EDC-K		Ban Thi	15	SPAX01
XS137	Panama	V263.T60	2	Freshly cut pork	11	Ban Thi	15	SPAX01
XS138	Panama	V266.T65	2	Freshly cut pork	13	Ban Thi	15	SPAX01
XS139	Panama	V266.T66	2	Freshly cut pork	13	Ban Thi	15	SPAX01
XS140	Panama	V114.T67	2	Freshly cut pork	15	Ban Thi	15	SPAX01
XS141	Panama	V114.T69	2	Freshly cut pork	15	Ban Thi	15	SPAX01
XS142	Panama	V114.T70	2	Freshly cut pork	15	Ban Thi	15	SPAX01
XS143	Panama	V181.T78	2	Transported po.	11	Ban Thi	15	SPAX01
XS144	Panama	V269.T86	2	Transported po.	14	Ban Thi	15	SPAX01
XS145	Panama	V269.T89	2	Transported po.	14	Ban Thi	15	SPAX01
XS146	Panama	V269.T90	2	Transported po.	14	Ban Thi	15	SPAX01
XS148	Panama	V270.T93	2	Transported po.	18	Ban Thi	15	SPAX01
XS149	Panama	V270.T94	2	Transported po.	18	Ban Thi	15	SPAX01
XS151	Panama	V271.T105	2	Retail pork		Ban Thi	15	SPAX01
XS153	Panama	V272.T108	2	Retail pork		Ban Thi	15	SPAX01
XS154	Panama	V272.T109	2	Retail pork		Ban Thi	15	SPAX01
XS150	Panama	V271.T104	2	Retail pork		Ban Thi	15	SPAX02
XS152	Panama	V271.T106	2	Retail pork		Ban Thi	16	SPAX03
XS155	Panama	V272.T111	2	Retail pork		Ban Thi	16	SPAX03
XS147	Panama	V270.T91	2	Transported po.	18	Ban Thi	15	SPAX04

Figure 4.7: Dendrogram of *Salmonella* Panama isolates from pork chain at different stages of production and environment from Northern Thailand. EBC: Environments before cutting; EDC: Environments during cutting; EAC: Environments after C&D; B: Cutting board; K: Knife; P: Personal hygiene swab.

4.6.5 *Salmonella* Krefeld and *Salmonella* Anatum

As shown in Figure 4.8A and B, respectively, the lowest number of restricted fragments (12 fragments) is observed in *S.* Krefeld. This serotype demonstrated the high clonal identity within the same cohort and also different cohort. All of them were obtained from farms (overshoe swabs) and faecal samples. 3 different patterns of pulsotypes from isolates were observed (Table 4.11).

Meanwhile, *Salmonella* Anatum isolates are shown in Figure 4.8B. 2 major pulsotypes (SANX01; FO and CC from cohort 20, and SANX03; FO and W3 from cohort 3) from different cohorts were clearly observed from 2 cohorts with a 78.1% similarity.

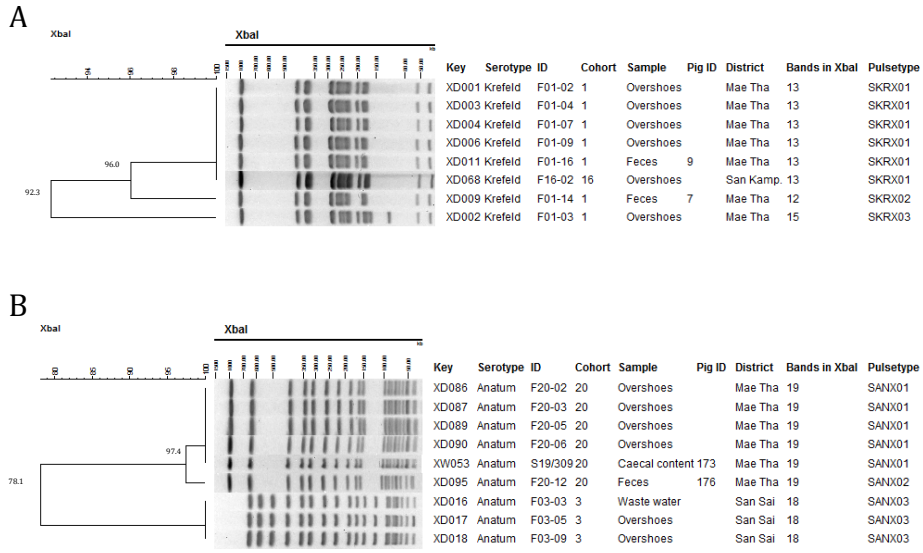
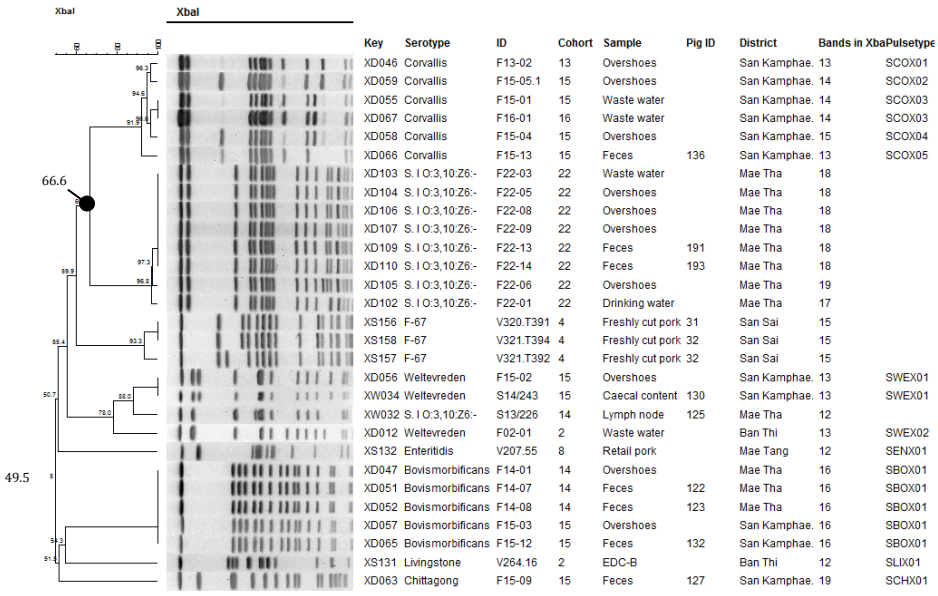


Figure 4.8: Dendrogram of the (A) *Salmonella* Krefeld and (B) *Salmonella* Anatum isolates

4.6.7 Other serotypes

Dendrograms of 29 isolates (17 known serovars and 12 unidentified serovars), are shown in Figure 4.9. The dendrogram shows clusters of these isolates classified according to percent similarity. Additional each cluster of pulsotypes was grouped within the same serovar group. It was found that 49.5% was the lowest similarity number whereas 66.6 % was the highest number of percent similarity that could cluster isolates into the same group of serotype.



Key	Serotype	ID	Cohort	Sample	Pig ID	District	Bands in Xba	Pulsetype
XD046	Corvallis	F13-02	13	Overshoes		San Kamphae.	13	SCOX01
XD059	Corvallis	F15-05.1	15	Overshoes		San Kamphae.	14	SCOX02
XD055	Corvallis	F15-01	15	Waste water		San Kamphae.	14	SCOX03
XD067	Corvallis	F16-01	16	Waste water		San Kamphae.	14	SCOX03
XD058	Corvallis	F15-04	15	Overshoes		San Kamphae.	15	SCOX04
XD066	Corvallis	F15-13	15	Feces	136	San Kamphae.	13	SCOX05
XD103	S. I O:3,10:Z6:-	F22-03	22	Waste water		Mae Tha	18	
XD104	S. I O:3,10:Z6:-	F22-05	22	Overshoes		Mae Tha	18	
XD106	S. I O:3,10:Z6:-	F22-08	22	Overshoes		Mae Tha	18	
XD107	S. I O:3,10:Z6:-	F22-09	22	Overshoes		Mae Tha	18	
XD109	S. I O:3,10:Z6:-	F22-13	22	Feces	191	Mae Tha	18	
XD110	S. I O:3,10:Z6:-	F22-14	22	Feces	193	Mae Tha	18	
XD105	S. I O:3,10:Z6:-	F22-06	22	Overshoes		Mae Tha	19	
XD102	S. I O:3,10:Z6:-	F22-01	22	Drinking water		Mae Tha	17	
XS156	F-67	V320.T391	4	Freshly cut pork	31	San Sai	15	
XS158	F-67	V321.T394	4	Freshly cut pork	32	San Sai	15	
XS157	F-67	V321.T392	4	Freshly cut pork	32	San Sai	15	
XD056	Weltevreden	F15-02	15	Overshoes		San Kamphae.	13	SWEX01
XW034	Weltevreden	S14/243	15	Caecal content	130	San Kamphae.	13	SWEX01
XW032	S. I O:3,10:Z6:-	S13/226	14	Lymph node	125	Mae Tha	12	
XD012	Weltevreden	F02-01	2	Waste water		Ban Thi	13	SWEX02
XS132	Enteritidis	V207.55	8	Retail pork		Mae Tang	12	SENX01
XD047	Bovismorbificans	F14-01	14	Overshoes		Mae Tha	16	SBOX01
XD051	Bovismorbificans	F14-07	14	Feces	122	Mae Tha	16	SBOX01
XD052	Bovismorbificans	F14-08	14	Feces	123	Mae Tha	16	SBOX01
XD057	Bovismorbificans	F15-03	15	Overshoes		San Kamphae.	16	SBOX01
XD065	Bovismorbificans	F15-12	15	Feces	132	San Kamphae.	16	SBOX01
XS131	Livingstone	V264.16	2	EDC-B		Ban Thi	12	SLIX01
XD063	Chittagong	F15-09	15	Feces	127	San Kamphae.	19	SCHX01

Figure 4.9: Dendrogram of *Salmonella* spp. isolates from the pork chain. EBC: Environments before cutting; EDC: Environments during cutting; EAC: Environments after C&D; B: Cutting board; K: Knife; P: Personal hygiene swab.

5. Discussion

It has been estimated that each year 93.8 million gastroenteritis cases from *Salmonella* species may occur, with 155,000 deaths. Of them, 80.3 million cases were associated with a foodborne incidence (Majowicz et al., 2010). Salmonellosis after pork consumption has been reported (Nielsen and Wegener, 1997);approximately 20% of salmonellosis cases originated from swine (Steinbach and Kroell, 1999). In Thailand, the gap of knowledge related to food borne diseases and effective measures to improve public health condition is still a problem (Padungtod et al., 2008).

The hypothesis, that animals and their surroundings as well as slaughter of animals and the slaughterhouse environment affected the microbiological status of meat, has been documented in a number of studies along the line between farms and abattoirs (Bahnson et al., 2006; De Busser et al., 2011; Kich et al., 2011; Visscher et al., 2011; Alban et al., 2012). Finishing pigs as functional carriers were observed in this study, too:22 cohorts of finishers were followed starting with farm surroundings throughout slaughter, and further products.

5.1 Finishing herds, finishers and slaughtered pigs

Salmonella prevalence data on farms and in the slaughterhouses were obtained in this study (Table 4.1 and 4.2):a higher level of *Salmonella* was found at the abattoir than at the farm level, which was in concordance with previous reports (Swanenburg et al., 2001; Hurd et al., 2002; Padungtod and Kaneene, 2006; Kich et al., 2011). Results from the literatures indicated that the *Salmonella* status of slaughtered pigs was not

only affected by the status of finishing at the fattening farm (Arguello et al., 2013). Transport (Berends et al., 1996) and lairage stress (Dorr et al., 2009; De Busser et al., 2011) (i.e. withdrawing feed, handling, mixing with unfamiliar pigs, high stock density and changing environment) may cause recrudescent infection during transport, routine resting or holding periods, when exposed to *Salmonella* organisms in the preslaughter surroundings (Hurd et al., 2001); influencing the amplitude of *Salmonella* infection/contamination (Berends et al., 1996; Patchanee et al., 2002; Kich et al., 2011).

The present study showed an increase of *Salmonella* in the ML and CC (63.9% and 83.1%, respectively) if compared ML and CC with *Salmonella* status at farm (61.4%), although the correlation between F (at farm) and ML samples was not significant (P = 0.07): A slight agreement (Kappa = 0.15) with no significant difference (McNemar $\chi^2 P$ = 0.42) was observed. This meant that results from the two samples within the same animal were not always consistent. The *Salmonella* status of finishing pigs was dynamic and Davies et al. (1999) indicated that sources of *Salmonella* in finishers presented in finishing period. Time spent in lairage increased *Salmonella* isolation rate in slaughtered pigs (Morgan et al., 1987; Warriss, 2003) whereas dose and serotype may affect results of *Salmonella* shedding (Ivanek et al., 2012).

In this study, positive faecal samples ranged from 25.0 - 87.5 %, with an overall prevalence of 61.4 %, which was higher than data from a previous study reporting 6% in Northern Thailand (Padungtod and Kaneene, 2006). Another study in Northern Thailand (Patchanee et al., 2002) reported a 54.9 % prevalence, which was comparable with the results in the present study, although the year of the two studies

was different. This indicated that, in this geographic area, *Salmonella* infection in animals may be stable in a high level. In consequence, the absence of regulations/measures applicable to farms with high infection, contagious animals may affect meat safety. Denmark was the first country which implemented control programs for *Salmonella*, based on the level of results from ELISA-Tests (level 1: no sanctions; level 2: implementation of *Salmonella* reducing actions on herd level; level 3: same as level 2 and slaughter of pigs under special hygienic precautions, including post slaughter microbial test and potential heat treatment of all meat products) (Wegener et al., 2003). Pre-harvest surveillance was required at the farms level in order to detect farm with infected pigs before slaughter and to manage hygienic slaughter procedures.

As for the pigs at the abattoir, mesenteric lymph nodes were used to determine pre-slaughter infection. Using these results, 63.9 % of pigs were infected with *Salmonella,* which were lower than they were in Brazil (67%) (Kich et al., 2011)and higher than it was reported from Portugal and Italy (Piras et al., 2011; Gomes-Neves et al., 2012). *Salmonella* in CC also significantly increased the odds ratio of *Salmonella* isolation in ML (OR = 2.3; 95% CI: 1.02 - 5.24) (P = 0.04), a slight agreement (κ= 0.13) was observed with significant difference (McNemar χ^2 $P < 0.01$). This meant that results from the two samples within the same animal were not always consistent. This observation was supported by the study of (Visscher et al., 2011). Moreover, Methner et al. (2011) recommended to use both ML and CC, to assess the *Salmonella* status in finishing pigs at the time of slaughter.

According to the diversity of *Salmonella* serotypes in this study, 27 serovars were discovered from 941 isolates. Ratios between isolates and serovar were found ranging between 3.3 (cohort 15) and 16.0 (cohort 6 and 13). Such ratios meant that the serotype was found in many samples. If the bacteria are genetically very diverse, this ratio should decrease and the number of samples that shared the same serotype should reduce and the number of serotype should increase. Comparing overshoe and faecal samples, serovar variability (ratio between isolates per serotype: 8.8) ranged within similar numbers (Table 4.9) with 15 and 13 serovars, respectively. Furthermore, a decreased serovar variability was observed after the animals were slaughtered (Table 4.10). This result was different from the previous study (Botteldoorn et al., 2003), which found that the highest variability of serotypes was found in the mesenteric lymph nodes. However, others (Jensen et al., 2004; Kich et al., 2011) reported that a higher incidence and a higher diversity of *Salmonella* serotypes were also observed in farms and environment (herd pen floors) where the pigs were raised, especially in outdoor production systems, which might be similar to open farms, from where samples mainly were obtained in this study.

Comparing *Salmonella* serotypes and molecular typing (PFGE) (Table 4.13), overshoe samples had a genotypic diversity with 1.8 isolates/pulsotype (32 pulsotypes among 58 isolates) this ratio was also found in faecal samples: in numerous cohorts, the strains detected in samples collected at farms showed more indistinguishable isolates between environment samples at farm and faecal samples. 6 isolates of *S.* Corvallis were classified into 5 pulsotypes, 2 indistinguishable isolates were available from wastewater of the 15th and 16th cohort and were found from the same district in

wastewater, pen swabs and faeces. Table 4.12 also showed examples of this phenomenon

- Cohort 1: serovars Krefeld (pulsotype SKRX01) and Stanley (pulsotype SSTX11);
- Cohort 9: serovar Rissen (pulsotype SRX13);
- Cohort 12: serovar Typhimurium (pulsotype SSTX08);
- Cohort 14 and 15: serovar Bovismorbificans (pulsotype SBOX01);
- Cohort 18: serovar Typhimurium (pulsotype SSTX03), Stanley (pulsotype SSTX01);
- Cohort 21: serovar Rissen (pulsotype SRX02);
- Cohort 22: *S.* 3,10:z_6:- (pulsotype U2).

Relation of *Salmonella* isolates between slaughtered pigs and environment at farms was not as apparent as those between faecal and environmental samples; nevertheless, it could also be seen from

- Cohort 15 (*S.* Weltevreden pulsotype SWEX01),
- Cohort 19 (*S.* Rissen pulsotype SRX16) and
- Cohort 21(*S.* Rissen pulsotype SRX02).

Additionally, from cohort 21, pulsotype SRX02, which was found in faecal samples of pig ID 180, could also be found in its mesenteric lymph nodes. However, genetic variation in evolutionary genetics might play a role in this method (van Belkum et al., 2001), closely and possibly related pulsotypes could be observed among isolates from farm and mesenteric lymph nodes and/or caecal samples which were only slightly different.

5.2 The slaughter process technology

After slaughter, carcasses were processed by scalding, dehairing and evisceration, with the application of heat and water, to reduce contamination from outer and inner contaminations. Arguello et al. (2013) suggested that scalding must be included as a verifiable control point to improve GMPs and HACCPs programs. However, the majority of increased contamination was associated with post-evisceration and splitting as reported by O'Connor et al. (2012).

In this study, 63.9 % (95 % CI: 52.9 – 74.9) and 83.1 % (95 % CI: 74.5 – 91.9) of slaughtered positive pigs with *Salmonella* were observed in ML and CC, respectively. A decrease in *Salmonella* prevalence (32.0 %; 95 % CI: 21.2 - 42.9) on CS was observed too. Processing procedures in place generally decreased the prevalence of *Salmonella* as carcasses were moved on their way to the cooler (O'Connor et al., 2012). Singeing and hot water decontamination (temperature 80 degrees C/176 degrees F) were reported as an effective risk-reducing way of slaughtering pigs for *Salmonella* reduction (Alban and Sorensen, 2010; da Silva et al., 2012). The consequence of washing carcasses with chlorinated (50-100 ppm) potable water in this slaughterhouse could decrease *Salmonella* to 12.9 % (95 % CI: 7.0 – 18.8). Such techniques might be an option to reduce high loads of contamination in slaughterhouses when facing high risks of *Salmonella* at herd level. Currently this treatment is not authorized in the EU countries, meanwhile, legally applied in the United States of America (Buncic and Sofos, 2012). Other techniques (such as vertical scalding, spraying, steam-treatment and processes after washing for decontamination) for intervention were reviewed and recommended (Buncic and Sofos, 2012).

