5. DISCUSSIONS AND CONLUSIONS

5.1 Prevalence of *Salmonella* in the slaughter pigs

5.1.1 Fecal prevalence

Salmonella prevalences of the finisher pork herds, followed through by this investigation from production to consumption, at farm level 1 to 2 days prior to slaughter, were 62.9% and 64.4%, by investigation of fecal and serum samples of the same animals, respectively (Dorn-in, 2005):

At slaughter, *Salmonella* were isolated in 83.4% from feces of the same pigs investigated before at farm level. The overall infection rate from farm via transport and lairage until at slaughter had increased by 20.1 percent.

So far, only one previous prevalence study investigated *Salmonella* in slaughter pigs and carcasses in Chiang Mai, Thailand (Patchanee *et al.*, 2002). That study revealed a farm prevalence estimate of 69.5%, which increased to 80.5%, just prior to the slaughtering of the pigs. Prevalences at source farms and their significant increase with slaughter of that study are therefore almost in total agreement with the results of this study. It can be postulated that this increase in prevalence of *Salmonella* was due to the new infection and/or cross-contamination during transportation and during the waiting phase at the slaughterhouse lairage. Transportation of pigs from farm to slaughterhouse took up to ½ day, the waiting period after unloading at the slaughterhouse typically lasted from the morning to the afternoon.

5.1.2 Mesenteric lymph node prevalence

The prevalence of *Salmonella* in the mesenteric lymph nodes of the study pigs was 64.1%; this percentage is significantly lower than that from the fecal samples, which were concurrently collected with the lymph node samples, but it is almost identical with the infection prevalence at farm level. Obviously isolations of *Salmonella* from lymph nodes immediately at slaughter almost perfectly reflect infection rates of finisher herds at farm level.

5.1.3 Danish Mix ELISA results of meat juice

Major disadvantages of *Salmonella* isolation by culture are the relatively low sensitivity of bacteriology and the complicated and time-consuming culture processes. Serological testing is inexpensive; a large number of samples can be rapid and at relatively low cost analyses. ELISA tests using muscle fluid samples from pigs taken at slaughter can be used as a practical alternative to serum to detect antibodies to *Salmonella* polysaccharide. The SALMOTYPE® Pig LPS ELISA (Labor Diagnostik, Leipzig, Germany) was used in this study. According to the manufacturer's instructions, the assay detects antibodies to the O-antigens 1, 4, 5, 6, 7 and 12, representing more than 90% of the most common *Salmonella* serotypes isolated from pigs in Europe. This assay is designed to measure the quantity of antibodies to *Salmonella* in pork meat juice or in pig serum. However, demonstrated serum- or meat juice antibodies do reflect only previous exposure rather than current infection with *Salmonella*.

Using the lymph node culture results as the gold standard, a sensitivity of 44.8% and a specificity of 69.2% were determined for the ELISA test. Respective test properties from a comparison of results with those of the fecal cultures were 41.7% and 70%, respectively.

At the individual pigs' level, results of the ELISA test did not demonstrate a strong agreement (kappa value = 0.057) between the *Salmonella* status in the meat juice of slaughter pigs and definite diagnosis from bacteriology. Test agreement between ELISA and lymph node culture results (kappa value = 0.122) was slightly better but still low. The 95% confidence intervals for the kappa values further indicate that these estimates did carry a large degree of uncertainty.

According to Lo Fo Wong *et al*. (2003), results from bacteriological and serological tests cannot be easily compared because of the different characteristics of both methods, such as their sensitivities and specificities on the one hand and on different sampling methods, such as different sample-sizes, -frequencies and –locations on the other hand.

A major complicating factor in bacteriology for detecting *Salmonella* organisms in individual pigs is the occurrence of apparently healthy carriers, which shed the organism intermittently in the feces, and silent carriers, which do not shed, but harbor the organism in mesenteric lymph nodes or in the mucosa of the cecum and colon. The difficulty varies according to *Salmonella* genotype. Serological tests like ELISA on the other hand are restricted to the herd level. Both tests aim at different study units (individual animals or herds) and at different stages or location of infection (carrier of organisms primarily in the intestines or systemic infection).

