CHAPTER V

DISCUSSION AND CONCLUSIONS

5.1 Discussion of Microbiological Test Results

5.1.1 TVC and *Enterobacteriaceae* Counts

Results indicate the average total bacterial load for all samples collected and tested for this study was 4.58±1.25 log cfu/cm^2. Meanwhile, the overall value of *Enterobacteriaceae* was 2.08±1.44 log cfu/cm^2 (n=180), which is slightly lower than in a similar study conducted in slaughterhouses in Hanoi, where an overall *Enterobacteriaceae* count of 2.7 log cfu/cm^2 was found (Long et al., 2009).

Exterior surfaces of healthy and live animals are naturally contaminated with large numbers of a variety of micro-organisms from contact sources such as soil which has comparable numbers (10^7) of bacteria per gram, and feces which are about 100x more contaminated and have an Aerobic Plate Count (APC) and “coliforms” of about 10^9 and 10^8 per gram of feces, respectively. All of these can act as a source of microbial contaminants of the meat with good shelf life which would normally have 10^2 -10^4 organisms per cm^2 (DVS, 2007). The analysis of data gathered from the study revealed total bacterial load and levels of hygiene indicator microorganisms to be within acceptable levels when held in contrast with the guidelines being followed by the NMIS. The mean value obtained for TVC in pig carcasses (see Table 1) was within the range set in Philippine guidelines from 4.0 – 5.0 log cfu/cm^2 (NMIS, 2008). Similarly, the mean count for *Enterobacteriaceae* in carcasses (see Table 1) was within the acceptable range of 2.0 – 3.0 log cfu/cm^2 based on the same guidelines (NMIS, 2008).

The study compared mean values of TVC and *Enterobacteriaceae* count in environmental sites and in pig carcasses between unaccredited and accredited slaughterhouses.

In comparing between environmental sites of the two abattoirs, results show that the difference between the mean TVC of all environmental samples in the unaccredited slaughterhouse and that of the accredited slaughterhouse was statistically significant (see Table 3). This is also reflected in comparing TVC and *Enterobacteriaceae* counts of each individual environmental sample, where there was a statistically significant difference in the TVC means of scalding vats from the unaccredited and accredited establishment (see Table 4). This can, however, be attributed to the irregular slaughter schedule of the unaccredited establishment which does slaughter anytime as the need arises (see Table 11). Consequently, during the time of most visits, the scalding vat had already been pre-heated prior to swabbing, which may be the reason for a consistently low level of TVC in each of the 5 visits.

Mean values for TVC were recorded to be highest in the floor, eviscerating table and butcher's knife at 5.76±0.71, 5.64±0.92 and 4.64±1.50 log cfu/cm^2 respectively for the unaccredited slaughterhouse. On the other hand, the butcher's knife, eviscerating table and dehairing table exhibited the highest count for the accredited facility at 5.56±0.40, 5.53±0.44 and 5.46±0.38 log cfu/cm^2, respectively (Table 4). However, apart from the scalding vat, no other site showed a statistical difference between the two slaughter facilities.

The values obtained for *Enterobacteriaceae* showed the highest numbers in the butcher's knife, splitting knife and the floor of the unaccredited slaughterhouse (3.11±1.52, 2.68±0.86 and 2.47±1.43 log cfu/cm^2, respectively) and on worker's hands, splitting knife and floor (3.01±1.41, 2.32±0.90 and 2.27±1.36 log cfu/cm^2, respectively). There was no statistically significant difference in the *Enterobacteriaceae* counts of any environmental site between the two slaughterhouses (Table 4). Meanwhile, the differences between means of samples for both TVC and *Enterobacteriaceae* in carcasses were found to be not statistically significant (see Table 3).

The high values recorded for TVC and *Enterobacteriaceae* in the abovementioned sampling sites may be attributed to the practices and slaughter habits of workers in both establishments (see Table 11). The consistently high values in both establishments particularly in utensils used for slaughter (butcher's knives and splitting knives) may be due to the lack of hand-washing and knife disinfection provisions in both slaughter lines. Furthermore, the lenient implementation and observance of hygiene by butchers especially when it comes to washing of knives before, during and after use may also contribute to the problem.

5.1.2 Detection of *E. coli* and Coliforms in Water Samples

Various problems and may also spur from unhygienic practices which are tolerated by the management of slaughterhouses. This is evident in the present study in the high level of contamination of coliforms and *E. coli* in water from the unaccredited slaughterhouse (4.51 to >2300 MPN/ml and 2.71 to 2300 MPN/ml, respectively) and the occurrence of contamination, albeit at lower levels (<0.90 to 16.67 MPN/ml and <0.90 to 11.61 MPN/ml, respectively) in the accredited facility.

A similar case was observed in a study in 2006 where high contamination and unhygienic conditions were observed in tank water at slaughterhouses in Hanoi with more than 62% of the water samples being positive for *Salmonella* (Le Bas et al., 2006). The study suggested that contamination at abattoirs may be a result of slaughtering practices and responsible for the high rate of pig carcass contamination. Among water samples obtained in the present study, the largest number of both coliforms and *E. coli* were detected in samples from the unaccredited slaughterhouse (see Figure 2). The cause of such values is probably the practice of gathering water in a fixed tub in the slaughter line which is the source of water for any and all activities in the facility from washing of hands, rinsing of knives, up to the final wash of the carcass (see Table 11). The water is replenished at the end of each operation and the tub is drained following no regular schedule.