In this study, a high positive correlation coefficient between *Salmonella* prevalence in lymph nodes and carcasses after splitting was observed. It was higher than the correlation coefficient between such prevalence in CC and CS. Nevertheless, relative risks between them were 1.04 and 1.49, respectively. On the other hand, a slight agreement ($\kappa = 0.03$) was observed between CS and CC, whereas that between ML and CS was poorly agreed ($\kappa < 0.00$), as indicated by others (Visscher et al., 2011) However, those two pairs were significantly different (McNemar $\chi^2 P < 0.01$). These inconsistencies of results in this study might arise from the techniques and types of samples used, including the study design, which might cause difficulty in drawing the conclusions (Padungtod and Kaneene, 2006), and latent undetectable carriers were reported as a natural element of *Salmonella enterica* epidemiology (Hurd et al., 2004).

In this study, about 65 -85 % of all carcass contaminations (after splitting) resulted from the animals themselves (infected animal and cross contamination). This indicated the effects of individual animals and/or the failure in evisceration procedure in the slaughter line, in conjunction with the infected batch of animals transferring the agents to the slaughterhouse. Some dressing activities could reduce carcass contamination, others were critical control points that jeopardized carcass hygiene (Arguello et al., 2013) such as inadequately cleaned polishing machines and inapt procedures during evisceration as previously reported (Berends et al., 1997). This information indicated the importance of slaughter and farm (fattening) stages in the pork production chain for controlling *Salmonella* in swine production.

5.3 Further processing and retail-ready pork

After chilling, no subsequent decontamination technique was applied. A 54.6 % prevalence of FP was observed in general when contaminated carcasses (12.9 % positive carcasses after washing) were delivered to the cutting unit. An even higher prevalence was obtained after cutting which increased in TP (70.2%). Processes after cutting and other subsequent operations such as gathering cut products in a container, keeping the product over night before packing, or transport condition might increase occasions for contamination of *Salmonella*-free pork. These results were in contrast to other results, where *Salmonella* in pork products was lower than that in carcasses (Padungtod and Kaneene, 2006; Gomes-Neves et al., 2012).

The two sample types, CW and FP, showed a statistically positive association (r_s= 0.4289, $P \leq 0.05$) in their prevalent results between them. Data on the relative risk showed a risk of having carcass in the cutting as affected by the *Salmonella* status in the pork (RR = 1.6; 95% CI: 1.3-2.1). Paired between CW and FP were similar to results of paired ML and FP in a slight agreement. These results were also supported by logistic regression: in this study, *Salmonella* status on CW and that of animal from ML affected the status of FP (Table 4.7). It emphasized that tissues from infected pigs as well as contaminated carcasses from inappropriate dressing were critical points for further processing and pork products, which were also reported by others (Berends et al., 1998; Vieira-Pinto et al., 2006; Gomes-Neves et al., 2012; van Hoek et al., 2012; Arguello et al., 2013).

Berends et al. (1998) described main risk factor. It took place when contaminated carcasses were entering a cutting unit: inappropriate cleaning and disinfection,

manipulation of contaminated materials and (re)contaminated surfaces. 14.0 % (95 % CI: 8.7-19.3) of *Salmonella* positive samples after cleaning and disinfection in this abattoir as well as environment prior to cutting indicated that the site was not properly cleaned and disinfected (4.0 %; 95% CI: 0.2-7.8). Hence, *Salmonella* became a resident flora on slaughter equipment (van Hoek et al., 2012). Not only contaminated carcasses, but also cutting boards were important sources to cross-contamination of foods with agents (Cliver, 2006). The present study also indicated that TP was associated with cutting boards during cutting (Table 4.7). Additionally, the workers in this slaughterhouse were the source of spreading the *Salmonella* (Gomes-Neves et al., 2012). This could be observed in the example of STYX09 in cohort 2 and 3, this strain survived in cutting after cleaning and disinfection in cohort 2, and was found on hands of staff prior to cutting in cohort 3 throughout the end of the day and other following cohorts, too. In consequence, pigs were an important source for the *Salmonella* contamination in line and for the carcasses produced. The other way round, if *Salmonella*-free pigs were produced, consumers could be provided with virtually *Salmonella*-free pork (Berends et al., 1997). In this study, the lowest prevalence on faecal samples occurred at farm (25%), could provide *Salmonella*-free pork at R-ready pork, too (Table 4.3: cohort 18).

Retail-ready pork samples from supermarket: in this study, individual samples could not be traced back to the individual pig. The prevalence of 31 % positive pork obtained here, was comparable with a report from the Netherlands (25-30%) (Berends et al., 1997). Retail-ready pork from supermarkets was statistically associated with FP and TP in this study (r_s = 0.54 and 0.52, respectively).

5.4 Characterization of *Salmonella* spp.

A higher diversity of serotypes was observed from pre-harvest samples (overshoe samples) than post-harvest (FP and TP) from the end of the production chain (Table 4.10). In total, 27 serovars were identified; main sources were especially from the environment (Table 4.10). The most frequent (45.9 %) serotype was *S*. Rissen, followed by *S*. Typhimurium (17.2%), *S*. Krefeld (5.0%), *S*. Panama (3.2%), and *S*. Weltevreden (2.9%). Only one serovar (*S*. Enteritidis) was found only in R-ready pork.

The strains found in this study were reported as common serotypes in Thai patients during 1993-2007 (Bangtrakulnonth et al., 2004; Hendriksen et al., 2009). The most common serovars obtained from Thai patients were *S*. Weltevreden (Bangtrakulnonth et al., 2004) and *S.* Enteritidis (Hendriksen et al., 2008). Bangtrakulnonth et al. (2004) reported that *S*. Enteritidis was the most common *Salmonella* isolate from frozen chicken whereas *S*. Weltevreden was the most common serovar in frozen seafood and frozen duck.

Among the serovars in this study, *S*. Rissen was the most predominant serovar in R-ready pork, which could be found in every cohort: 46% of all isolates were *S*. Rissen. This serovar could be observed from the farm where the animals were fattened. This result supported other studies, which reported *S*. Rissen as the most frequently isolated serotype in living pigs and slaughtered pigs in Thailand (Padungtod and Kaneene, 2006; Padungtod et al., 2008). Angkititrakul et al. (2005) reported that *S*. Rissen was the most common serovar in pork from Northeast Thailand. However, another study (Vindigni et al., 2007) demonstrated that *S.* Anatum was the most

frequent isolated from pork in Bangkok, followed by *S*. Rissen. It might indicate that *S*. Rissen was a common serotype in pork in Thailand and which may indicate other trade chains in the capital city.

Here, many pulsotypes of *S.* Rissen found in retail-ready pork were indistinguishable from pulsotypes in swine. Results of this study revealed that swine was a possible source of *S.* Rissen. The significance of the present results indicated that retail-ready pork was an important source of *Salmonella.* In addition, animals, slaughterhouses and cutting could instigate its contamination.

Characteristics of *Salmonella* spp. on carcasses after splitting mostly related to serovars of *Salmonella* in slaughtered pigs (Table 4.8). Keeping track of the killed animals and the carcass after washing process, it was found that 82% of *Salmonella* serovars were seen in the animals from the same cohort and 18% of them were not found. However, *Salmonella* still remained on the carcasses after washing. This was not different from previous stages and from the animals in the same cohort. Pulsotyping might be another way to find outsources of contamination in the slaughterhouse. The confidence in the initial hypothesis that animals carried infectious agents into the production system was even higher than genotyping and pulsotyping: about 80% of pulsotype was similar to those found in the animals. For the serovars which were not obtained from the animals in this study, resident slaughterhouse flora played a rule here (Berends et al., 1996; Swanenburg et al., 2001; Smid et al., 2012). This study supported others; *Salmonella* Rissen pulsotype SRX27, which might remain in the slaughterhouse, have been found after washing the carcass repeatedly.

The technique of characterization of *Salmonella* with serotyping, an important tool in public health, could generally be divided into common serovars in different geographic areas (Yan et al., 2004). A diversity of serotypes in pig production was shown in various studies (Inthavong et al., 2006; Vieira-Pinto et al., 2006; Patchanee, 2008; Visscher et al., 2011; Schmidt et al., 2012). Reports of dominating serovars in pork production were different in different countries:

- *S.* Derby in USA (Schmidt et al., 2012); Italy (Piras et al., 2011); Ireland (Prendergast et al., 2009)
- *S.* Typhimurium in Germany (Methner et al., 2011; Visscher et al., 2011); Portugal (Vieira-Pinto et al., 2006; Gomes-Neves et al., 2012); Brazil (Kich et al., 2011)
- *S.* Agona in Japan (Futagawa-Saito et al., 2008)
- *S.* Rissen in Lao PDR (Inthavong et al., 2006)

Conventional serotyping was widely used and its acceptance as a method to differentiate the agent was not to discuss. However, the limitation of *Salmonella* serotyping existed. In this study, some isolates could not be given the name of serovar (i.e. S. O:3,10 H:e,h; S. O:3,10 H:z_6; S. O:3,15 H:f,g,r). In addition, some isolates could not be serotyped. Hence, another technique for typing was required for further specific investigation (Yan et al., 2004).

Molecular typing analysis of bacterial genome was important to an assessment of relationships among foodborne pathogens. From 327 available isolates, 83 patterns of pulsotype were obtained Using XbaI for genotyping *S.* Rissen was reported to be

.

unsuitable to distinguish different pulsotypes of serovar Rissen (De Busser et al., 2011) However, this study found *Xba*I was useful. The highest number of pulsotype using XbaI was found in *S.* Rissen (31 pulsotypes from 169 isolates), followed by *S.* Stanley (15 pulsotypes from 36 isolates) and *S.* Typhimurium (10 pulsotypes from 53 isolates). PFGE pattern between 100kb – 48.5 kb by XbaI was the most distinguishable areas. The similarities were high among them (84.2 - 100 % similarity), compared with other serovars. *S.* Rissen was recognized as a genetically diverse serotype (Vieira-Pinto et al., 2006; Hendriksen et al., 2008), which was also shown in this study. The predominant pattern of *S.* Rissen was R2 (SRX02) from 27 isolates was observed from various cohorts and various sample types among the same cohort indicating that *Salmonella* was transferred from farm to pork (R). However, other enzymes have been recommended for further subtyping for an effective molecular epidemiological investigation (Kerouanton et al., 2007; Goering, 2010; De Busser et al., 2011; Trujillo et al., 2011).

Non-typable strains without the use of Thiourea were observed from *S.* Panama in this study. The problem could be solved when adding Thiourea at a final concentration of 50-70 μM (Silbert et al., 2003; Goering, 2010), which might effect on other non-typeable serotypes (*S.* Livingstone) too, as reported by others (Liebana et al., 2001; De Busser et al., 2011).

λDNA ladders did not represent the most optimum standard since larger fragments tended to co-migrate near to the top of the gel (Goering, 2010); the lowest 48.5-kb fragment was used as an initial size reference. In this study, *S.* Rissen, *S.* Stanley and *S.* Typhimurium showed fragments between 48.5kb – 100.0 kb. A universal size

standard strain, *Salmonella* Braenderup H9812, for standardized PFGE protocols was established, covering a wide range of DNA fragment sizes (Hunter et al., 2005).

6. Summary

Molecular Epidemiology and Serodiversity of *Salmonella enterica* in a Pork Chain "From Farm to Fork" in Northern Thailand

This study was conducted from December 2004 – May 2005 in Chiang Mai and Lamphun provinces in Northern Thailand. In a larger framework, 193 live pigs (from 22 cohorts) were sampled individually, followed up into an abattoir and further investigated on cutting and R for *Salmonella enterica*. The dynamic of agent transfer in a pork chain including pork products in Northern Thailand was studied. For that, 1,000 isolates of *Salmonella enterica* were available.

Overall prevalence of *Salmonella* in samples from pigs and associated environments of the pork chain was 48.9 % (971/1982 samples). Drinking and cleaning water from farms had a similar prevalence (13.6 %). Wastewater was *Salmonella* positive almost every time and overshoe samples indicated comparable high positive results (95.5 % and 94.8 %, respectively). At slaughterhouse level, the highest percentage was found during cutting procedures, 23% of samples were positive,

At individual pig level, the lowest prevalence was obtained from carcasses after washing (12.9 %). Between farm faeces and mesenteric lymph nodes samples, no considerable difference was noticed, 61.4 % and 63.9 %, respectively. The prevalence after splitting was about 2.5 times higher than that after washing. Caecal content yielded the highest percentage of positive samples (83.1 %). The number of

Salmonella positive results was different depending on sites and cohorts of investigation.

The highest positive correlation coefficient was found between carcasses after washing and FP (r_s = 0.66; P = 0.0014), indicating that the carcass quality after splitting related to FP quality. Here, a relative risk (1.64; 95%CI: 1.294 - 2.089) was observed with statistical significance.

Detecting of *Salmonella* on CW increased the odds (OR = 3.9; P-value = 0.039) of FP. *Salmonella* from cutting boards increased odds of TP (OR = 3.9; P = 0.042) significantly. Additionally, *Salmonella* on overshoes at fattening farms increased odds of *Salmonella* being positive in TP (OR = 5.5; P = 0.015). The detection of *Salmonella* in mesenteric lymph nodes increased the odds of *Salmonella* findings in CC (OR = 2.3; P = 0.045) and of contaminated FP (OR = 2.0, P = 0.030).

Overall, 26 serovars were identified. *Salmonella* Rissen was the predominant serovar (45.9%). 7 serovars (*S.* Anatum, Krefeld, Panama, Rissen, Stanley, Typhimurium and Weltevreden) were identified throughout the complete chain (farms, slaughterhouses and R samples). 11 serovars (*Salmonella* Afula, Agona, Alfort, Bovismorbificans, Chittagong, Corvallis, Derby, Hato, Israel, Langensalza, Regent and Rideau) were detected only in farm samples including faeces from animals entering slaughterhouse, whereas 3 serovars (*Salmonella* Eppendorf, Livingstone and Tsevie) were detected only from samples from the slaughterhouses. One serovar (*S.* Enteritidis) was isolated only from 1 R product sample and could not be detected from somewhere else.

Dendrograms of PFGE patterns (pulsotypes) of *S.* Krefeld, *S.* Panama and *S.* Bovismorbificans were highly similar, while those of *S.* Stanley, *S.* Typhimurium, *S.* Rissen and *S.* Corvallis were highly diverse. The highest number of isolates was obtained from overshoes. The ratio of 3.1 isolates per pulsotype indicated the smallest variability, which was observed in isolates from FP.

Transfer/ trace back to the farm and evidence for contamination during slaughter could be observed. The pulsotype SRX02 could be traced back to the farm of origin; it was obtained from wastewater, overshoes, F, CC, ML, CS, CW, FP, and R samples.

Retail: frequently, *Salmonella* isolates from R pork were more closely related to mesenteric lymph nodes and/or samples from environment during cutting and/or FP and/or TP.

7. Zusammenfassung

Molekulare Epidemiologie und Serodiversität von Salmonella enterica in einer Schweineproduktionskette „from Farm to Fork“ in Nord- Thailand

Diese Untersuchung erfolgte innerhalb eines größeren Vorhabens vom Dezember 2004 bis Mai 2005 in Chiang Mai und der Provinz Lamphun in Nord- Thailand. 193 Schlachtschweine (22 unterschiedliche Mastgruppen) wurden individuell bereits in der Herkunft beprobt und bis in den Schlachtbetrieb hinein individuell verfolgt. Die Probenahme erfolgte weiterhin auf individueller Basis über die Schlachtung und Bearbeitung, Zerlegung, Transport und Verkauf. Hierfür standen 1.000 Isolate zur Verfügung.

Die Gesamtprävalenz von Salmonella in allen Proben lag bei 48,9 % (971 von 1982 Proben). Tränkwasser und Wasser zur Reinigung lagen beide bei 13,6 %. Schmutzwasser war fast vollständig Salmonella- positiv (95,5 %), ebenso wie die eingesetzten Sockenproben (94,8 %). Im Schlachtbetrieb und Schlachtgruppen-bezogen, wurde der höchste Prozentsatz positiver Proben in der Zerlegung mit 23 % gefunden.

Einzeltierbezogen wurde die niedrigste Quote mit 12,9 % nach dem Abwaschen der Tierkörper erzielt. Zwischen den Fäkalproben nach dem Transport und den Mesenterial-Lymphknotenproben war der Unterschied nur gering (61,4 % und 63,9 %). Nach dem Spalten der Tierkörper war die Prävalenz ca. 2,5 mal höher als nach

dem Waschen. Der Caecum-Inhalt erbrachte die höchste Prävalenz (83,1 %). Die Nachweisrate war abhängig von der Probenahmestelle und der Sendung.

Der höchste Korrelationskoeffizient fand sich zwischen den Positionen, nach dem Waschen“ und „Frischfleisch nach dem Zerlegen“ (r_s = 0,66; *P* = 0,0014), was auf Zusammenhänge zwischen den Positionen hindeutet. Das Relative Risiko war hier signifikant (1,64; 95% CI: 1,294 – 2,089).

Der Nachweis von Salmonellen nach dem Waschen erhöhte die Wahrscheinlichkeit (OR = 3,9; *P* = 0,039), daß auch Frischfleisch nach dem Zerlegen Salmonella- positiv war. Die Wahrscheinlichkeit positiver Proben nach dem Transport erhöhte sich, wenn auch die Zerlege-Unterlagen positiv waren (OR = 3,9; *P* = 0,042), auch, wenn die Sockenproben bereits positiv waren (OR = 5,5; *P* = 0,01). Der Nachweis in den Mesenterial- Lymphknoten erhöhte die Wahrscheinlichkeit des Nachweises von positivem Caecal-Inhalt (OR = 2,3; *P* = 0,045) und von positivem Frischfleisch nach dem Zerlegen (OR = 2,0; *P* = 0,03).

Insgesamt wurden 26 Serovaren identifiziert. Salmonella Rissen war vorherrschend mit 45,9 % der Isolate. 7 Serovaren (*S*. Anatum, Krefeld, Panama, Rissen, Stanley, Typhimurium und Weltevreden) wurden entlang der gesamten Kette nachgewiesen (Farm, Schlachtbetrieb, Vertrieb), darunter die am häufigsten nachgewiesenen Serovaren (*S*. Rissen, Typhimurium, Stanley). 11 Serovaren (*S*. Afula, Agona, Alfort, Bovismorbificans, Chittagong, Corvallis, Derby, Hato, Israel, Langensalza, Regent und Rideau) wurden nur in Farm- Proben einschließlich der Faeces der Tiere nach dem Transport nachgewiesen. 3 Serovaren (*S*. Eppendorf, Livingstone und

Tsevie) wurden nur aus Proben im Schlachtbetrieb, eine Serovar (*S*. Enteritidis) wurde nur aus dem Vertrieb und nur aus einer Probe isoliert.

Die Dendrogramme der PFGE („Pulsotypen“) von *S*. Krefeld, *S*. Panama und *S*. Bovismorbificans ähnelten einander stark, während *S*. Stanley, *S*. Typhimurium, *S*. Rissen und *S*. Corvallis sehr unterschiedlich waren. Die höchste Variabilität wurde in den Sockenproben gefunden, die Relation von 3,1 per Pulsotyp weist auf die geringste Variabilität hin, gefunden bei Proben von Frischfleisch nach der Zerlegung.