Considering these limitations, investigations of pooled serum or meat juice samples by ELISA are suitable, fast and cheap for screening for the presence of infection with *Salmonella* on a herd basis.

5. 2 *Salmonella* prevalence of the pig carcasses

5.2.1 Prevalence in carcass swabs before chlorinated-water spray

The prevalence of *Salmonella* obtained from the surfaces of carcasses of pigs after evisceration indicates contamination or cross-contamination by fecal contents, infected tissues, and by the slaughterhouse environment (Oosterom, 1991). Slaughter pigs themselves are believed to be the main sources of contamination of carcasses, with improper slaughtering processes or unhygienic technical handling adding to such surface contamination during slaughter. Carcass swabbing is used to assess *Salmonella* carcass contamination/cross-contamination, summarized under slaughtering hygiene.

Salmonella were detected in carcass swabs of 33.1% of pig carcass' surfaces before the carcasses were washed with chlorinated water. With about 1/3rd of the carcasses being contaminated, the high level of obviously poor slaughtering hygiene is indicated. Borch *et al.* (1996) mentioned that in a slaughtering line, evisceration is the most important stage for hygienic awareness. Enclosure of the rectum and continuous disinfection of handling tools are major preventive measures, which have to be applied at this slaughter stage. *Salmonella* isolations from carcass swabs consequently point to the need to review the slaughter process and to take corrective actions. Practical standards such as Hazard Analysis Critical Control Points (HACCP) should be strictly applied.

5.2.2 Prevalence in carcass swabs after overnight chilling

Carcasses before chilling were washed and sprayed with 50-100 ppm chlorinated-water to reduce carcass surface contamination. Carcasses then were shock-frozen for 2 hours at –18 to 20°C, followed by storage and overnight cooling at 4°C. The remaining 13.3% *Salmonella* prevalence of carcasses after chilling indicates that chlorinated water spraying did reduce carcass contamination by about 20%, from

33.1% prior to spraying to 13.3 % after overnight chilling, but in combination with chilling was by far insufficient to reduce carcass contamination to truly low levels. About 1 out of 8 pigs remained to be *Salmonella*-infected and did enter the subsequent processing line.

Reasons for decreases or increases in the amounts of contaminating microorganisms on carcass surfaces are manifold. Gill and Bryant (1992) observed a reduction in the levels of gram-negative bacteria during chilling. In contrast, Bolton *et al.* (2002) found that final washing did increase bacterial counts, and chilling led to a small but statistically significant increase in total viable cell counts. Such observations may lead to the conclusion that the observed decrease of *Salmonella* prevalence, apart from the use of chlorinated water spray, may be due to further factors. Effective chilling may be a particular point to consider because it should prevent the proliferation of bacteria on warm carcass surfaces.

5.3 Pre-slaughter factors effecting *Salmonella* prevalence

Many studies corroborate on the effects of factors on the increase of *Salmonella* prevalence at slaughterhouse level. Hald *et al.* (2003) indicated that infected pigs are mostly unapparently infected; these clinically normal carrier pigs are considered to be the main source of *Salmonella* shedding. Dickson *et al.* (2003) summarize that shedding of *Salmonella* may be exacerbated by a long list of stressors, including noise, unfamiliar smells, vibration, changes in temperature, breakdown of social groupings or food deprivation. It is important to consider that stresses principally may affect the hosts' immune system. However, no conclusive report so far exists which demonstrates a direct association between stress or immune status and increased shedding or susceptibility to *Salmonella* infection in pigs (Dickson *et al.*, 2003).

