5. DISCUSSION AND CONCLUSIONS

5.1. Discussion

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

5.1.1. Materials and Methods

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

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

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

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

5.1.2. Results of Isolations

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

This result is similar to investigations of Patchanee *et al.* (2002). The authors did determine an average herd-level prevalence of 69.5% for slaughter pigs from investigations of mesenteric lymph nodes of pigs slaughtered at the slaughterhouses in Chiang Mai. Patchanee *et al.* (2002) did attribute this high prevalence though particularly to effects of transport and lairage prior to slaughter. As the study pigs at farm level still had transport and lairage ahead of them, the mean *Salmonella* prevalence of 62.9% indicates that pigs throughout farms are already infected to a degree higher than expected so far. This already high farm-level infection rate probably will be further exacerbated by stress factor during transport and lairage and by handling during the slaughter process.

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

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

5.1.3. Serotypes of Isolates

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

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

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

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

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

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

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

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

5.1.4. Results of Serological Tests

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

5.1.5. Correlation between Isolation and Serological Tests Results

A total of 189 pigs were examined both blood serum and faecal samples. *Salmonella* prevalences from investigations of faecal samples and of serum in total were not different (62.4% and 60.8%, respectively). However, 45% (85/189) of pigs were found positive only in one but negative in the other test. This result explains the low correlation (kappa=0.0492, p=0.05399) between results of faecal isolation and serological testing. Such result was also found by Davies *et al.* (2003) who also established a poor correlation between bacteriological and serological test results. Other investigations, in contrast (Lo Fo Wong *et al.*, 2003, Sorensen *et al.*, 2004, Rajic *et al.*, 2005, and Funk *et al.*, 2005) established a moderate to strong correlation between *Salmonella* culture-positive and sero-positive results at herd level. Lo Fo Wong *et al.* (2003) found, that the correlation coefficient between bacteriological and serological results were 62% and 58% at cut-off values of >10 and > 40 OD% of the serological test, respectively. Sorensen *et al.* (2004) found the odds for being culture positive for *Salmonella* to increase 1.3- to 1.5-fold with each increase of 10% in herd serology. Funk *et al*. (2005) reported correlations between faecal culture and the Danish Mix-ELISA of 0.40, 0.36, 0.43 and 0.43 ($p<0.0001$) for OD% cut-offs $\geq$ 10, 20, 30 and 40, respectively. Funk *et al*. (2005) also concluded to recommend a higher OD% cut-off if more approximate estimations of the faecal prevalence are desired. It has to be kept in mind, that both test systems not necessarily principally measure the same substrate. Reducing both test systems to their major substrates, cultures of faeces at the minimum indicate that animals carry agents in the intestines, while detection of antibodies points to more systemic carriers of the organism.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5.2. Conclusion

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

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

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