Die Rückverfolgung auf die Farm aus dem Schlachtbetrieb war möglich. Der Pulostyp SRX02 wurde im Schmutzwasser, Sockenproben, Fäkal- und Caecalproben, Mesenterial- Lymphknoten, den Schweinehälften nach dem Spalten, nach dem Waschen, auf Frischfleisch nach dem Zerlegen und in Proben des Handels wiedergefunden

Handel: Die Isolate Aus dem Handel ähnelten häufiger den Proben aus den Mesenterial- Lymphknoten und/ oder denen von der Umgebung der Zerlegung und/ oder dem Frischfleisch nach der Zerlegung und/ oder dem Transportierten Fleisch.

8. References

Alban, L., Baptista, F.M., Mogelmose, V., Sorensen, L.L., Christensen, H., Aabo, S., Dahl, J., 2012. Salmonella surveillance and control for finisher pigs and pork in Denmark - A case study. Food Research International 45, 656-665.

Alban, L., Sorensen, L.L., 2010. Hot-water decontamination is an effective way of reducing risk of Salmonella in pork. Fleischwirtschaft 90, 109-113.

Algino, R.J., Badtram, G.A., Ingham, B.H., Ingham, S.C., 2009. Factors associated with Salmonella prevalence on pork carcasses in very small abattoirs in Wisconsin. J Food Prot 72, 714-721.

Angkititrakul, S., Chomvarin, C., Chaita, T., Kanistanon, K., Waethewutajarn, S., 2005. Epidemiology of antimicrobial resistance in Salmonella isolated from pork, chicken meat and humans in Thailand. The Southeast Asian journal of tropical medicine and public health 36, 1510-1515.

Applied Maths NV, 2011. BioNumerics Manual Version 6.6. Applied Maths NV Sint-Martens-Latem.

Arguello, H., Alvarez-Ordonez, A., Carvajal, A., Rubio, P., Prieto, M., 2013. Role of slaughtering in Salmonella spreading and control in pork production. J Food Prot 76, 899-911.

Bahnson, P.B., Fedorka-Cray, P.J., Ladely, S.R., Mateus-Pinilla, N.E., 2006. Herd-level risk factors for Salmonella enterica subsp. enterica in U.S. market pigs. Prev Vet Med 76, 249-262.

Bangtrakulnonth, A., Pornreongwong, S., Pulsrikarn, C., Sawanpanyalert, P., Hendriksen, R.S., Lo Fo Wong, D.M., Aarestrup, F.M., 2004. Salmonella serovars from humans and other sources in Thailand, 1993-2002. Emerg Infect Dis 10, 131-136.

Beloeil, P.A., Fravalo, P., Fablet, C., Jolly, J.P., Eveno, E., Hascoet, Y., Chauvin, C., Salvat, G., Madec, F., 2004. Risk factors for Salmonella enterica subsp. enterica shedding by market-age pigs in French farrow-to-finish herds. Prev Vet Med 63, 103-120.

Ben Aissa, R., Al-Gallas, N., Troudi, H., Belhadj, N., Belhadj, A., 2007. Trends in Salmonella enterica serotypes isolated from human, food, animal, and environment in Tunisia, 1994-2004. The Journal of infection 55, 324-339.

Berends, B.R., Urlings, H.A.P., Snijders, J.M.A., VanKnapen, F., 1996. Identification and quantification of risk factors in animal management and transport regarding Salmonella spp in pigs. Int J Food Microbiol 30, 37-53.

Berends, B.R., Van Knapen, F., Mossel, D.A.A., Burt, S.A., Snijders, J.M.A., 1998. Salmonella spp. on pork at cutting plants and at the R level and the influence of particular risk factors. Int J Food Microbiol 44, 207-217.

Berends, B.R., VanKnapen, F., Snijders, J.M.A., Mossel, D.A.A., 1997. Identification and quantification of risk factors regarding Salmonella spp. On pork carcasses. Int J Food Microbiol 36, 199-206.

Boonmar, S., Bangtrakulnonth, A., Pornrunangwong, S., Marnrim, N., Kaneko, K., Ogawa, M., 1998. Salmonella in broiler chickens in Thailand with special reference to contamination of R meat with Salmonella enteritidis. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 60, 1233-1236.

Botteldoorn, N., Herman, L., Rijpens, N., Heyndrickx, M., 2004. Phenotypic and molecular typing of Salmonella strains reveals different contamination sources in two commercial pig slaughterhouses. Appl Environ Microbiol 70, 5305-5314.

Botteldoorn, N., Heyndrickx, M., Rijpens, N., Grijspeerdt, K., Herman, L., 2003. Salmonella on pig carcasses: positive pigs and cross contamination in the slaughterhouse. J Appl Microbiol 95, 891-903.

Boyen, F., Haesebrouck, F., Maes, D., Van Immerseel, F., Ducatelle, R., Pasmans, F., 2008. Non-typhoidal Salmonella infections in pigs: a closer look at epidemiology, pathogenesis and control. Vet Microbiol 130, 1-19.

Buncic, S., Sofos, J., 2012. Interventions to control Salmonella contamination during poultry, cattle and pig slaughter. Food Research International 45, 641-655.

Cardinale, E., Maeder, S., Porphyre, V., Debin, M., 2010. Salmonella in fattening pigs in Reunion Island: herd prevalence and risk factors for infection. Prev Vet Med 96, 281-285.

Chantong, W., 2005. Salmonella isolation from slaughter pigs and carcasses in a slaughterhouse in Chiang Mai, Thailand. Chiang Mai University and Freie University of Berlin, Chiang Mai, 72.

Chen, M.H., Chiou, C.S., Chiang, Y.C., Chen, P.H., Tsai, S.W., Tsen, H.Y., 2012. Comparison of the pulsed field gel electrophoresis patterns and virulence profiles of the multidrug resistant

strains of Salmonella enterica serovar Schwarzengrund isolated from chicken meat and humans in Taiwan. Food Research International 45, 978-983.

Cliver, D.O., 2006. Cutting boards in Salmonella cross-contamination. J AOAC Int 89, 538-542.

D'Aoust, J.-Y., 1997. Salmonella Species. In: Doyle, M.P., Beuchat, L.R., Montville, T.J. (Eds.), Food Microbiology Fundamentals and Frontiers. American Society for Microbiology, Washington, DC, 129-158.

da Silva, L.E., Dias, V., Ferronatto, A., Guerra, P., Berno, L., Triches, N., Kich, J.D., Corbellini, L.G., Cardoso, M., 2012. Longitudinal dissemination of Salmonella enterica clonal groups through the slaughter process of Salmonella-positive pig batches. J Food Prot 75, 1580-1588.

Davies, P., Funk, J., Morrow, W.E.M., 1999. Fecal shedding of Salmonella by a cohort of finishing pigs in North Carolina. Swine Health Prod 7, 231-234.

De Busser, E.V., Maes, D., Houf, K., Dewulf, J., Imberechts, H., Bertrand, S., De Zutter, L., 2011. Detection and characterization of Salmonella in lairage, on pig carcasses and intestines in five slaughterhouses. Int J Food Microbiol 145, 279-286.

Dorn-In, S., Fries, R., Padungtod, P., Kyule, M.N., Baumann, M.P., Srikitjakarn, L., Chantong, W., Sanguangiat, A., Zessin, K.H., 2009. A cross-sectional study of Salmonella in pre-slaughter pigs in a production compartment of northern Thailand. Prev Vet Med 88, 15-23.

Dorr, P.M., Tadesse, D.A., Zewde, B.M., Fry, P., Thakur, S., Gebreyes, W.A., 2009. Longitudinal study of Salmonella dispersion and the role of environmental contamination in commercial swine production systems. Appl Environ Microbiol 75, 1478-1486.

Edwards, P.R., Ewing, W.H., 1972. Identification of Enterobacteriacecae. Burgess Publishing Company Minneapolis, Minnesota.

Elwood, M., 2000. Critical appraisal of epidemiological studies and clinical trials. Oxford University Press Oxford, UK.

Fablet, C., Robinault, C., Jolly, J.P., Collet, M., Chemaly, M., Labbe, A., Madec, F., Fravalo, P., 2006. Salmonella enterica level in French pig farms effluents: Experimental and field data. Livestock Science 102, 216-225.

Farzan, A., Friendship, R.M., Dewey, C.E., Warriner, K., Poppe, C., Klotins, K., 2006. Prevalence of Salmonella spp. on Canadian pig farms using liquid or dry-feeding. Prev Vet Med 73, 241-254.

Fedorka-Cray, P.J., Whipp, S.C., Isaacson, R.E., Nord, N., Lager, K., 1994. Transmission of Salmonella typhimurium to swine. Vet Microbiol 41, 333-344.

Foley, S.L., Zhao, S., Walker, R.D., 2007. Comparison of molecular typing methods for the differentiation of Salmonella foodborne pathogens. Foodborne Pathog Dis 4, 253-276.

Freeman, M., 2011. PFGE: Tips and Tricks to Success and Interpretation of Results for Foodborne Outbreak Investigations. In, BioNumerics Workshop for PulseNet Participants.

Futagawa-Saito, K., Hiratsuka, S., Kamibeppu, M., Hirosawa, T., Oyabu, K., Fukuyasu, T., 2008. Salmonella in healthy pigs: prevalence, serotype diversity and antimicrobial resistance observed during 1998-1999 and 2004-2005 in Japan. Epidemiol Infect 136, 1118-1123.

Garcia-Feliz, C., Carvajal, A., Collazos, J.A., Rubio, P., 2009. Herd-level risk factors for faecal shedding of Salmonella enterica in Spanish fattening pigs. Prev Vet Med 91, 130-136.

Gaul, S.B., Wedel, S., Erdman, M.M., Harris, D.L., Harris, I.T., Ferris, K.E., Hoffman, L., 2007. Use of pulsed-field gel electrophoresis of conserved XbaI fragments for identification of swine Salmonella serotypes. J Clin Microbiol 45, 472-476.

Gebreyes, W.A., Thakur, S., Morrow, W.E., 2006. Comparison of prevalence, antimicrobial resistance, and occurrence of multidrug-resistant Salmonella in antimicrobial-free and conventional pig production. J Food Prot 69, 743-748.

Goering, R.V., 2010. Pulsed field gel electrophoresis: a review of application and interpretation in the molecular epidemiology of infectious disease. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 10, 866-875.

Gomes-Neves, E., Antunes, P., Tavares, A., Themudo, P., Cardoso, M.F., Gartner, F., Costa, J.M., Peixe, L., 2012. Salmonella cross-contamination in swine abattoirs in Portugal: Carcasses, meat and meat handlers. Int J Food Microbiol 157, 82-87.

Gotter, V., Klein, G., Koesters, S., Kreienbrock, L., Blaha, T., Campe, A., 2012. Main risk factors for Salmonella-infections in pigs in north-western Germany. Prev Vet Med 106, 301-307.

Gracey, J.F., Collins, D.S., 1992. Meat Hygiene. Baillière Tindall London.

Grimont, P.A.D., Weill, F.-X., 2007. Antigenic Formulae of the Salmonella Serovars (9th ed.) WHO Collaborating Center for Reference and Research on Salmonella, Institut Pasteur, Paris.

Hansen, T.B., Christensen, B.B., Aabo, S., 2010. Salmonella in pork cuttings in supermarkets and butchers' shops in Denmark in 2002 and 2006. Zoonoses and public health 57 Suppl 1, 23-29.

Hauser, E., Tietze, E., Helmuth, R., Junker, E., Blank, K., Prager, R., Rabsch, W., Appel, B., Fruth, A., Malorny, B., 2010. Pork contaminated with Salmonella enterica serovar 4,[5],12:i:-, an emerging health risk for humans. Appl Environ Microbiol 76, 4601-4610.

Hendriksen, R.S., Bangtrakulnonth, A., Pulsrikarn, C., Pornreongwong, S., Hasman, H., Song, S.W., Aarestrup, F.M., 2008. Antimicrobial resistance and molecular epidemiology of Salmonella Rissen from animals, food products, and patients in Thailand and Denmark. Foodborne Pathog Dis 5, 605-619.

Hendriksen, R.S., Bangtrakulnonth, A., Pulsrikarn, C., Pornruangwong, S., Noppornphan, G., Emborg, H.D., Aarestrup, F.M., 2009. Risk factors and epidemiology of the ten most common Salmonella serovars from patients in Thailand: 2002-2007. Foodborne Pathog Dis 6, 1009-1019.

Hosmer, D., Lemeshow, S., 2000. Applied Logistic Regression, 2nd edition. John Wiley and Sons New York.

Hotes, S., Kemper, N., Traulsen, I., Rave, G., Krieter, J., 2010. Risk factors for Salmonella infection in fattening pigs - an evaluation of blood and meat juice samples. Zoonoses and public health 57 Suppl 1, 30-38.

Hunter, P.R., Gaston, M.A., 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J Clin Microbiol 26, 2465-2466.

Hunter, S.B., Vauterin, P., Lambert-Fair, M.A., Van Duyne, M.S., Kubota, K., Graves, L., Wrigley, D., Barrett, T., Ribot, E., 2005. Establishment of a universal size standard strain for use with the PulseNet standardized pulsed-field gel electrophoresis protocols: converting the national databases to the new size standard. J Clin Microbiol 43, 1045-1050.

Hurd, H.S., Gailey, J.K., McKean, J.D., Griffith, R.W., 2005. Variable abattoir conditions affect Salmonella enterica prevalence and meat quality in swine and pork. Foodborne Pathog Dis 2, 77-81.

Hurd, H.S., Gailey, J.K., McKean, J.D., Rostagno, M.H., 2001. Rapid infection in market-weight swine following exposure to a Salmonella typhimurium-contaminated environment. Am J Vet Res 62, 1194-1197.

Hurd, H.S., McKean, J.D., Griffith, R.D., Rostagno, M.H., 2004. Estimation of the Salmonella enterica prevalence in finishing swine. Epidemiol Infect 132, 127-135.

Hurd, H.S., McKean, J.D., Griffith, R.W., Wesley, I.V., Rostagno, M.H., 2002. Salmonella enterica infections in market swine with and without transport and holding. Appl Environ Microbiol 68, 2376-2381.

ICMSF, 1996. Salmonellae. In: Roberts, T.A., Barid-Parker, A.C., Tompkin, R.B. (Eds.), Microorganisms in foods 5: Mircobiological specifications of food pathogens. Blackie Academic & Professional, London.

Inthavong, P., Srikitjakarn, L., Kyule, M., Zessin, K.H., Baumann, M., Douangngeun, B., Fries, R., 2006. Microbial contamination of pig carcasses at a slaughterhouse in Vientiane capital, Lao PDR. The Southeast Asian journal of tropical medicine and public health 37, 1237-1241.

Ivanek, R., Osterberg, J., Gautam, R., Sternberg Lewerin, S., 2012. Salmonella fecal shedding and immune responses are dose- and serotype- dependent in pigs. PLoS One 7, e34660.

Jensen, A.N., Lodal, J., Baggesen, D.L., 2004. High diversity of Salmonella serotypes found in an experiment with outdoor pigs. Njas-Wagen J Life Sc 52, 109-117.

Kerouanton, A., Marault, M., Lailler, R., Weill, F.X., Feurer, C., Espie, E., Brisabois, A., 2007. Pulsed-field gel electrophoresis subtyping database for foodborne Salmonella enterica serotype discrimination. Foodborne Pathog Dis 4, 293-303.

Kich, J.D., Coldebella, A., Mores, N., Nogueira, M.G., Cardoso, M., Fratamico, P.M., Call, J.E., Fedorka-Cray, P., Luchansky, J.B., 2011. Prevalence, distribution, and molecular characterization of Salmonella recovered from swine finishing herds and a slaughter facility in Santa Catarina, Brazil. Int J Food Microbiol 151, 307-313.

Kranker, S., Alban, L., Boes, J., Dahl, J., 2003. Longitudinal study of Salmonella enterica aerotype Typhimurium infection in three Danish farrow-to-finish swine herds. J Clin Microbiol 41, 2282-2288.

Krumkamp, R., Reintjes, R., Dirksen-Fischer, M., 2008. Case-case study of a Salmonella outbreak: an epidemiologic method to analyse surveillance data. Int J Hyg Environ Health 211, 163-167.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159-174.

Liebana, E., Guns, D., Garcia-Migura, L., Woodward, M.J., Clifton-Hadley, F.A., Davies, R.H., 2001. Molecular typing of Salmonella serotypes prevalent in animals in England: assessment of methodology. J Clin Microbiol 39, 3609-3616.

Lo Fo Wong, D.M., Dahl, J., Stege, H., van der Wolf, P.J., Leontides, L., von Altrock, A., Thorberg, B.M., 2004. Herd-level risk factors for subclinical Salmonella infection in European finishing-pig herds. Prev Vet Med 62, 253-266.

Magistrali, C., Dionisi, A.M., De Curtis, P., Cucco, L., Vischi, O., Scuota, S., Zicavo, A., Pezzotti, G., 2008. Contamination of Salmonella spp. in a pig finishing herd, from the arrival of the animals to the slaughterhouse. Res Vet Sci 85, 204-207.

Mannion, C., Fanning, J., McLernon, J., Lendrum, L., Gutierrez, M., Duggan, S., Egan, J., 2012. The role of transport, lairage and slaughter processes in the dissemination of Salmonella spp. in pigs in Ireland. Food Research International 45, 871-879.

McDowell, S.W., Porter, R., Madden, R., Cooper, B., Neill, S.D., 2007. Salmonella in slaughter pigs in Northern Ireland: prevalence and use of statistical modelling to investigate sample and abattoir effects. Int J Food Microbiol 118, 116-125.

Methner, U., Rammler, N., Fehlhaber, K., Rosler, U., 2011. Salmonella status of pigs at slaughter--bacteriological and serological analysis. Int J Food Microbiol 151, 15-20.

Michael, G.B., Cardoso, M., Schwarz, S., 2006. Molecular analysis of Salmonella enterica subsp. enterica serovar Agona isolated from slaughter pigs. Vet Microbiol 112, 43-52.

Minami, A., Chaicumpa, W., Chongsa-Nguan, M., Samosornsuk, S., Monden, S., Takeshi, K., Makino, S., Kawamoto, K., 2010. Prevalence of foodborne pathogens in open markets and supermarkets in Thailand. Food Control 21, 221-226.

Molla, B., Sterman, A., Mathews, J., Artuso-Ponte, V., Abley, M., Farmer, W., Rajala-Schultz, P., Morrow, W.E., Gebreyes, W.A., 2010. Salmonella enterica in commercial swine feed and subsequent isolation of phenotypically and genotypically related strains from fecal samples. Appl Environ Microbiol 76, 7188-7193.

Morgan, I.R., Krautil, F.L., Craven, J.A., 1987. Effect of time in lairage on caecal and carcass salmonella contamination of slaughter pigs. Epidemiol Infect 98, 323-330.

Nicolay, N., Thornton, L., Cotter, S., Garvey, P., Bannon, O., McKeown, P., Cormican, M., Fisher, I., Little, C., Boxall, N., De Pinna, E., Peters, T.M., Cowden, J., Salmon, R., Mason, B., Irvine, N., Rooney, P., O'Flanagan, D., 2011. Salmonella enterica serovar Agona European outbreak associated with a food company. Epidemiol Infect 139, 1272-1280.