Moreover, Stärk *et al.* (2002) stressed that experts from different countries failed to come to total agreement on probable sources of *Salmonella* introduction in slaughter pigs. Consensus though existed that typically between 21 and 33% of pigs coming from a chronically infected farm would be infected with *Salmonella*, but only

one-third of these infected pigs would be shedders. Differences in opinion regarding *Salmonella* dynamics could be due to either true differences in risks as a consequence of distinct management and transport practices in variable sites or to a difference in perception. Hence, further research and studies concerning the actual causes of *Salmonella* occurrence in slaughter pigs are required.

5.4 *Salmonella* serotype distribution in the slaughter pigs and carcasses

The most frequent serotype identified in this study was *S.* Rissen (45.8%), in which there were 54.3% obtained in mesenteric lymph nodes and 41.7 % in feces, similar to the 45.4% obtained from total samples and 53.7% from feces of the finisher pigs at farm level (Dorn-in, 2005). The next most prevalent serotypes found in this study were *S.* Stanley (11.7%) and *S.* Typhimurium (10.8%). Of those, 16.6% of *S.* Stanley was obtained from feces and 8.6% from mesenteric lymph nodes, while 9.3% of *S.* Typhimurium was found in mesenteric lymph nodes and 9.5% in feces. Serotypes found in those samples were closely related to 15.7% of *S.* Stanley and 9.9% of *S.* Typhimurium obtained in feces of finisher pigs at farm level by Dorn-in (2005).

Based on various proportions of serotypes found in this study, most of them gradually decreased in magnitudes on finished carcasses, but still existed with low proportions in the final carcasses. Only some serotypes, e.g. *S.* Anatum, Panama, Krefeld, Weltevreden, and *Salmonella* serogroup II (F-67), disappeared on the final carcass surfaces after they were sprayed with chlorinated-water and were chilled overnight. *Salmonella* spp. Weltevreden, Saintpoul, and Eppendorf, which were already found in the lymph nodes and faeces, had non cross-contaminated pork carcasses either before use of chlorinated water or after chilling. No emerging serotype was found on the final carcasses, reflecting absence of additional contamination on carcasses from handling in this slaughterhouse.

According to the study of *Salmonella* serogroups in Chiang Mai slaughterhouse by Pachanee *at el.* (2002), the most frequent serogroup was C. This group was also the most prevalent in this study.

Occurrences of *Salmonella* serotypes for Thailand have been summarized by Bangtrakulnonth *et al*. (2004). Their report only includes serotype distribution from human food-borne gastrointestinal infections and from different foods, but does not include pork. Nevertheless, the most five common serotypes found during the past 10 years (1993-2002) according to that report were *S.* Weltevreden, Enteritidis, Anatum, Derby, and 1, 4, 4, 12:i:-sspI.

5.5 Conclusions

The incidence of salmonellosis in man has increased in recent years and animals, particularly pigs, are incriminated as the principal reservoir. *Salmonella* monitoring in many countries is a prerequisite to enter global pork markets today. For Chiang Mai, Thailand, where pig production and pork consumption are predominant and widespread, essentially no baseline data concerning *Salmonella* occurrence through the pork production chain were available prior to this study.

This work is part of the first *Salmonella* investigation conducted along the entire pork production chain in Chiang Mai, Thailand. The particular focus of this study was on establishing *Salmonella* prevalences in slaughter pigs. Bacteriological laboratory investigation for *Salmonella* infections of individual slaughter pigs did follow international standard methods (ISO 6579). A commercial meat juice ELISA test additionally was used for serological screening of *Salmonella* infection at herd (slaughter batch) level.

The study revealed high levels of *Salmonella* in pigs during slaughter and on their carcasses with variable serotype distributions. At the end of the slaughtering process, *Salmonella* contamination still was present despite disinfection with chlorinated-water spray and chilling.

No particular and practical suggestions are made to remedy the situation in light of the actual management of the slaughter process. Considering the high infection levels, a comprehensive program seems the only viable option to reduce *Salmonella.* Such a program will have to contain multiple components of individual good hygienic practices and standard measures in the slaughtering system or, even better, involve the establishment of a HACCP system throughout the slaughter process.