5. DISCUSSION AND CONCLUSION

5.1. Discussion

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

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

Aerobic plate counts and *Enterobacteriaceae* counts on carcasses swabs

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

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

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

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

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

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

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

Salmonella isolation

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

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

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

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

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

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

***Salmonella* serotype distribution**

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

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

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

5.2. Conclusions

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

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

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