Nielsen, B., Wegener, H.C., 1997. Public health and pork and pork products: regional perspectives of Denmark. Rev Sci Tech 16, 513-524.

Nollet, N., Maes, D., De Zutter, L., Duchateau, L., Houf, K., Huysmans, K., Imberechts, H., Geers, R., de Kruif, A., Van Hoof, J., 2004. Risk factors for the herd-level bacteriologic prevalence of Salmonella in Belgian slaughter pigs. Prev Vet Med 65, 63-75.

O'Connor, A.M., Wang, B., Denagamage, T., McKean, J., 2012. Process mapping the prevalence of Salmonella contamination on pork carcass from slaughter to chilling: a systematic review approach. Foodborne Pathog Dis 9, 386-395.

Olaimat, A.N., Holley, R.A., 2012. Factors influencing the microbial safety of fresh produce: a review. Food Microbiol 32, 1-19.

Padungtod, P., Kadohira, M., Hill, G., 2008. Livestock production and foodborne diseases from food animals in Thailand. The Journal of veterinary medical science / the Japanese Society of Veterinary Science 70, 873-879.

Padungtod, P., Kaneene, J.B., 2006. Salmonella in food animals and humans in northern Thailand. Int J Food Microbiol 108, 346-354.

Pang, J.C., Chiu, T.H., Helmuth, R., Schroeter, A., Guerra, B., Tsen, H.Y., 2007. A pulsed field gel electrophoresis (PFGE) study that suggests a major world-wide clone of Salmonella enterica serovar Enteritidis. Int J Food Microbiol 116, 305-312.

Patchanee, P., 2008. Epidemiology of Salmonella enterica related to swine production system and food safety. The Graduate School of the Ohio State University. The Ohio State University, 201.

Patchanee, P., Zessin, K.H., Staak, C., Srikitjakarn, L., Taravijitkul, P., Tesaprateep, T., 2002. Pre slaughter infection of Salmonella spp. and consideration of using the Danish mix ELISA for monitoring Salmonella in pigs. Chiangmai Veterinary Journal 1, 33-38.

Piras, F., Brown, D.J., Meloni, D., Mureddu, A., Mazzette, R., 2011. Investigation of Salmonella enterica in Sardinian slaughter pigs: prevalence, serotype and genotype characterization. Int J Food Microbiol 151, 201-209.

Pornruangwong, S., Hendriksen, R.S., Pulsrikarn, C., Bangstrakulnonth, A., Mikoleit, M., Davies, R.H., Aarestrup, F.M., Garcia-Migura, L., 2011. Epidemiological investigation of Salmonella enterica serovar Kedougou in Thailand. Foodborne Pathog Dis 8, 203-211.

Pornruangwong, S., Sriyapai, T., Pulsrikarn, C., Sawanpanyalert, P., Boonmar, S., Bangtrakulnonth, A., 2008. The epidemiological relationship between Salmonella enterica serovar typhimurium

and Salmonella enterica serovar 4,[5],12:i:- isolates from humans and swine in Thailand. The Southeast Asian journal of tropical medicine and public health 39, 288-296.

Prendergast, D.M., Duggan, S.J., Gonzales-Barron, U., Fanning, S., Butler, F., Cormican, M., Duffy, G., 2009. Prevalence, numbers and characteristics of Salmonella spp. on Irish R pork. Int J Food Microbiol 131, 233-239.

Rajic, A., Keenliside, J., McFall, M.E., Deckert, A.E., Muckle, A.C., O'Connor, B.P., Manninen, K., Dewey, C.E., McEwen, S.A., 2005. Longitudinal study of Salmonella species in 90 Alberta swine finishing farms. Vet Microbiol 105, 47-56.

Refsum, T., Heir, E., Kapperud, G., Vardund, T., Holstad, G., 2002. Molecular epidemiology of Salmonella enterica serovar typhimurium isolates determined by pulsed-field gel electrophoresis: comparison of isolates from avian wildlife, domestic animals, and the environment in Norway. Appl Environ Microbiol 68, 5600-5606.

Ribot, E.M., Fair, M.A., Gautom, R., Cameron, D.N., Hunter, S.B., Swaminathan, B., Barrett, T.J., 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7, Salmonella, and Shigella for PulseNet. Foodborne Pathog Dis 3, 59-67.

Rostagno, M.H., Callaway, T.R., 2012. Pre-harvest risk factors for Salmonella enterica in pork production. Food Research International 45, 634-640.

Rostagno, M.H., Hurd, H.S., McKean, J.D., Ziemer, C.J., Gailey, J.K., Leite, R.C., 2003. Preslaughter holding environment in pork plants is highly contaminated with Salmonella enterica. Appl Environ Microbiol 69, 4489-4494.

Sanguankiat, A., Pinthong, R., Padungtod, P., Baumann, M.P., Zessin, K.H., Srikitjakarn, L., Fries, R., 2010. A cross-sectional study of Salmonella in pork products in Chiang Mai, Thailand. Foodborne Pathog Dis 7, 873-878.

Schmidt, J.W., Brichta-Harhay, D.M., Kalchayanand, N., Bosilevac, J.M., Shackelford, S.D., Wheeler, T.L., Koohmaraie, M., 2012. Prevalence, enumeration, serotypes, and antimicrobial resistance phenotypes of salmonella enterica isolates from carcasses at two large United States pork processing plants. Appl Environ Microbiol 78, 2716-2726.

Silbert, S., Boyken, L., Hollis, R.J., Pfaller, M.A., 2003. Improving typeability of multiple bacterial species using pulsed-field gel electrophoresis and thiourea. Diagnostic Microbiology and Infectious Disease 47, 619-621.

Smid, J.H., Heres, L., Havelaar, A.H., Pielaat, A., 2012. A biotracing model of Salmonella in the pork production chain. J Food Prot 75, 270-280.

Sorensen, L.L., Alban, L., Nielsen, B., Dahl, J., 2004. The correlation between Salmonella serology and isolation of Salmonella in Danish pigs at slaughter. Vet Microbiol 101, 131-141.

StataCorp, 2009. Stata Statistical Software. Stata Corporation, College Station, Texas.

Steinbach, G., Kroell, U., 1999. [Salmonella infections in swine herds--epidemiology and importance for human diseases]. DTW. Deutsche tierarztliche Wochenschrift 106, 282-288.

Stevens, A., Kerouanton, A., Marault, M., Millemann, Y., Brisabois, A., Cavin, J.F., Dufour, B., 2008. Epidemiological analysis of Salmonella enterica from beef sampled in the slaughterhouse and Rers in Dakar (Senegal) using pulsed-field gel electrophoresis and antibiotic susceptibility testing. Int J Food Microbiol 123, 191-197.

Stevens, M.P., Humphrey, T.J., Maskell, D.J., 2009. Molecular insights into farm animal and zoonotic Salmonella infections. Philosophical transactions of the Royal Society of London. Series B, Biological sciences 364, 2709-2723.

Swaminathan, B., Barrett, T.J., Hunter, S.B., Tauxe, R.V., 2001. PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 7, 382-389.

Swanenburg, M., van der Wolf, P.J., Urlings, H.A., Snijders, J.M., van Knapen, F., 2001. Salmonella in slaughter pigs: the effect of logistic slaughter procedures of pigs on the prevalence of Salmonella in pork. Int J Food Microbiol 70, 231-242.

Tenover, F.C., Arbeit, R.D., Goering, R.V., Mickelsen, P.A., Murray, B.E., Persing, D.H., Swaminathan, B., 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33, 2233-2239.

Trujillo, S., Keys, C.E., Brown, E.W., 2011. Evaluation of the taxonomic utility of six-enzyme pulsed-field gel electrophoresis in reconstructing Salmonella subspecies phylogeny. Infection, genetics and evolution : journal of molecular epidemiology and evolutionary genetics in infectious diseases 11, 92-102.

Utrarachkij, F., Pornraungwong, S., Siripanichgon, K., Nakajima, C., Suzuki, Y., Suthienkul, O., 2012. Possible horizontal transmission of Salmonella via reusable egg trays in Thailand. Int J Food Microbiol 154, 73-78.

van Belkum, A., Struelens, M., de Visser, A., Verbrugh, H., Tibayrenc, M., 2001. Role of genomic typing in taxonomy, evolutionary genetics, and microbial epidemiology. Clinical microbiology reviews 14, 547-560.

van Hoek, A.H., de Jonge, R., van Overbeek, W.M., Bouw, E., Pielaat, A., Smid, J.H., Malorny, B., Junker, E., Lofstrom, C., Pedersen, K., Aarts, H.J., Heres, L., 2012. A quantitative approach towards a better understanding of the dynamics of Salmonella spp. in a pork slaughter-line. Int J Food Microbiol 153, 45-52.

Vieira-Pinto, M., Tenreiro, R., Martins, C., 2006. Unveiling contamination sources and dissemination routes of Salmonella sp. in pigs at a Portuguese slaughterhouse through macrorestriction profiling by pulsed-field gel electrophoresis. Int J Food Microbiol 110, 77-84.

Vigo, G.B., Cappuccio, J.A., Pineyro, P.E., Salve, A., Machuca, M.A., Quiroga, M.A., Moredo, F., Giacoboni, G., Cancer, J.L., Caffer, I.G., Binsztein, N., Pichel, M., Perfumo, C.J., 2009. Salmonella enterica subclinical infection: bacteriological, serological, pulsed-field gel electrophoresis, and antimicrobial resistance profiles--longitudinal study in a three-site farrow-to-finish farm. Foodborne Pathog Dis 6, 965-972.

Vindigni, S.M., Srijan, A., Wongstitwilairoong, B., Marcus, R., Meek, J., Riley, P.L., Mason, C., 2007. Prevalence of foodborne microorganisms in R foods in Thailand. Foodborne Pathog Dis 4, 208-215.

Visscher, C.F., Klein, G., Verspohl, J., Beyerbach, M., Stratmann-Selke, J., Kamphues, J., 2011. Serodiversity and serological as well as cultural distribution of Salmonella on farms and in abattoirs in Lower Saxony, Germany. Int J Food Microbiol 146, 44-51.

Warriss, P.D., 2003. Optimal lairage times and conditions for slaughter pigs: a review. The Veterinary record 153, 170-176.

Wegener, H.C., Hald, T., Lo Fo Wong, D., Madsen, M., Korsgaard, H., Bager, F., Gerner-Smidt, P., Molbak, K., 2003. Salmonella control programs in Denmark. Emerg Infect Dis 9, 774-780.

WHO, 2005. The Treatment of diarrhoea : a manual for physicians and other senior health workers In: rev, t. (Ed.) WHO Press.

Yan, S.S., Pendrak, M.L., Abela-Ridder, B., Punderson, J.W., Fedorko, D.P., Foley, S.L., 2004. An overview of Salmonella typing: Public health perspectives. Clinical and Applied Immunology Reviews 4, 189-204.

Zou, W., Lin, W.J., Foley, S.L., Chen, C.H., Nayak, R., Chen, J.J., 2010. Evaluation of pulsed-field gel electrophoresis profiles for identification of Salmonella serotypes. J Clin Microbiol 48, 3122-3126.

9. Appendix

9.1 Cohort and location of farm origin

Cohort	Farm type	District	Province
1	Open	Mae Tha	Lamphun
2	Open	Ban Thi	Lamphun
3	Closed	San Sai	Chiang Mai
4	Open	San Sai	Chiang Mai
5	Open	San Sai	Chiang Mai
6	Open	NA	NA
7	Open	Pa Sang	Lamphun
8	Open	Mae Tang	Chiang Mai
9	Open	Mae Rim	Chiang Mai
10	Open	Pa Sang	Lamphun
11	Open	Mae Tang	Chiang Mai
12	Open	Mae Wang	Chiang Mai
13	Closed	San Kamphaeng	Chiang Mai
14	Open	Mae Tha	Lamphun
15	Closed	San Kamphaeng	Chiang Mai
16	Closed	San Kamphaeng	Chiang Mai
17	Open	Mae On	Chiang Mai
18	Open	San Kamphaeng	Chiang Mai
19	Open	Mae Tang	Chiang Mai
20	Open	Mae Tha	Lamphun
21	Open	Mae Tha	Lamphun
22	Open	Mae Tha	Lamphun

NA = Not available

9.2 PFGE technique for salmonellae

Day -1

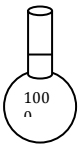
1. Subculture salmonellae from stock-culture (Glycerol) on Standard Iagar. Incubate at 37°C for 14-18 h.

Day 0

2. Streak a single colony from the first Standard Iagarplate to a second Standard Iagarplate and Standard II slant agar. Incubate at 37°C for 14-18 h.

Day 1

3. Weigh90 mg (0.09 g) ofCertified Megabase Agaroseinto 15 ml centrifuge tube (for preparing 2% Certified™ Megabase Agarose, see 7.)
4. Prepare **TE-Buffer** (10 mM Tris :1 mM EDTA, pH 8.0) as follows:



   a. 10 ml of 1 M Tris, pH 8.0
   b. 5 ml of 0.2 M EDTA**,** pH 8.0
   c. Dilute to 1000 ml with double-distilled water (ddH_2O)
   d. Sterilization* for using in "washing step" on DAY 2

5. Prepare **Cell Suspension Buffer** (100 mM Tris:100 mM EDTA, pH 8.0) as follow:

   a. 10 ml of 1 M Tris, pH 8.0
   b. 50 ml of 0.2 M EDTA (or 20 ml of 0.5 M EDTA), pH 8.0
   c. 40 ml of steriledouble-distilled water
   d. Keep it in refrigerator before use

6. Turn on shaker water bath (54ºC), hot plate (100 ºC) and spectrophotometer with **630** nm wavelength
7. Prepare **2% Certified™ Megabase Agarose**in TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0) for PFGE plugs as follows:
   a. 90 mg (0.09 g) of Cert. megabase-agarose(from 3.)
   b. Add 4.5 ml sterileTE-Buffer; swirl gently to disperse agarose.
   c. Loosen cap and heat at 100°C in beaker of water on the hot plate until agarose is *completely dissolved*. !!!!! (about 15-20 min)
   d. Check the temperature of water bath (54 ºC) and cooling the agarose in the water bath (54 ºC) (about 10 min) before use the agarose.

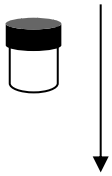
8. Label 10-ml glass tubes with the lab numbers of the culture.
9. Transfer **2 ml of Cell Suspension Buffer (CSB**) to 10-ml labeled glass tubes. Use a sterile loop to remove some colonies from agar plate; suspend cells in CSB by spinning loop gently so cells will evenly disperse.
10. Adjust concentration of cell suspensions
    a. Pipette 100 µl of sterile water into 12 wells in the first row of microtiter plate.
    b. Pipette 100 of each sample into well in 2nd and 3rd row.
    c. Measure the value from 630nm wavelength. The absorbance (optical density) result should be between **0.55-0.60**

d. In case of the optical density is too high, dilute with sterile CSB as following table;

Optical density	CSB
0.601-0.620	50 µL
0.621-0.630	100 µL
0.631-0.640	200 µL
0.641-0.650	300 µL
0.651-0.680	400 µL
0.681-0.700	500 µL
0.701-0.720	600 µL
0.721-0.740	700 µL
0.741-0.760	800 µL

e. In case of the optical density is too low, add additional cell from culture plate

11. Label 1.5-ml micro-centrifuge tube with PFGE culture number.
12. Label each well plug mold with PFGE culture number.
13. Transfer **200 µl (0.2 ml**) **adjusted cell suspensions** to labeled 1.5-ml micro-centrifuge tube. If cell suspensions are at room temperature, agarose can be added directly without pre-warming cell suspensions, if cell suspensions are cold, place tubes containing cell suspensions in plastic holder (floats); incubate in a 37°C water bath for a few minutes.
14. Add **10 µl of Proteinase K (20 mg/ml stock)** to each micro-centrifuge tube and mix gently with pipette tip. (120 µl are needed for 12 cell suspensions.)
15. Add **200 µl (0.2 ml)melted of 2%** Certified™ Megabase Agaroseto 0.2-ml cell suspension; mix by gently pipetting mixture up and down a few times. Maintain temperature of melted agarose by keeping flask in beaker of warm water (55-60°C)
16. **Immediately**, dispense part of mixture into the appropriate well(s) of plug mold. Do not allow bubbles to form. Up to four plugs of each sample can be made from those amounts of cell suspension and agarose. Allow plugs to solidify at room temperature for 10-15 min. They can also be placed in the refrigerator (4°C) for 5 minutes.
17. Label 20-ml glass tube with PFGE culture numbers and place tubes in rack.
18. **Prepare Cell Lysis Buffer** (50 mM Tris:50 mM EDTA , pH 8.0 + 1% Sarcosyl) as follows:



Ingredient	***Cell Lysis Buffer***	
	5 ml for 1 Sample	***60 ml for 12 Samples***
Cell Suspension Buffer	2.5 ml	30 ml
10 % Sarcosyl	0.5 ml	6 ml
Sterile double-distilled water *	2 ml	24 ml
Proteinase K	25 µl	300 µl

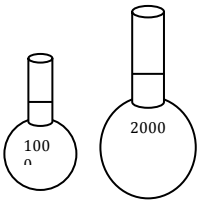
19. Remove white tape from bottom of mold and push out plug(s) into appropriately labeled glass tube.
20. Add **5 ml of Cell lysis bufferwith Proteinase K** to each labeled glass tube. *Be sure plugs are under buffer and do not stick on one side of tube*.
21. Incubate in a 54°C shaker water bath for **20 h (overnight)** with *constant and vigorous agitation* (150-175 rpm). If lysing in water bath, make sure *water level is above level of lysis buffer* in tubes.

DAY 2

22. Remove glass tubes from water bath.
23. Set temperature of shaker water bath to 50°C.
24. **Washing step**: Pre-heat sterile water (240-360 ml for 12 tubes) and TE-Buffer (480-720 ml for 12 tubes) in a 50°C water bath.
25. Carefully pour off lysis buffer into an appropriate discard container; plugs can be held in tubes with a small glass spatula and use plastic sieve for securing the plugs *. (*Use ethanol absolute GR for cleaning equipment)
26. Add 10-15 sterile water that has been pre-heated to each tube and shake the tubes in a 50°C water bath for 10-15 min.
27. Pour off water from the plugs and repeat wash step with pre-heated water one more time.
28. Pour off water, add 10-15 ml pre-heated (50°C) sterile TE Buffer, and shake the tubes in 50°C shaker water bath for 10-15 min.
29. Pour off TE and repeat wash step with pre-heated TE three more times.
30. Descant last wash and add 5-10 ml sterile TE. Continue with step 31. or store plugs in TE Buffer at 4°C until needed. Plugs can be transferred to smaller tubes for storage that contain 500 µl.
31. Turn on water bath (55°C-60°C)
32. Prepare 3000 ml of **0.5X Tris-Borate EDTA Buffer (TBE)** that is needed for both the gel and electropholysis running buffer. (in 1000 and 2000 ml volumetric flask)

2x water

4x TE



Reagent	Volume		
10x TBE	**50 ml**	**100 ml**	**150 ml**
0.5 M Thiourea	**200 µl**	**400 µl**	**600 µl**
Dilute with double-distilled water to	**1000 ml**	**2000 ml**	**3000 ml**

33. **Preparation of Agarose gel**: Prepare a 1.2% (100 ml for 14×21 cm gel) agarose gel with 0.5X TBE-Buffer and load plugs and.

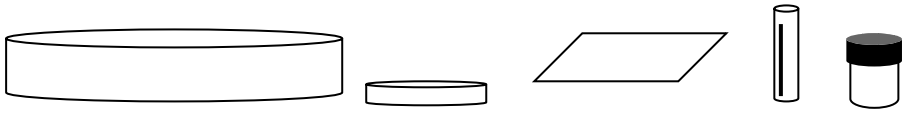
    a. Weigh 1.2 g of Pulsed-field Certified Agaroseinto 250 ml screw-cap flask.
    b. Add 100 of 0.5x TBE; swirl gently to disperse agarose.
    c. Loosen cap and heat it on hot plate at 300°C (about 20-25 min) until agarose is completely dissolved.
    d. Recap flask and then place in 55-60°C water bath for 15-20 min.
    e. Place the gel form on a leveling table. Position the comb holder to the gel form (the bottom edge of comb is 2-mm above the surface of the gel platform)
    f. Carefully pour agarose into gel form. Be sure there are no bubbles(the rest of agarose gel keep it in 55°C-60°C water bath for seal slots propose)
    g. Leave gel solidified at room temperature about 30 min.
    h. After solidified, keep inrefrigerator (4°C) before using for electrophoresis
34. Label 1.5-ml microcentrifuge tubes with PFGE culture number.
35. **Pre-Restriction Incubation Step**: Dilute 10x H buffer 1:10 with sterile double-distilled water into into 15 ml centrifuge tube according to the following table.

Reagent	µl/plug Slice	µl/ 12plug Slices
Sterile double-distilled water	180µl	**2160 µl**
H Buffer (Roche Applied Science)	20 µl	**240 µl**
Total Volume	200 µl	**2400 µl**

36. Carefully remove plug from TE with glass spatula, use plastic sieve for securing the plugs, and place in a sterile disposable Petri dish or on large glass slide.
37. Cut a 1.0-2.0-mm-wide slice from test samples with a single edge razor blade (or scalpel, cover slip, etc.) and transfer to labeled 1.5-ml micro-centrifuges. Replace rest of plug into the original tube that contains 0.5 ml TE buffer. Store at 4ºC.
38. Cut 1.0-2.0-mm-wide slices from plug of the ***S.* Braenderup STSAL 82** standard and transfer to labeled 1.5-ml micro-centrifuges tubes. Replace rest of plug in original tube that contains 5 ml TE buffer. Store at 4ºC.
39. Add 200 µl diluted H buffer (1x) to labeled 1.5-ml microcentrifuge tubes. Make sure that plug slice is under buffer.
40. Incubate plugs at 37 ºC for 10-15 min.
41. Prepare enzyme **for restriction incubation step**: Dilute 10x H Buffer 1:10 with sterile water and add *Xba*I restriction enzyme (50U/sample)* according to the following table. Mix in the same centrifuge tube that was used for the diluted H buffer.

Reagent	µl/plug Slice	µl/12 plug Slices
Double-distilled water	43.5 µl	**522µl**
H Buffer (Roche Applied Science)	5 µl	**60 µl**
Enzyme (*40 U/µl*)	1.5 µl	**18 µl**
Total Volume	50 µl	**600 µl**

42. After incubation, **remove buffer** from plug slice using a pipette fitted with 200-250 µl tip. ***Be careful not to damagethe plug slice*** with the pipette tip and that the plug slice is not discarded with pipette tip.



43. Add 200µl restriction enzyme mixture to each tube. Close the tube and mix by tapping gently; be sure slices are under enzyme mixture.
44. Incubate sample and control plug slice in Thermo mixer 37°C for 2hr.
45. Turn on and confirm that water bath is equilibrated to 55-60°C.

46. Put black gel frame in electrophoresis chamber. Add 3.9 L prepared 0.5x TBE (from 32.). Close cover of unit.
47. Turn on cooling module (14°C), power supply, and pump approximately 30 min before gel is to be run.
48. Preparing ES Solution (EDTA-Sarcosyl Solution) for 9 ml, 50 µl / sample.
    a. 1.46 g of 0.5 M EDTA (Na-free)
    b. 9 ml of sterile double-distilled water
    c. 3 pieces of NaOH and adjust pH to 9.0 with pH Indicator strip (pH 7.5-14)
    d. 1 ml of 10% Sarkosyl
49. Preparing loading buffer solution (100 µl / sample) (or use from last time preparation that was kept in refrigerator)
    a. 0.04 g of EDTA in 10 ml of sterile double-distilled water
    b. 4 g of Saccharose
    c. 0.003 g of Bromophenol blue
50. Remove restricted plug sliced from 37°C water bath
51. Add 25 µl of ES Solution (EDTA-Sarcosyl Solution)
52. Add 50 µl of loading buffer
53. Remove comb from gel after solidification (about 3-4 hr) .
54. Cut marker (Pulse Marker™ 50-1000 kb) as thin as possible and load the marker in wells (lanes) 1,7,15. After finish, keep the rest of marker in temperature 2-8°C
55. Remove restricted plug slices from tubes with tapped end of spatula, cut a pieces of 2-mm off and load into appropriate well. Gently push plugs to bottom and front of well with wide end of spatula. Manipulate position with spatula and be sure that are no bubbles.
    a. Load *S.* Braenderup STSAL82 in wells (lanes) 14^{th}
    b. Load samples in remaining wells 2^{nd} -6^{th} and 8^{th} -13^{th}
56. Fill in well of gel with meltedPulsed-field Cert.-agarose (equilibrated to 55-60°C) (put pipette tip as low as possible but not to damage the plugs). Allow hardening for 3-5 min. Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.
57. Set electrophoresis condition on CHEF DR-II
    a. Initial A time: 2.2s
    b. Final A time: 63.8 s
    c. Start Ratio: 1.0 (if applicable)
    d. Voltage: 200 V (6 V/cm)
    e. Run time: 19-20 h
58. Start electrophoresis

DAY 3

59. When electrophoresis run is over, turn off equipment; remove and put the gel into covered container
60. Stain gel with **ethidium bromide** (dilute 90µl of ethidium bromide stock solution (10 mg/ml) with 1000 ml of distilled water). Stain gel for 20 – 30 min in covered container on horizontal shaker (Certomat®U) with speed 40 min^{-1}.
61. Pour off all ethidium bromide into specific container.
62. Destain gel in approximately 500 ml distilled water for 20 min on horizontal shaker with speed 40 min^{-1}.
63. Capture image with imaging equipment. If background interferes with resolution, destain for an additional 20 min on horizontal shaker with speed 40 min^{-1}.
64. Follow direction given with the ***imaging equipment*** to serve gel image as an ".img" or ".1sc" file; convert this file to "**.tif**" file for analysis with the BioNumerics®software program.
65. Cleaning chamber of electrophoresis by draining buffer from electrophoresis chamber and discard into sink. Rinse chamber with 2 L distilled water and flush hoses with distilled water by letting pump run before draining water from chamber and hoses.

REMARK

* Sterilization in a hot air oven at 100°C for 6 h

9.3 Preparation of stock solutions

1 M Tris, pH 8.0

Tris base (1M of Tris : 121.14 g)	121.1	gram
Double-distilled water (ddH_2O) to	1000.0	milliliter

Note: dissolve in 800 ml of ddH_2O, adjust to desired pH with concentrated HCl and add ddH_2O to 1 liter. Autoclave to sterilize

0.2 M EDTA, pH 8.0

Na_2EDTA (1M of EDTA : 372.3g)	74.46	gram
Double-distilled water (ddH_2O) to	1000.0	milliliter

Note: Dissolve in approx. 800 ml ddH2O, adjust pH to 8.0 with NaOH, and adjust to 1 liter final volume with distilled water. Autoclave to sterilize

10x Tris-Borate EDTA buffer

Composition for 1000 ml

0.9 M Tris (Hydroxymethyl) – aminomethan	109.0	gram/liter
0.9 M Boric acid	55.6	gram/liter
0.025 EDTA with Na	9.3	gram/liter

Note: dissolve in 800 ml of ddH_2O and add ddH2O to 1 liter. Autoclave to sterilize

10% Sarkosyl

Sadium Lauroyl Sarcosinate	10.0	gram
Double-distilled water	100.0	milliliter

Proteinase K (20 mg/ml)

Proteinase K	25.0	mg
Double-distilled water	12.5	milliliter

Note: Dissolve 12.5 ml of ddH_2O into vial of Proteinase K powder, mix and transfer into small tubes and store in a freezer (-20°C)

0.5 M Thiourea

Thiourea (1M**:** 76.12)	38.06	g
Double-distilled water	100.0	milliliter

9.4 Protocol for imaging equipmentDIAS-II (Digital Imaging and Analysis System II)

SERVA Electrophoresis GmBH

1. Turn on computer.
2. Double click icon program "**Remote Capture DC**" desktop.
3. Put the gel into the imaging equipment and close the door.
4. Turn on UV Light.
5. At program "Remote Capture DC" , adjust zoom to the right position
6. Set "Maro" to ON, "AF-assist light and Flash" to OFF, "AF operation" to AF unlock
7. Under "Shooting Setting" choose Metering Mode Evaluative, "ISO Speed" to 200 and "AE Mode" to Program AE.
8. Set size and resolution of the pictures to Medium/Normal M1
9. Change "Belichtungskorrektur" to 0
10. Click "Sucher starten" 2 times
11. Click "Suchereinstellengen ändern"
12. Click "Auslösen", the image will be saved in the local computer with filename *.jpg
13. Double click icon "Zoom Browser EX" at desktop.
14. Find the gel picture that has already taken.
15. Click "Datei" > "Speichern unter…"
16. Insert file name and change format to *.tif
17. Open program "Adobe Photoshop 7.0.1
18. Click "Datei" > "Offen"., chose the file name that has to be adjusted.
19. Rotate picture by click "Bild" > Arbeitfläch drehen > Per Eingabe… > put number to rotate the picture > OK
20. Click Freistellungswerkzeug (C) to crop the picture
21. Click "Datei" > Speichern.

9.5 Comparison of PFGE: Pulse Net USA and In-house protocol

Pulse Net USA (Version May, 2007) protocol	*In-house protocol*
Day 0	**Day 0**
1. Streak an isolated colony from test cultures onto **Trypticase Soy Agar with 5% defibrinated sheep blood (TSA-SB) plates**(or comparable media) for confluent growth; stab or streak small screw cap tubes of TSA, HIA, or similar medium, using the same inoculating needle/loop. This will ensure that the same colony can be retested if necessary. Incubate cultures at 37ºC for 14-18 h.	2. Streak an isolated colony from test culture to **Standard I**plates (or comparable media) for confluent growth. Ensure that the same colony can be retested if necessary. Incubates at 37°C for 14-18 h.
Day 1	**Day 1**
1. Turn on shaker water bath or incubator (54ºC), stationary water baths (55- 60ºC) and spectrophotometer (or equivalent instrument such as the Dade Microscan Turbidity meter or bioMérieux Vitek colorimeter).	1. Turn on shaker water bath or incubator (54ºC), stationary water baths (55- 60ºC) and spectrophotometer
2. Prepare TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0)1 as follows: 10 ml of 1 M Tris, pH 8.0 **2 ml of 0.5 M EDTA**, pH 8.0 Dilute to 1000 ml with sterile Ultrapure water (Clinical Laboratory Reagent Water (CLRW)) **Note**: The TE Buffer is used to make the plug agarose and also to wash lysed PFGE plugs.	2. **Prepare TE-Buffer** (10 mM Tris :1 mM EDTA, pH 8.0) as follows: **10 ml of 1 M Tris, pH 8.0** **5 ml of 0.2 M EDTA (**or 1 ml of 1 M EDTA), pH 8.0 **Dilute to 1000 ml with sterile Ultrapure water** **Note**: The TE Buffer is used to make the plug agarose and also to wash lysed PFGE plugs.
3. Prepare **1% SeaKem Gold agarose** in TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0) for PFGE plugs as follows: a. Weigh **0.50 g (or 0.25 g) SeaKem Gold (SKG)** agarose into **250 mlscrew-cap flask**. b. Add **50.0 ml (or 25.0 ml)** TE Buffer; swirl gently to disperse agarose. c. Loosen or remove cap and cover loosely with clear film, and **microwave for 30-se**c; mix gently and repeat for **10-sec intervals** until agarose is completely dissolved. d. Recap flask and return to 55- 60ºC water bath and equilibrate the agarose in the water bath for 15 minutes or until ready to use.	3. **Prepare 2% Cert. Megabase – agarose** in TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0) for PFGE plugs as follows: a. **Weigh 90 mg (0.09 g) of megabase-agarose** into 12-40 ml screw-cap tube b. **Add 4.5 ml TE-Buffer**; swirl gently to disperse agarose. c. Loosen or remove cap and cover loosely with clear film, and heat at **100°C in Beaker of water** until agarose is ***completely dissolved***. !!!!! d. Recap flask and return to 55-60°C water bath and equilibrate the agarose in the water bath for 15 minutes or until ready to use.
4. Label small tubes (12-mm x 75-mm Falcon tubes or equivalent) with culture numbers.	4. Label small tubes with culture numbers.
5. Prepare Cell Suspension Buffer (100 mM Tris:100 mM EDTA, pH 8.0) as follows: 10 ml of 1 M Tris, pH 8.0 20 ml of 0.5 M EDTA, pH 8.0 Dilute to 100 ml with sterile Ultrapure water (CLRW)	5. **Prepare Cell Suspension Buffer** (100 mM Tris:100 mM EDTA, pH 8.0) as follow: **10 ml of 1 M Tris, pH 8.0** **50 ml of 0.2 M EDTA** (or 20 ml of 0.5 M EDTA**)**, pH 8.0 **Dilute to 100 ml with sterile Ultrapure water**
6. Transfer ≈2 ml of Cell Suspension Buffer(CSB) to small labeled tubes. Use a **sterile polyester-fiberor cotton swab** that has been moistened with sterile CSB to remove some of the growth from the agar plate; suspend cells in CSB by spinning **swab** gently so cells will be evenly dispersed and formation of aerosols is minimized.	6. Transfer ≈2 ml of Cell Suspension Buffer (CSB) to small labeled tubes. Use a **sterile loop** to remove some of the growth from agar plate; suspend cells in CSB by spinning loop gently so cells will evenly dispersed and formation of aerosols is minimized.
7. Adjust concentration of cell suspensions to one of values given below by diluting with sterile CSB or by adding additional cells. a. Spectrophotometer: **610** nm wavelength, absorbance (Optical Density) of **1.00** b. Dade Microscan Turbidity Meter: **0.40 - 0.45** (measured in Falcon 2054 tubes) **0.58 - 0.63** (measured in Falcon 2057 tubes) c. bioMérieux Vitek colorimeter: **≈17-18%** transmittance (measured in Falcon 2054 tubes)	7. Adjust concentration of cell suspensions to value given below by diluting with sterile CSB or by adding additional cells. • Spectrophotometer: **630** nm wavelength, absorbance (Optical Density) of **0.550-0.600**
CASTING PLUGS	
1. Transfer **400 µl (0.4 ml)** adjusted cell suspensions to labeled 1.5-ml microcentrifuge tubes. If cell suspensions are at room temperature, agarose can be added directly without pre-warming cell suspensions. If cell suspensions are cold, place tubes containing cell suspensions in plastic holders (floats); incubate in a 37ºC water bath for a few minutes.	1. Transfer **200 µl (0.2 ml**) **adjusted cell suspensions** to labeled 1.5-ml micro-centrifuge tube. If cell suspensions are at room temperature, agarose can be added directly without pre-warming cell suspensions, if cell suspensions are cold, place tubes containing cell suspensions in plastic holder (floats); incubate in a 37°C water bath for a few minutes.
2. Add **20 µl of Proteinase K (**20 mg/ml stock) to each tube and mix gently with pipet tip. (200 µl are needed for 10 cell suspensions.)	2. Add **10 µl of Proteinase K (20 mg/ml stock)** to each tube and mix gently with pipet tip. (120 µl are need for 12 cell suspensions.) !!!!!
3. Add **400 µl (0.4 ml)** melted **1% SeaKem Gold agarose** to the	3. Add **200 µl (0.2 ml)melted of 2% Cert. Megabase –**

0.4-ml cell suspension; mix by gently pipetting mixture up and down a few times. Maintain temperature of melted agarose by keeping flask in beaker of warm water (55-60ºC).	**Agarose**to 0.2-ml cell suspension; mix by gently pipetting mixture up and down a few times. Maintain temperature of melted agarose by keeping flask in beaker of warm water (55-60°C)
4. Immediately, dispense part of mixture into appropriate well(s) of reusable plug mold. Do not allow bubbles to form. Two plugs of each sample can be made from these amounts of cell suspension and agarose. Allow plugs to solidify at room temperature for 10-15 min. They can also be placed in the refrigerator (4ºC) for 5 minutes.	4. Immediately, dispense part of mixture into appropriate well(s) of plug mold. Do not allow bubbles to form. Up to four plugs of each sample can be made form those amounts of cell suspension and agarose. Allow plugs to solidify at room temperature for 10-15 min. They can also be placed in the refrigerator (4°C) for 5 minutes.
LYSIS OF CELLS IN AGAROSE PLUGS	
1. Label 50-ml polypropylene screw-cap or 50-ml Oak Ridge tubes with culture numbers.	1. Label 50-ml polypropylene screw-cap with culture numbers.
2. Prepare Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) as follows: 25 ml of 1 M Tris, pH 8.0 50 ml of 0.5 M EDTA, pH 8.0 50 ml of 10 % Sarcosyl (N-Lauroylsarcosine, Sodium salt) Dilute to 500 ml with sterile Ultrapure water (CLRW)	2. **Prepare Cell Lysis Buffer** (50 mM Tris:50 mM EDTA , pH 8.0 + 1% Sarcosyl) as follows: 25 ml of 1 M Tris, pH 8.0 **125 ml of 0.2 M EDTA (**or 50 ml of 0.5 M EDTA), pH 8.0 50 ml of 10% Sarcosyl (N-Lauroylsarcosine, Sodium salt) Dilute to 500 ml with sterile Ultrapure water
3. Calculate the total volume of Cell Lysis/Proteinase K Buffer needed as follows: a. 5 ml Cell Lysis Buffer(50 mM Tris:50 mM EDTA,pH 8.0 + 1% Sarcosyl) is needed per tube (e. g., 5 ml x 10 tubes = 50 ml). b. 25 µl Proteinase K stock solution (20 mg/ml) is needed per tube of the cell lysis buffer (e. g., 25 µl x 10 tubes = 250 µl). c. Measure correct volumes into appropriate size test tube or flask and mix well.	3. Calculate the total volume of Cell Lysis/Proteinase K Buffer needed as follows: a. 5 ml Cell Lysis Buffer (50 mM Tris:50 mM EDTA, pH 8.0 + 1% Sarcosyl) is needed per tube. (e.g., 5 ml × 10 tubes = 50 ml). b. 25 µl Proteinase K stock solution (20 mg/ml) is needed per tube of the cell lysis buffer.(e.g. 25 µl × 12 tubes = 300 µl). c. Measure correct volumes into appropriate size test tube or flask and mix well.
4. Add 5 ml of Proteinase K/Cell Lysis Buffer to each labeled 50 ml tube.	4. Add 5 ml of Proteinase K/Cell Lysis Buffer to each labeled 50 ml tube.
5. Trim excess agarose from top of plugs with scalpel, razor blade or similar instrument. Open reusable plug mold and transfer plugs from mold with a 6-mm wide spatula to appropriately labeled tube. If disposable plug molds are used, remove white tape from bottom of mold and push out plug(s) into appropriately labeled tube. Be sure plugs are under buffer and not on side of tube.	
6. **Remove tape from reusable mold.** Place both sections of the plug mold, spatulas, and scalpel in 70% isopropanol (Utrarachkij et al.), ethanol or other suitable disinfectant. **Soak them for 15 minutes before washing them.** Discard disposable plug molds or disinfect them in 10% bleach for 30-60 minutes if they will be washed and reused.	5. Remove white tape form bottom of mold and push out plug(s) into appropriately labeled tube. Be sure plugs are under buffer and not on side of tube.
7. Place tubes in rack and incubate in a 54ºC shaker water bath or incubator for **1.5 - 2 h** with constant and vigorous agitation (150-175 rpm). If lysing in water bath, be sure water level is above level of lysis buffer in tubes.	6. Place tubes in rack and incubate in a 54°C shaker water bath or incubator for **20 h (overnight)** with constant and vigorous agitation (150-175 rpm). If lysing in water bath, be sure water level is above level of lysis buffer in tubes.
8. Pre-heat enough sterile Ultrapure water (CLRW) to 50ºC so that plugs can be washed two times with 10-15 ml water (200-250 ml for 10 tubes).	**Day 2**
WASHING OF AGAROSE PLUGS AFTER CELL LYSIS	
1. Remove tubes from water bath or incubator, and carefully pour off lysis buffer into an appropriate discard container; plugs can be held in tubes with a screened cap or spatula.	1. Remove tubes from water bath or incubator, and carefully pour off lysis buffer into an appropriate discard container; plugs can be held in tubes with a screened cap or spatula*. * For cleaning proposes use ethanol absolute GR for analysis
2. Add at 10-15 ml sterile Ultrapure water (CLRW) that has been pre-heated to 50ºC to each tube and shake the tubes in a 50ºC water bath or incubator for 10-15 min.	2. Add at 10-15 sterile water that has been pre-heated to 50°C to each tube and shake the tubes in a 50°C water bath or incubator for 10-15 min.

	1 Sample	6 Samples	12 Samples
Cell Lysis Buffer	**5 ml**	**30 ml**	**60 ml**
Cell Suspension Buffer	2.5 ml	15 ml	30 ml
10 % Sarcosyl	0.5 ml	3 ml	6 ml
A. Bidest	2 ml	12 ml	24 ml
Proteinase K	25 µl	150 µl	300 µl

3. Pour off water from the plugs and repeat wash step with pre-heated water (Step 2) one more time.

   a. Pre-heat enough sterile TE Buffer (10 mM Tris:1 mM EDTA, pH 8.0) in a 50ºC water bath so that plugs can be washed four times with 10-15 ml TE (300-350 ml for 10 tubes) after beginning last water wash.

4. Pour off water, add 10-15 ml pre-heated (50ºC) sterile TE Buffer, and shake the tubes in 50ºC water bath or incubator for 10-15 min.

5. Pour off TE and repeat wash step with pre-heated TE three more times.

6. Decant last wash and add 5-10 ml sterile TE. Continue with step 1 in "Restriction Digestion" section or store plugs in TE Buffer at 4ºC until needed. Plugs can be transferred to smaller tubes for storage.

RESTRICTION DIGESTION OF DNA IN AGAROSE PLUGS WITH *XbaI*

1. Label 1.5-ml microcentrifuge tubes with culture numbers; label 3 (10-well gel) or 4 (15-well gel) tubes for *Salmonella* ser. Braenderup H9812 standards.

   a. **Optional Pre-Restriction Incubation Step**: Dilute 10X H buffer (Roche Applied Science or equivalent) 1:10 with sterile Ultrapure water (CLRW) according to the following table.

Reagent	µl/Plug Slice	µl/10 Plug Slices	µl/15 Plug Slices
A.Bidest	180µl	1800 µl	2700 µl
H Buffer	20 µl	200 µl	300 µl
Total Volume	200 µl	2000 µl	3000 µl

   b. Add 200 µl diluted H buffer (1X) to labeled 1.5-ml microcentrifuge tubes.
   c. Carefully remove plug from TE with spatula and place in a sterile disposable Petri dish or on large glass slide.
   d. Cut a 2.0- to 2.5-mm-wide slice from test samples with a single edge razor blade (or scalpel, cover slip, etc.) and transfer to tube containing diluted H buffer. Be sure plug slice is under buffer. Replace rest of plug into the original tube that contains 5 ml TE buffer. Store at 4ºC.
   e. Cut three or four 2.0-mm-wide slices from plug of the ***S.* ser. Braenderup H9812** standard and transfer to tubes with diluted H buffer. Be sure plug slices are under buffer. Replace rest of plug in original tube that contains 5 ml TE buffer. Store at 4ºC.
   f. Incubate sample and control plug slices in 37ºC water bath for 5-10 min or at room temperature for 10-15 min.
   g. After incubation, remove buffer from plug slice using a pipet fitted with 200-250 µl tip all the way to bottom of tube and aspirate buffer. Be careful not to damage the plug slice with pipet tip and that plug slice is not discarded with pipet tip.

3. Pour off water from the plugs and repeat wash step with pre-heated water (Step 2) one more time.

   a. Pre-heat enough sterile TE-Buffer (10 mM Tris:1 mM EDTA, pH8.0) in a 50°C water bath so that plugs can be washed four times with 10-15 ml TE (300-350 ml for 10 tubes) after beginning last water wash.

4. Pour off water, add 10-15 ml pre-heated (50°C) sterile TE Buffer, and shake the tubes in 50°C water bath or incubator for 10-15 min.

5. Pour off TE and repeat wash step with pre-heated TE three more times.

6. Descant last wash and add 5-10 ml sterile TE. Continue with step 1 in "Restriction Digestion" sectionor store plugs in TE Buffer at 4°C until needed. Plugs can be transferred to smaller tubes for storage.

Before restriction with Enzyme,
prepare 0.5X Tris-Borate EDTA Buffer (TBE) that is needed for both the gel and electropholysis running buffer (see page 5)

Prepare gel (see page 5)

1. Label 1.5-ml microcentrifuge tubs with culture number Pre-Restriction Incubation Step: Dilute 10X H Buffer 1:10 with sterile water according to the following table

   a. **Optional Pre-Restriction Incubation Step**: Dilute 10X H buffer (Roche Applied Science or equivalent) 1:10 with sterile Ultrapure water (CLRW) according to the following table.

Reagent	µl/Plug Slice	µl/12 Plug Slices
A.Bidest	180µl	**2160 µl**
H Buffer	20 µl	**240 µl**
Total Volume	200 µl	**2400 µl**

   b. Add 200 µl diluted H buffer (1X) to labeled 1.5-ml microcentrifuge tubes.
   c. Carefully remove plug from TE with spatula and place in a sterile disposable Petri dish or on large glass slide.
   d. Cut a 2.0- to 2.5-mm-wide slice from test samples with a single edge razor blade (or scalpel, cover slip, etc.) and transfer to tube containing diluted H buffer. Be sure plug slice is under buffer. Replace rest of plug into the original tube that contains 5 ml TE buffer. Store at 4ºC.
   e. Cut three or four 2.0-mm-wide slices from plug of the ***S.* ser. Braenderup** standard and transfer to tubes with diluted H buffer. Be sure plug slices are under buffer. Replace rest of plug in original tube that contains 5 ml TE buffer. Store at 4ºC.
   f. Incubate sample and control plug slices in 37ºC water bath for 5-10 min or at room temperature for 10-15 min.
   g. After incubation, remove buffer from plug slice using a pipet fitted with 200-250 µl tip all the way to bottom of tube and aspirate buffer. Be careful not to damage the plug slice with pipet tip and that plug slice is not discarded with pipet tip.

2. Dilute 10X H buffer 1:10 with sterile Ultrapure water (CLRW) and add *XbaI* restriction enzyme (50 U/sample) according to the following table. Mix in the same tube that was used for the diluted H buffer

Reagent	µl/Plug Slice	µl/10 Plug Slices	µl/15 Plug Slices
A.Bidest	175µl	1750 µl	2625 µl
H Buffer	20 µl	200 µl	300 µl
Enzyme (*10 U/µl*)	5 µl	50 µl	75 µl
Total Volume	200 µl	2000 µl	3000 µl

3. Add 200 µl restriction enzyme mixture to each tube. Close tube and mix by tapping gently; be sure plug slices are under enzyme mixture.

4. Incubate sample and control plug slices in 37°C water bath for 1.5-2 h.

5. If plug slices will be loaded into the wells (Option B), continue with Steps 1-4 of the next section (**CASTING AGAROSE GEL**) approximately 1 h before restriction digest reaction is finished so the gel can solidify for at least 30 minutes before loading the restricted PFGE plugs.

CASTING AGAROSE GEL

A. Loading Restricted Plug Slices on the Comb:

B. Loading Restricted Plug Slices into the Wells:

1. Confirm that water bath is equilibrated to 55- 60ºC.

2. Make volume of 0.5X Tris-Borate EDTA Buffer (TBE) that is needed for both the gel and electrophoresis running buffer according to one of the following tables.

5X TBE:

Reagent	Volume in milliliters (Methner et al.)					
5X TBE	200	210	220	230	240	250
Reagent Grade Water	1800	1890	1980	2070	2160	2250
Total Volume of 0.5 X TBE	2000	2100	2200	2300	2400	2500

10X TBE:

Reagent	Volume in milliliters (Methner et al.)					
5X TBE	100	105	110	115	120	125
Reagent Grade Water	1900	1995	2090	2185	2280	2375
Total Volume of 0.5 X TBE	2000	2100	2200	2300	2400	2500

3. Make **1% SeaKem Gold (SKG)agarose** in 0.5X TBE as follows:

   a. Weigh appropriate amount of **SKG** into 500 ml screw-cap flask.
   b. Add appropriate amount of 0.5X TBE; swirl gently to disperse agarose.
   c. Loosen or remove cap and cover loosely with clear film, and microwave for 60-sec; mix gently and repeat for 15-sec intervals until agarose is completely dissolved.
   d. Recap flask and return to 55- 60ºC water bath and equilibrate the agarose in the water bath for 15 minutes or until ready to use.

Mix **1.0** g agarose with 100 ml 0.5X TBE for 14-cm-wide gel form (10 or 15 wells)

4. A small volume (2-5 ml) of melted and cooled (50-60ºC) 1% SKG agarose may be wanted to seal wells after plugs are loaded. Prepare 50 ml by melting 0.5 g agarose with 50 ml 0.5X TBE in 250 ml screw-cap flask as described above. Unused SKG agarose can be kept at room temperature, melted,

2. Dilute 10X H Buffer 1:10 with sterile water and add *XbaI* restriction enzyme (50U/sample) according to the following table. Mix in the same tube that was used for the diluted H buffer

Reagent	µl/Plug Slice	µl/12 Plug Slices
A.Bidest	178.5 µl	**2142 µl**
H Buffer	20 µl	**240 µl**
Enzyme (*40 U/µl*)	1.5 µl	**18 µl**
Total Volume	200 µl	**2400 µl**

3. Add 200µl restriction enzyme mixture to each tube. Close tube and mix by tapping gently; be sure slices are under enzyme mixture.

4. Incubate sample and control plug slice in 37°C shaker for 20-24 **(Overnight)**

Day 3

-

B. Loading Restricted Plug Slices into the Wells

1. Confirm that water bath is equilibrated to 55-60°C

2. Make volume of 0.5X Tris-Borate EDTA Buffer (TBE) that is needed for both the gel and electropholysis running buffer according to one of the following table

10X TBE:

Reagent	Volume in milliliters (Methner et al.)					
10X TBE	**100**	105	110	115	120	**150**
Reagent Grade Water	**1900**	1995	2090	2185	2280	**2850**
Total Volume of 0.5 X TBE	**2000**	2100	2200	2300	2400	**3000**

3. Preparation of agarose gel: **Prepare a 1.2%** (100 ml for 14×21 cm gel) **Agarose gel** with 0.5X TBE-Buffer and load plugs and seal slots.

   a. Weigh appropriate amount of **Pulsed-field Cert.-Agarose** into 500 ml screw-cap flask.
   b. Add appropriate amount of 0.5X TBE; swirl gently to disperse agarose.
   c. Loosen or remove cap and cover loosely with clear film, and heat it until agarose is completely dissolved.
   d. Recap flask and place in 55-60°C water bath until ready to use.

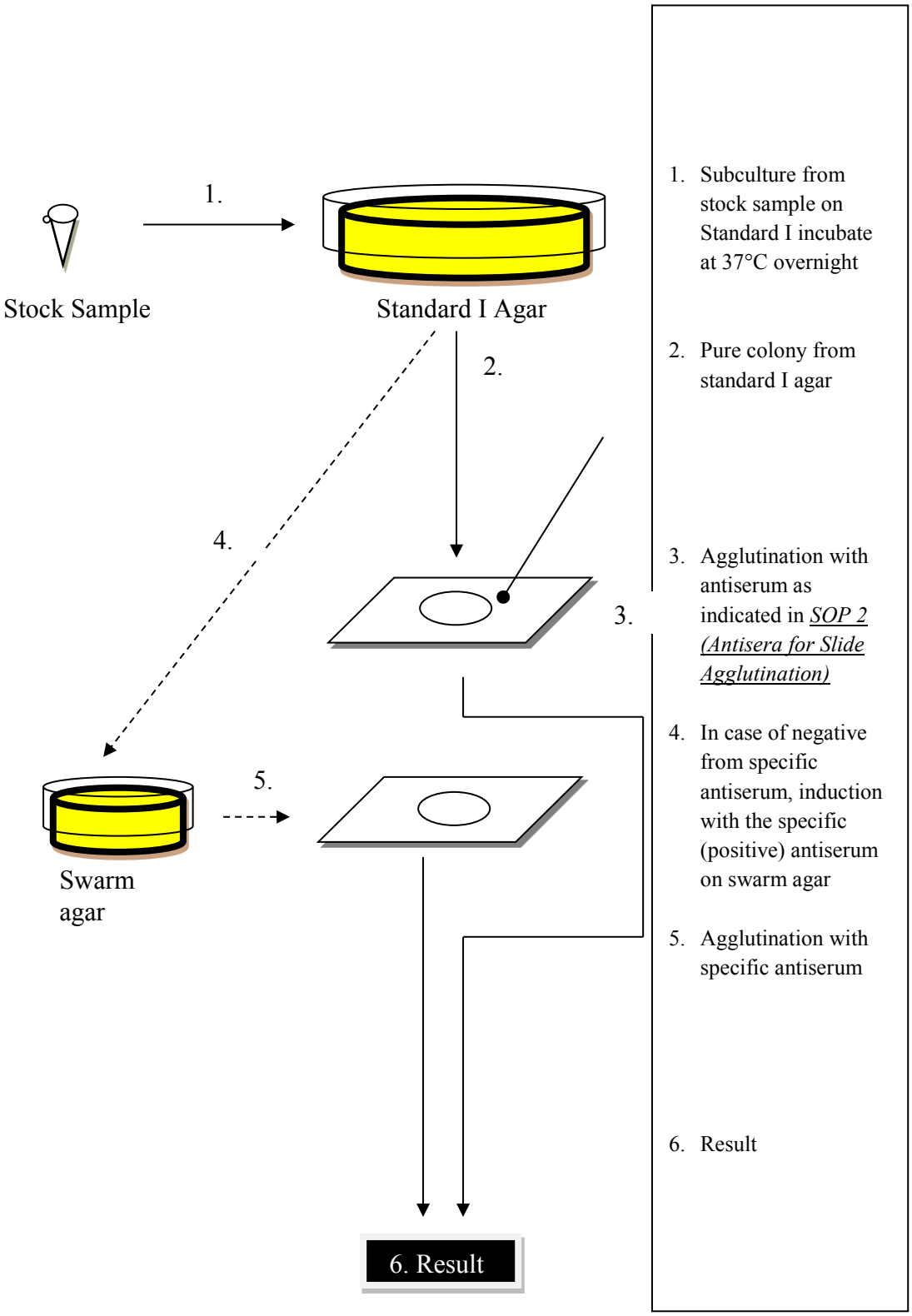
Mix **1.2** g agarose with 100 ml 0.5X TBE for 14-cm-wide gel form (10 or 15 wells)

4. Cool melted agarose in 55-60°C water bath for 15-20 min; carefully pour agarose into gel form (casting stand) fitted with comb, Be sure there are no bubbles.

and reused several times. Microwave for 15-20 sec and mix; repeat for 10-sec intervals until agarose is completely melted. Place in 55-60ºC water bath until ready to use. Alternatively, save approximately 5 ml of the melted agarose used to cast the gel in a pre-heated (55-60ºC) 50 ml flask and place in 55-60ºC water bath until used. **Note**: Place the gel form on a leveling table and adjust until perfectly leveled before pouring gel. Position the comb holder so that front part (side with small metal screws) and teeth face the bottom of gel and the bottom edge of the comb is 2 -mm above the surface of the gel platform.	
5. Cool melted SKG agarose in 55-60ºC water bath for 15-20 min; carefully pour agarose into gel form (casting stand) fitted with comb. Be sure there are no bubbles.	5. A small volume (2-5 ml) of melted and cooled (50-60°C) agarose may be wanted to seal well after plugs are loaded.
	Electrophoresis
6. Put black gel frame in electrophoresis chamber. Add 2-2.2 L freshly prepared 0.5X TBE. Close cover of unit. (The amount of buffer depends on whether residual buffer was left in tubing, or if unit was flushed with water after the last gel was run.)	6. Turn on cooling module (14°C), power supply, and pump (setting at ≈ 70 for a flow of 1 liter/minute) approximately 30 min before gel is to be run.
7. Turn on cooling module (14ºC), power supply, and pump (setting at ≈70 to achieve a flow rate of 1 liter/minute) approximately 30 min before gel is to be run.	7. Put black gel frame in electrophoresis chamber. Add 2-2.2 L freshly prepared 0.5X TBE. Chose cover of unit. The amount of buffer depends on whether residual buffer was left in tubing, or if unit was flushed with water after the last gel was run.
8. Remove restricted plug slices from 37ºC water bath. Remove enzyme/buffer mixture and add 200 µl 0.5X TBE. Incubate at room temperature for 5 minutes.	8. **Preparing ES Solution (Stop Buffer) for 9 ml, 50 µl / sample** • 1.46 g of 0.5 M EDTA (Na-free) • 9 ml of A. Bidest • 3 pieces of NaOH and adjust pH to 9.0 • 1 ml of 10% Sarcosyl
	9. **Preparing loading buffer solution (100 µl / sample)** • 40 mg of EDTA in 10 ml of A. Bidest • 4 g of Saccharose • 3 mg of Bromphenolblau
	10 **Remove restricted plug sliced from 37°C water bath** • Add 50 µl of ES Solution • Add 100 µl of loading buffer
9. Remove comb after gel solidifies for at least 30 minutes.	11. Remove comb after gel solidifies for at least 30 minutes
10. Remove restricted plug slices from tubes with tapered end of spatula and load into appropriate wells. Gently push plugs to bottom and front of wells with wide end of spatula. Manipulate position with spatula and be sure that are no bubbles. a. Load *S.* ser. Braenderup H9812 standards in wells (lanes) 1, 5, 10 (10-well gel) or in wells 1, 5, 10, 15 (15-well gel). b. Load samples in remaining wells.	12. Remove restricted plug slices from tubes with tapped end of spatula and load into appropriate well. Gently push plugs to bottom and front of well with wide end of spatula. Manipulate position with spatula and be sure that are no bubbles. a. Load marker in wells (lines) 1,7,15. b. Load *S.* ser. Braenderup standards in wells (lanes) 14 c. Load samples in remaining wells.
11. Fill in wells of gel with melted **1% SKG agarose** (equilibrated to 55- 60ºC). Allow to harden for 3-5 min. Unscrew and remove end gates from gel form; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.	13. Fill in well of gel with meltedPulsed-field Cert.-agarose (equilibrated to 55-60°C). Allow to harden for 3-5 min. Unscrew and remove end gates from gel from; remove excess agarose from sides and bottom of casting platform with a tissue. Keep gel on casting platform and carefully place gel inside black gel frame in electrophoresis chamber. Close cover of chamber.
ELECTROPHORESIS CONDITIONS	
1. Select following conditions on **CHEF DR-II**. Initial A time: 2.2s Final A time: 63.8 s Start Ratio: 1.0 (if applicable) Voltage: 200 V Run time: 19-20 h	1. Select following conditions on **CHEF DR-II**. Initial A time: 2.2s Final A time: 63.8 s Start Ratio: 1.0 (if applicable) Voltage: 200 V (6 V/cm) Run time: 19-20 h
Day 2	**Day 4**
STAINING AND DOCUMENTATION OF PFGE AGAROSE GEL	
1. When electrophoresis run is over, turn off equipment; remove and stain gel with ethidium bromide. Dilute 40 µl of ethidium bromide stock solution (10 mg/ml) with 400 ml of reagent grade water (this volume is for a staining box that is approximately 14-cm x 24-cm; a larger container may require a larger amount of staining solution). Stain gel for 20 - 30 min in covered container.	1. When electrophoresis run is over, turn off equipment; remove and stain gel with ethidium bromide. Dilute 40µl of ethidium bromide stock solution (10 mg/ml) with 400 ml of reagent grade water (this volume is for staining box that is approximately 14-cm × 24-cm; a larger container may require a larger amount of staining solution). Stain gel for 20 – 30 min in covered container.
2. Destain gel in approximately 500 ml reagent grade water for 60 - 90 min; change water every 20 minutes. Capture image a Gel Doc 1000, 2000, EQ, or XR, or equivalent documentation system. If too much background is observed destain for an additional 30-60 min.	2. Destain gel in approximately 500 ml reagent grade water for 60-90 min; change water every 20 minutes. Capture image. If background interferes with resolution, destain for an additional 30-60 min.

3. Follow directions given with the imaging equipment to save gel image as an *.img or *.1scfile; convert this file to *.tiffile for analysis with the BioNumerics®software program.	3. Follow direction given with the imaging equipment to serve gel image as an *.img or *.1sc file; convert this file to *.tif file for analysis with the BioNumerics®software program.
4. Drain buffer from electrophoresis chamber and discard. Rinse chamber with 2 L reagent grade water or, if unit is not going to be used for several days, flush lines with water by letting pump run for 5-10 min before draining water from chamber and hoses.	4. Drain buffer from electrophoresis chamber and discard. Rinse chamber with 2 L reagent grade water or; if unit is not going to be used for several days, flush lines with water by letting pump run for 5-10 min before draining water from chamber and hoses.

9.6 SOP 1: Slide Agglutination for Confirmation

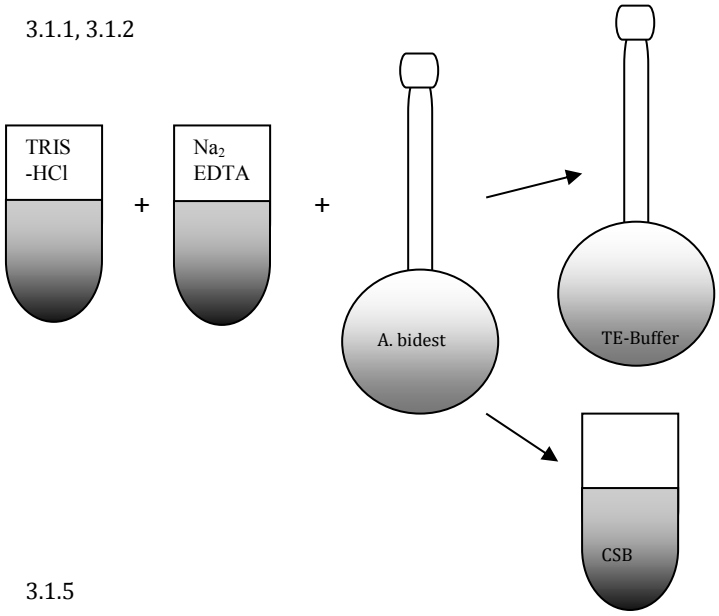


9.7 SOP 2: List of *Salmonella* serovars and specific antisera

***Salmonella* Serovar**	**Formular**	**Group**	**Specific Antisera I**	**Specific Antisera II**
Afula	6,7:f,g,t:e,n,x	C	t	x
Agama	4,12:i:1,6	B	I	6
Agona	1,4,[5],12:f,g,s:[1,2]	B	s	-
Alfort	3,10:f,g:e,n,x	E	g	x
Anatum	3,{10}{15}{15,34}:e,h:1,6	E	e	6
Bardo	8:e,h:1,2	C	e	2
Bovismorbificans	6,8,20:r,[i]:1,5	C	r	5
Bredeney	1,4,12,27:l,v:1,7	B	l	7
Brunei	8,20:y:1,5	C	y	5
Chester	1,4,[5],12:e,h:e,n,x	B	e	x
Corvallis	8,20:z_4,z_{23}:[z_6]	C	z_4,z_{23}	-
Derby	1,4,[5],12:f,g:[1,2]	B	f	-
Elisabethville	3,{10},{15}:r:1,7	E	r	7
Emek	8,20:g,m,s:-	C	s	-
Enteritidis	1,9,12:g,m:-	D	m	-
Eppendorf	1,4,12,27:d:1,5	B	d	5
Galiema	6,7,14:k:1,2	C	k	2
Give	3,{10}{15}{15,34}:l,v:1,7	E	l	7
Gloucester	1,4,12,27:i:l,w	B	i	w
Haardt	8:k:1,5	C	k	5
Hadar	6,8:z_{10}:e,n,x	C	z_{10}	x
Haifa	1,4,[5],12:z_{10}:1,2	B	z_{10}	2
Hato	1,4,[5],12:g,m,s:[1,2]	B	g	-
Hindmarsh	8,20:r:1,5	C	r	5
Hongkong	1,3,19:z:z_6	E	z	z_6
Indiana	1,4,12:z:1,7	B	z	7
Infantis	6,7,14:r:1.5	C	r	5
Krefeld	1,3,19:y:l,w	E	y	w
Lagos	1,4,[5],12:i:1,5	B	i	5
Langensalza	3,10:y:l,w	E	y	w
Lexington	3,{10}{15}{15,34}:z_{10}:1,5	E	z_{10}	5
London	3,{10}{15}:l,v:1,6	E	l	6
Mbandaka	6,7,14:z_{10}:e,n,z_{15}	C	z_{10}	z_{15}
Montevideo	{6,7,14}{54}:g,m,[p],s:[1,2,7]	C		
Nchanga	3,{10}{15}:l,v:1,2	E	l	2
Newport	6,8,20:e,h:1,2	C	e	2
Panama	1,9,12:l,v:1,5	D	v	5
Paratyphi B	1,4,[5],12:b:1,2	B	b	2
Regent	3,10:f,g,[s]:[1,6]	E	f	-
Reubeuss	8,20:g,m,t:-	C	t	-
Rideau	1,3,19:f,g:-	E	f	-
Rissen	6,7,14:f,g:-	C	f	-
Saintpaul	1,4,[5],12:e,h:1,2	B	e	2
Schwarzengrund	1,4,12,27:d:1,7	B	d	7
Senftenberg	1,3,19:g,[s],t:-	E	t	-
Stanley	1,4,[5],12,27:d:1,2	B	d	2
Tsevie	1,4,12:i:e,n,z_{15}	B	i	z_{15}
Thompson	6,7,14:k:1,5	C	k	5
Tumodi	1,4,12:i:z_6	B	i	z_6
Typhimurium	1,4,[5],12:i:1,2	B	i	2
Virchow	6,7,14:r:1,2	C	r	2
Weltevreden	3,{10}{15}:r:z_6	E	r	z_6

9.8 SOP 3: Solutions for PFGE

3.1.1, 3.1.2



3.1.5

	1 Sample	6 Samples	12 Samples
Cell Lysis Buffer	**5 ml**	**30 ml**	**60 ml**
Cell Suspension Buffer	2.5 ml	15 ml	30 ml
10 % Sarcosyl	0.5 ml	3 ml	6 ml
A. Bidest	2 ml	12 ml	24 ml
Proteinase K	25 µl	150 µl	300 µl

3.1.6

Reagent	**µl/Plug Slice**	**µl/6 Plug Slices**	**µl/12 Plug Slices**
A.bidest	180µl	1080 µl	2160 µl
H Buffer	20 µl	120 µl	240 µl
Total Volume	200 µl	1200 µl	2400 µl

3.1.7

Reagent	**µl/Plug Slice**	**µl/6 Plug Slices**	**µl/12 Plug Slices**
A.bidest	175µl	1050 µl	2142 µl
H Buffer	20 µl	120 µl	240 µl
Enzyme (40 U/µl)	1.5 µl	9 µl	18 µl
Total Volume	200 µl	1200 µl	2400 µl

3.1.1 TE-Buffer: (10 mM Tris:100 mM EDTA, pH 8.0)

- 10 ml of Tris, pH 8.0
- 5 ml of 0.2 M EDTA, pH 8.0
- 1000 ml of sterile Ultrapure water

3.1.2 Cell Suspension Buffer: (100 mM Tris:100 mM EDTA, pH 8.0)

- 10 ml of 1 M Tris, pH 8.0
- 50 ml of 0.2 M EDTA, pH 8.0
- 100 ml of sterile Ultrapure water

***Note**: Keep suspension on ice when more than 6 cultures to process at one time.*

3.1.3 Proteinase K (20 mg/ml stock)
***Note**: For best results, aliquot in 300-500 µl into small tubes and store in a freezer (-20°C) until ready to use.*

3.1.4 2% Agarose in TE Buffer

- 90 mg (0.09 g) Agarose
- 4.5 ml TE-Buffer
- Heat at 100°C

3.1.5 Cell lysis buffer: (50 mM Tris:50 mM EDTA, pH8.0 +1% Sarcosyl)

- 25 ml of 1 M Tris, pH 8.0
- 50 ml of 0.5 EDTA, pH 8.0
- 50 ml of 10% Sarcosyl (N-Lauroylsarcosine, Sodium salt)
- 500 ml of sterile Ultrapure water

3.1.6 Reagent buffer

3.1.7 Restriction enzyme in Reagent Buffer
***Note:** Keep vial of restriction enzyme on ice or insulates storage box (-20°C) at all time.*

3.1.8 ES Solution (Stop Buffer) for 9 ml 50 µl/sample

- 1.46 g of 0.5 M EDTA (Na-free)
- 9 ml of A.Bidest
- ≈ 3 pieces of NaOH ↔ pH 9.0
- 1 ml of 10 % Sarcosyl

3.1.9 Loading Buffer solution (100 µl/sample)

- 40 mg EDTA in 10 ml A. Bidest
- 4 g Saccharose
- 3 mg Bromphenolblau

3.1.10 Gel preparation (1.2 % Agarose) and Tri-Borate EDTA Buffer (3000 ml)

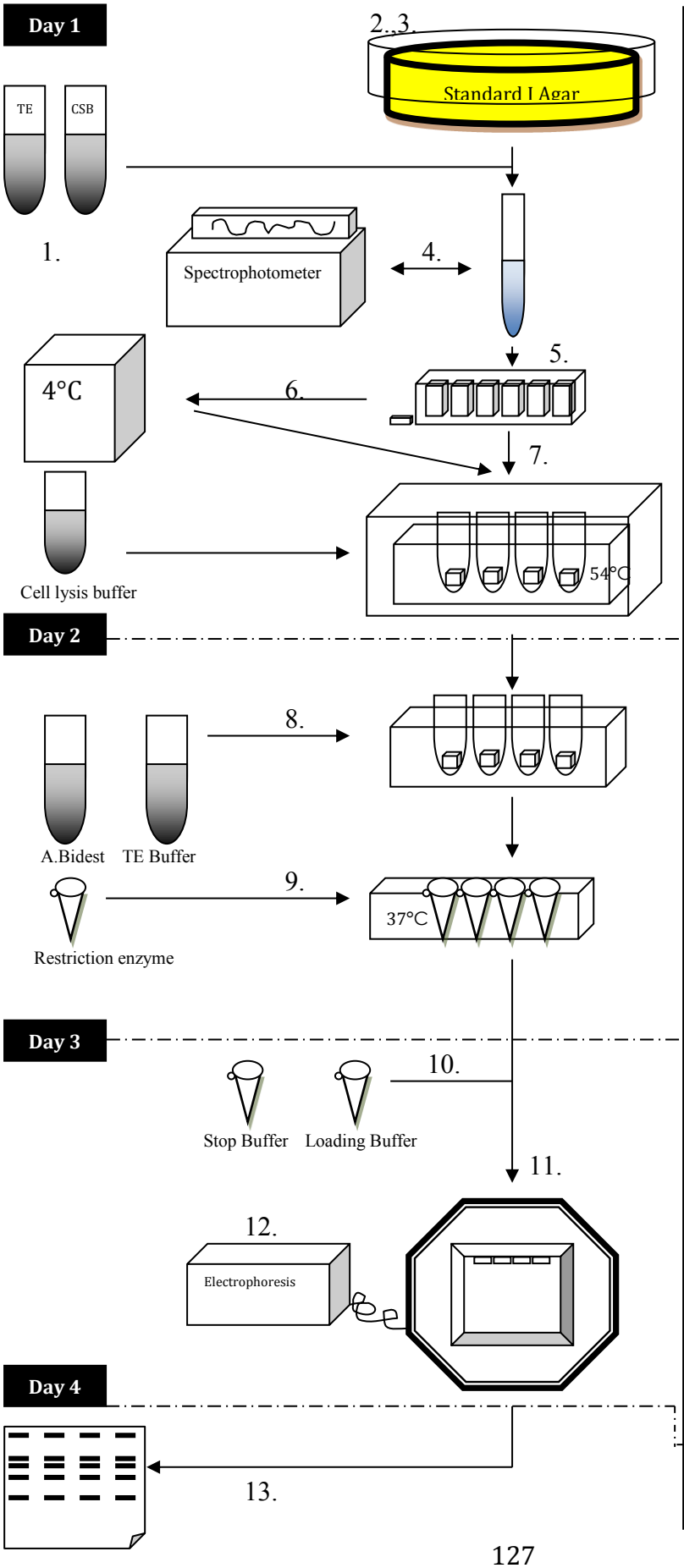
- 1.2 g Agarose
- 100 ml 0.5X TBE

***Note**: Place the gel form on leveling table and adjust until perfectly leveled before pouring gel. Position the comb holder so that front part (slide with small metal screws) and teeth face the bottom of gel and the bottom edge of the comb is 2-mm above the surface of the gel platform.*

3.1.10

Reagent	**Volume in milliliters (Methner et al.)**	
10X TBE	50	100
Reagent Grade Water	950	1900
Total Volume of 0.5 X TBE	1000	2000

9.9 PFGE technique: Flow chart



1. *TE*[3.1.1)] and *CSB*[3.1.2)] Buffer preparation
2. Fresh and pure culture on Standard I
3. Incubate at 37°C, overnight
4. Adjust concentration of cell suspension
   - Spectrophotometer: 630 nm (OD 0.550-0.600)
5. Casting plugs
   - 200 µl adjusted cell suspension
   - 10 µl *Proteinase K*[3.1.3)]
   - 200 µl of *2% Agarose*[3.1.4)]
6. Refrigerate ≈ 15 min
7. Lysis of cell in agarose plugs in shaker water bath at 54°C 20 h
   a. 5ml of *Cell lysis buffer*[3.1.5)] /sample
8. Washing of agarose plug after cell lysis with
   a. 1X 10 ml of A.Bidest
   b. 4X 10 ml of TE-Buffer
9. Restriction digestion of DNA in agarose plugs with *Xba*I or other enzymes overnight
   9.1 Pre-incubation step with *Reagent Buffer*[3.1.6)] ≈ 15 min
   9.2 Incubation step with *Restriction enzyme*[3.1.7)] at 37°C overnight
10. Adding *Stop Buffer*[3.1.8)] and *Loading Buffer solution*[3.1.9)]
11. Loading the plugs into *1.2% Agarose Gel* in *TBE Buffer*[3.1.10)] chamber
12. Start gel electrophoresis in 2-2.2 L and select following condition on **CHEF-DR II**
    a. Initial switch time: 0.5 s
    b. Final switch time: 60s
    c. Voltage 6V
    d. Run time: 20 h
    e. Temperature: 14°C
13. Gel Staining and Photograph (save in .tif file)

9.10 The laboratory record for PFGE

Origin of isolates:															
Subculturing Date:								Name:							
	M	1	2	3	4	5	6	M	7	8	9	10	11	R	M
Number of the sample															
Serotype, originally															
Confirmation															
Laboratory number, Microbiology															
Transfer of pure cultures in the molecular biology laboratory (Date / signature):															
	M	1	2	3	4	5	6	M	7	8	9	10	11	R	M

Page 1

	M	1	2	3	4	5	6	M	7	8	9	10	11	R	M
Number of the sample															
Laboratory Number, Molecular Biology															
Restriction Enzyme :	M	1	2	3	4	5	6	M	7	8	9	10	11	R	M

File Name:															
BioNumberic	M	1	2	3	4	5	6	M	7	8	9	10	11	R	M
Number of the sample															

9.11 List of media, chemical reagents and equipment

9.11.1 Media and reagents for microbiological analysis

Media and reagents	Article number	Company
Brain Heart infusion broth (BHI)	48200	Serva
NaCl Peptone solution		
• NaCl	1.06404.0500	Merck
• Peptone	1.07213.1000	Merck
Standard I Nutrient Agar	1.07881.0500	Merck
Standard II Nutrient Agar	1.07883.0500	Merck
Brilliant-green Phenol-red Lactose Sucrose Agar (BPLS)	1.07232.0500	Merck
Buffer Peptone Water	1.07228.0500/5007	Merck
Xylose Lysine Tergitol 4 agar (XLT4)		
• XLT4 Agar, Base	1.13919.0500	Merck
• XLT4 Agar Supplement (Sodium tetradecylsulfate solution 26 – 28 %)	1.08981.0100	Merck
Muller Kaufman Tetrathionate broth (MKTT)	1.05878.0500	Merck
Rappaport-Vassiliadis broth (RV)	1.07700.0500	Merck
Triple Sugar Iron Agar (TSI)	1.03915.0500	Merck
Urea agar		
• Urea agar Base acc. To CHRISTENSEN	1.08492.0500	Merck
• Urea	1.08487.0500	Merck
Motility Indole Lysine Decarboxylation (Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)(Stevens et al.)		
Voges-Proskauer broth		
• Peptone from meat	48620	Serva
• Glucose	1.04074.1000	Merck
• NaCl	1.06404.1000	Merck
Polyspecific Enteroclone Anti-Salmonella I (A-E)	TR 1111	Sifin
Polyspecific Enteroclone Anti-Salmonella I (A-E)	TR 1115	Sifin
Polyspecific Enteroclone Anti-Salmonella II (F-67)	TR 1121	Sifin
Polyspecific Enteroclone Anti-Salmonella II (F-67)	TR 1125	Sifin
Enteroclon Anti-Salmonella B	TR 1201	Sifin
Enteroclon Anti-Salmonella B	TR 5201	Sifin
Enteroclon Anti-Salmonella C	TR 1202	Sifin
Enteroclon Anti-Salmonella D	TR 1203	Sifin
Enteroclon Anti-Salmonella D	TR 5203	Sifin
Enteroclon Anti-Salmonella E	TR 1204	Sifin
Monospecific Enteroclones Anti-Salmonella and test sera Aniti-Salmonella O,Vi		
Anti-Salmonella O 2	TR 1301	Sifin
Anti-Salmonella O 4	TR 1302	Sifin
Anti-Salmonella O 4	TR 5302	Sifin
Anti-Salmonella O 5	TR 1303	Sifin
Anti-Salmonella O 5	TR 5303	Sifin
Anti-Salmonella O 6_1	TR 1304	Sifin
Anti-Salmonella O 7	TR 1305	Sifin
Anti-Salmonella O 8	TR 1306	Sifin
Anti-Salmonella O 9	TR 1307	Sifin
Anti-Salmonella O 9	TR 5307	Sifin
Anti-Salmonella O 10	TR 1308	Sifin
Anti-Salmonella O 11	TR 1323	Sifin
Anti-Salmonella O 13	TR 1325	Sifin
Anti-Salmonella O 14	TR 1309	Sifin
Anti-Salmonella O 15	TR 1310	Sifin
Anti-Salmonella O 16	TR 1328	Sifin
Anti-Salmonella O 17	TR 1329	Sifin
Anti-Salmonella O 18	TS 1330	Sifin
Anti-Salmonella O 19	TR 1311	Sifin
Anti-Salmonella O 20	TR 1312	Sifin
Anti-Salmonella O 21	TR 1331	Sifin
Anti-Salmonella O 22	TS 1332	Sifin
Anti-Salmonella O 25	TR 1335	Sifin
Anti-Salmonella O 27	TR 1313	Sifin
Anti-Salmonella O 28	TR 1336	Sifin
Anti-Salmonella O 30	TR 1339	Sifin

Media and reagents	Article number	Company
Anti-Salmonella O 34	TR 1314	Sifin
Anti-Salmonella O 35	TR 1341	Sifin
Anti-Salmonella O 38	TR 1344	Sifin
Anti-Salmonella O 39	TR 1345	Sifin
Anti-Salmonella O 40	TR 1346	Sifin
Anti-Salmonella O 41	TR 1347	Sifin
Anti-Salmonella O 42	TR 1348	Sifin
Anti-Salmonella O 43	TR 1349	Sifin
Anti-Salmonella O 44	TR 1350	Sifin
Anti-Salmonella O 45	TR 1351	Sifin
Anti-Salmonella O 46	TR 1315	Sifin
Anti-Salmonella O 47	TR 1353	Sifin
Anti-Salmonella O 48	TR 1354	Sifin
Anti-Salmonella O 50	TR 1355	Sifin
Anti-Salmonella O 51	TR 1356	Sifin
Anti-Salmonella O 52	TR 1357	Sifin
Anti-Salmonella O 53	TR 1358	Sifin
Anti-Salmonella O 54	TR 1359	Sifin
Anti-Salmonella O 55	TR 1360	Sifin
Anti-Salmonella O 56	TR 1361	Sifin
Anti-Salmonella O 57	TR 1362	Sifin
Anti-Salmonella O 58	TR 1363	Sifin
Anti-Salmonella O 59	TR 1364	Sifin
Anti-Salmonella O 60	TR 1365	Sifin
Anti-Salmonella O 61	TR 1366	Sifin
Anti-Salmonella O 62	TR 1367	Sifin
Anti-Salmonella O 63	TR 1368	Sifin
Anti-Salmonella O 65	TR 1369	Sifin
Anti-Salmonella O 66	TR 1370	Sifin
Anti-Salmonella O 67	TR 1371	Sifin
Anti-Salmonella Vi	TR 1316	Sifin
Monospecific Enteroclons and test sera Anti-Salmonella H		
Anti-Salmonella H a	TR 1401	Sifin
Anti-Salmonella H b	TR 1402	Sifin
Anti-Salmonella H c	TR 1403	Sifin
Anti-Salmonella H d	TR 1404	Sifin
Anti-Salmonella H E	TR 1405	Sifin
Anti-Salmonella H E	TR 5405	Sifin
Anti-Salmonella H f	TR 1407	Sifin
Anti-Salmonella H g	TR 1406	Sifin
Anti-Salmonella H g	TR 5406	Sifin
Anti-Salmonella H g,m	TR 1408	Sifin
Anti-Salmonella H g,m	TR 5408	Sifin
Anti-Salmonella H h	TR 1409	Sifin
Anti-Salmonella H i	TR 1410	Sifin
Anti-Salmonella H i	TR 5410	Sifin
Anti-Salmonella H k	TS 1411	Sifin
Anti-Salmonella H L	TR 1412	Sifin
Anti-Salmonella H L	TR 5412	Sifin
Anti-Salmonella H m	TS 1413	Sifin
Anti-Salmonella H n	TR 1438	Sifin
Anti-Salmonella H p	TS 1414	Sifin
Anti-Salmonella H q	TS 1415	Sifin
Anti-Salmonella H r	TR 1416	Sifin
Anti-Salmonella H s	TS 1417	Sifin
Anti-Salmonella H t	TS 1418	Sifin
Anti-Salmonella H u	TS 1419	Sifin
Anti-Salmonella H v	TS 1420	Sifin
Anti-Salmonella H w	TS 1421	Sifin
Anti-Salmonella H x	TS 1422	Sifin
Anti-Salmonella H y	TR 1423	Sifin
Anti-Salmonella H z	TR 1424	Sifin
Anti-Salmonella H $z_{4,z23}$	TS 1425	Sifin
Anti-Salmonella H z_{6}	TS 1426	Sifin
Anti-Salmonella H z_{10}	TR 1427	Sifin
Anti-Salmonella H z_{15}	TS 1428	Sifin
Anti-Salmonella H z_{23}	TR 1440	Sifin
Anti-Salmonella H z_{24}	TS 1429	Sifin
Anti-Salmonella H z_{29}	TS 1430	Sifin

Media and reagents	Article number	Company
Anti-Salmonella H z_{32}	TS 1431	Sifin
Anti-Salmonella H z_{35}	TR 1445	Sifin
Anti-Salmonella H z_{38}	TR 1447	Sifin
Anti-Salmonella H z_{41}	TR 1448	Sifin
Anti-Salmonella H 1	TR 1437	Sifin
Anti-Salmonella H 1	TR 5437	Sifin
Anti-Salmonella H 2	TR 1433	Sifin
Anti-Salmonella H 2	TR 5433	Sifin
Anti-Salmonella H 5	TS 1434	Sifin
Anti-Salmonella H 6	TS 1435	Sifin
Anti-Salmonella H 7	TS 1436	Sifin

9.11.2 Media and reagents for molecular analysis

Media and reagents	Article number	Company
ESP solution • 0.5 EDTA • 1% N-Lauroyl-Sarcosine Sarkosly • 1 mg/ml Proteinase K, pH 9	 E 2.628-2 L-9150 03 115 801 001	 Sigma-Aldrich Sigma-Aldrich Roche
Ethanol	9065.4	Carl Roth
Ethidium bromide	E-8751	Sigma-Aldrich
Megabase - Agarose	161-3108	Biorad
Pulse marker 50-1,000 kb	D-2416	Sigma-Aldrich
TBE Buffer (10X) • 0.9 M Tris (Hydroxymethyl)-aminomethan • 0.9 M Boric acid • 0.025 M EDTA with Na	 5429.3 15165 39760	 Carl Roth Serva Serva
TE Buffer • 10 mM Tris • 1 mM EDTA	 4855.2 39760	 Carl Roth Serva
Ultra pure DANN grade Agarose	9012-36-6	Biorad
Restriction Endonuclease Xba I	11 047 663 001	Roche
SuRE/Cut Buffer H for Restriction Enzymes	11 417 991 001	Roche
N- Lauroyl Sarcosine sodium salt	L9150-1000	Sigma-Aldrich
Sodium hydroxide pellets	1.06498.1000	Merck
Saccharose		
Bromphenolblau		
Agarose		
Na_2EDTA		
Thiourea (1M: 76.12)		

9.11.3 Equipment

9.11.3.1 Equipment for microbiological analysis

Equipment	Article number/ model	Company
Freezer (-30°C)	Premium	Liebherr
Balance	L2200S-D	Sartorius
Incubator for 37°C	Kelvitron®t	Heraerus
Incubator for 42°C		Melag
Laboratory blender	Stomacher 400	Seward
Refrigerator	Standard 430	Kirsch
Refrigerator	Export	Bosch
Refrigerator	Profi line FKS2600	Liebherr
Thermometer/pH meter	CG804	Schott

9.11.3.2 Equipment for molecular analysis

Equipment	Article number/ model	Company
Autopipette, 0.5 - 10 µl	4910 000.018	Eppendorf
Autopipette, 10 - 100 µl	4910 000.042	Eppendorf
Autopipette, 100 -1,000 µl	4910 000.069	Eppendorf
Balance	LP2200P	Sartorius
Balance	A200S	Sartorius
CHEF-DR®II System	170-3612	Bio-Rad
CHEF-DR Disposable Plug Mold	170-3713	Bio-Rad
15 Well Comb		Bio-Rad
Digital Imaging and Analysis System • Cabinet incl. Power cable • Canon PowerShot G9 12.1 MP digital camera incl. power adaptor • CD Gelscan 6.0 Software incl. manual	 DIAS-II GS-V60	Serva
Magnetic Stirrer	MR2002	Heidolph
Magnetic Stirrer	VMS-A	VWR
Refrigerator 4/-20°C	KGE2612	Bosch
Refrigerator 4°C	Laber-461	Bosch
Refrigerator -20°C	ARCTIS JUMBO	AEG
Spectrophotometer	Multiskan®Plus	Titertek

9.12 List of abbreviations

%	Percentage
°C	Degree Celsius
°F	Fahrenheit
µg	Microgram (10^{-6} g)
µl	Microliter (10^{-6} L)
µM	Micromolar (10^{-6} M)
95%CI	95% confidence interval
APC	Aerobic plate count
$BaCl_2$	Barium chloride
BHI	Brain Heart Infusion broth
bp	Base pair
BPLS	Brilliant-greeb Phenol-red Lactose Sucrose Agar
BPW	Buffered peptone water
$CaCl_2$	Calcium chloride
CC	Caecal contents
CFU	Colony-forming unit
CDC	Centers for disease control and prevention
CHEF	Contour-clamped homogeneous electric field
cm	Centimeter
cm^2	Square centimeter
CS	Carcass after splitting
CSB	Cell Suspension Buffer
CW	Carcass after washing
DNA	Deoxyribonucleic acid
e.g.	exemplīgrātia (for example)
EAC	Environment after cleaning and disinfection
EBC	Environment before cutting
EDC	Environment during cutting
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
et al.	et alii (and others)
EU	European Union
F	Faeces
FP	Freshly cut pork
FO	Overshoe swabs
g	Gram
h	Hour
H_2SO_4	Sulphuric acid
HCl	Hydrogen chloride
i.e.	id est(that is)
IgG	Immunoglobulin G
ISO	International Organization for Standardization
KCl	Potassium Chloride
kg	Kilogram
Kp	Kilo base
L	Liter
mg/ml	Milligram per milliliter
$MgCl_2$	Magnesium Chloride
min	Minute
MKTTn	Muller Kauffman Tetrathionate Broth with Nivobiocin
mL	Milliliter (10^{-3} L)
ML	Mesenteric lymph nodes
mm	Milimeter (10^{-3} m)
mM	Milomolar (10^{-3} M)
NA	Not avalivable
Na	Sodium
NaCl	Sodium Chloride
NaOH	Sodium Hydroxide

ND	not determined or not done
O_2	Oxygen
OD	Optical Density
OR	Odds Ratio
P	Probability
PBS	Phosphate Buffer Solution
PE	Pre-enrichment
PFGE	Pulse Field Gel Electrophoresis
pH	Negative logarithm of hydrogen ion concentration
ppm	Parts per million
rpm	Round per minute
R	Retail-ready pork
RR	Relative Risk, Risk Ratio
r_s	Spearman's rank correlation coefficient
TBE	Tris-Borate EDTA Buffer
TE	Tris EDTA Buffer
TP	Transported pork
TSI	Triple Sugar Iron
TTB	Tetrathinate broth
UPGMA	Unweighted Pair Group Method with Arithmetic mean
V	Volt
w/v	Weight by Volume
WHO	World Health Organization
WHOCC-Salm	World Health Organization collaboration Center for Reference and Research on *Salmonella*
XLT4	Xylose Lactose Tergitol^{TM} 4
κ	Kappa
χ^2	Chi-squared

10. The publication list during study

Paper publication

- Dorn-In, S., R. Fries, P. Padungtod, M.N. Kyule, M.P.O. Baumann, L. Srikitjakarn, W. Chantong, **A. Sanguangiat**, K.H. Zessin(2009). A cross-sectional study of *Salmonella* in pre-slaughter pigs in a production compartment of Northern Thailand. Preventive Veterinary Medicine 88. p. 15-23.
- **Sanguankiat, A**., H. Irsigler,K.-H. Zessin, L. Srikitjakarn, R. Fries(2010). *Salmonella* Isolates from Different Localisations in a Pork Deboning Area. 10. Fachtagung Fleisch- und Geflügelfleischhygiene für Angehörige der Veterinärverwaltung. p.49-54, ISBN 978-3-00-031226-7 Berlin, Koserstr. 20, 2-3 März 2010.
- **Sanguankiat, A**., R. Pinthong, P. Padungtod, M.P.O. Bauman, K.H. Zessin, L. Srikijakarn, R. Fries (2010). A Cross-Sectional Study of *Salmonella* in Pork Products in Chiang Mai, Thailand. Foodborne Pathogens and Disease 7, 873-878.

Oral and Poster Presentation

- **Sanguankiat, A**., H. Irsigler, K.-H. Zessin, L. Srikitjakarn, R. Fries. *Salmonella* Isolates from Different Localisations in a Pork Deboning Area. 10. Fachtagung Fleisch- und Geflügelfleischhygiene für Angehörige der Veterinärverwaltung. 2./3. März 2010, Berlin, Germany.
- **Sanguankiat, A**., S. Dorn-in, W. Chantong, R. Pingtong, P. Padungtod, L. Srikitjakarn, K.H. Zessin, R. Fries. Pig farm – to – pork: Risk factors of *Salmonell*a *enterica* subsp. *enterica* comtamination in freshly cut pork in Northern Thailand. ISVEE 13th, 20th-24th August 2012, Maastricht, Netherlands.
- **Sanguankiat, A**.: Identische *Salmonella*-Isolate entlang einer Schweinekette. 13. Fachtagung Fleisch- und Geflügelfleischhygiene: Lebensmittelketten: Techniken, Hygiene, Biosicherheit. 5./6. März 2013, Berlin, Germany.
- **Sanguankiat, A**., S. Dorn-in, W. Chantong, R. Pingtong, P. Padungtod, L. Srikitjakarn, K.H. Zessin, R. Fries. Serodiversity of *Salmonella* on farms, in an abattoir and pork in Northern Thailand. The 3rd Food Safety and Zoonoses Symposium for Asia Pacific, 3rd-6th July 2013, Chiang Mai, Thailand.

11. Acknowledgments

First of all, I would like to express a deep sense of gratitude towards to Herr. Univ.-Prof. Dr. Reinhard Fries, my doctorate thesis supervisor, for his patient guidance, enthusiastic encouragement and useful critiques of this research work and provide me with strength and opportunity during staying in Germany.

I would like to thank all the people involved in my achievement, thanks to the instructors who provide me with strength and opportunity. Special thanks to Assistant Professor Dr. Thavatchai Sakpuaram, former dean of Faculty of Veterinary Medicine, and Assistant Professor Suwicha Kasemsuwan, head of department of Veterinary Public Health, for the opportunity to undertake this course. I would like to attribute my success to my first academic chief, Associate Professor Dr. Thaweesak Songserm for his support and assistance.

Special thank for Dr. Samart Dorn-In and Dr. Wasan Chantong, who were my classmates in the master course of Veterinary Public Health, for everything that we had done together during study design, data collection and management of our work. We are the “Thai pork chain team”.

I would like to acknowledge present staffs and doctorate students of Institute of Meat Hygiene and Technology, Freie Universität Berlin, Lieselotte Bräutigam, Rosi Ludewig, Herlinde Irsigler, Dorothea Jaeger, Ilona Kern, Yvonne Schneider, Dr. Tobias Gäng, Dr. Nina Langkabel, Maria Kohnen, Dr. Tongkorn Meeyam and Iman Sharief for all technical supports and friendships, which are truly grateful for me.

Acknowledgments

I am extremely thankful to Dr. Atthaporn Roongsitthichai, who has scrutinized the final of the publication drafts especially for English style and grammar, correcting both and offering suggestions for improvement, and also Dr. Suneerat Wuttichindanon, who advise me for statistical analysis.

My lovely appreciation goes to my father, mother, sisters for their spiritual support and assistance. Thank you for my nieces, Claire and Kylie, even though you don't know how cheerful you give to me. Thanks to my colleagues, friends and students for happiness of my life. Finally, thanks to my aunts, grandfather as well as my lovely grandmother for bringing up me [the author] to be mature and strong.

Lastly, I would like to thank the Faculty of Veterinary Medicine, Kasetsart University and the Institute of Meat Hygiene and Technology, Free University of Berlin for funding my study aboard.

12. Selbständigkeitserklärung

Hiermit erkläre ich, dass die vorliegende Arbeit selbständig angefertigt habe. Ich versichere, dass ich ausschließlich die angegebenen Quellen und Hilfen in Anspruch genommen habe.

Berlin, den 27.01.2014 Arsooth Sanguankiat