On the other hand, the least number was detected in the accredited establishment at <0.90 MPN/ml in both coliform and *E. coli* counts. However, coliforms and *E. coli* were detected on two occasions in the said facility (see Figure 3) at levels higher than that prescribed in the country standards.

It is necessary to regularly monitor water quality in slaughterhouses as it can serve as a source of bacterial, viral, and chemical contamination of slaughtered meat and meat by-products, and when the bacterial content of water exceeds the minimum standard, disinfection is required (Maranan et al., 2008). The NMIS already conducts such checking of water quality in slaughterhouses as part of requirements for the yearly accreditation process and collects water samples as part of their monitoring program which to some extent includes unaccredited slaughterhouse in cooperation with local government sanitation authorities.

5.1.3 Detection of *Salmonella*

The overall *Salmonella* prevalence for samples taken from environmental sites and pig carcass samples for this study was 15.6%. *Salmonella* was detected in 17.9% (25 out of 140) of environmental samples which were obtained prior to start of operations. This is comparable with a study in Thailand where 16% (16 out of 100) prevalence was recorded in the environment after disinfection (Sanguankiat et al., 2010). Higher levels of contamination were detected in a study in Belgium (Botteldoorn et al., 2003) in which 25% of environmental samples were contaminated with *Salmonella*, and in Spain in 42 out of 66 samples (63.6%) collected after cleaning procedures (Arguello et al., 2012).

Prevalence of *Salmonella* in carcasses was 7.5% with 3 out of a total of 40 carcass swab samples tested positive. This documented prevalence is lower than those found in other papers of similar objectives. A study by Chantong in 2005 attributed

Salmonella contamination to a high level of poor slaughtering hygiene. The same study found 33.1% of pig carcass surfaces being contaminated with *Salmonella*. before washing with chlorinated water (Chantong, 2005). In the same year, 48.9% *Salmonella* spp. prevalence in carcasses was reported in a slaughterhouse in Hanoi, Vietnam (Thai, 2007). *Salmonella* contamination was detected in 37% of the pig carcasses in commercial slaughterhouses in Belgium (Botteldoorn et al., 2003).

The prevalence in carcasses in this study is not within the value set for fresh meat as per Philippine guidelines which require total absence of *Salmonella* in a sample. This underscores the need for stricter observance of hygiene in the slaughter process or the implementation of additional measures or steps as proposed by past studies to reduce carcass contamination, whenever possible and feasible. Carcass decontamination with 2% lactic acid has been shown to reduce *Salmonella* prevalence on pig carcasses by two-fold, from 14% to 7% (Larsen et al., 2003). Chilling of pork carcasses to ≤7 °C within 24 hours is also an effective tool to suppress Salmonella growth and reduce their counts in order to prevent their entry to further steps of the meat chain (Gill and Quessy, 2000). Carcass chilling facilities are notably absent in both involved facilities.

A comparison of results from the two involved abattoirs in this study revealed a higher overall prevalence of *Salmonella* in the unaccredited slaughterhouse at a rate of 21.1% than in the accredited establishment which was 11.1%. A comparable trend was observed in comparing prevalence in these facilities specifically for environmental samples and for carcass samples. As seen in Table 6, a prevalence of 21.4% was established for environmental samples from the unaccredited abattoir while a lower prevalence of 14.3% was recorded in the accredited slaughterhouse. Similarly, for carcass samples collected after the final wash, a prevalence of 15% was determined for the unaccredited establishment while no *Salmonella* was detected in any of the 20 carcass samples collected from the accredited slaughterhouse.

Investigating occurrence of *Salmonella* in the specific environmental sites along the slaughter line, the study reveals that one of the highest occurrences of

Salmonella was on the slaughterhouse floors at 28%. This may not be deemed as much of a problem when it comes to direct carcass contamination since government regulations stipulate that no floor dressing should be allowed in any slaughter facilities, even in unaccredited abattoir or locally registered meat establishments. Moreover, the characteristics of the basic slaughter line in the Philippines feature a tiered or terraced system, which facilitates simple but efficient carcass movement off the floor (Heinz, 2008). However, an equal percentage of 28% prevalence was detected in butcher's knives and also in samples from worker's hands (20%) which are both direct contact with meat in almost the entire slaughter process.

A more detailed examination of these results for specific slaughterhouses shows a much higher percentage of *Salmonella* positive samples from the floor in the accredited slaughterhouse (60%) compared to the unaccredited facility (7%). Meanwhile, occurrence of *Salmonella* was equal in worker's hands in both facilities, both at 20% but was much higher in other meat contact surfaces in the unaccredited abattoir. A higher percentage of butcher's knives were found to be contaminated in the unaccredited (40%) compared to the accredited slaughterhouse (10%). Moreover, *Salmonella* was also detected in splitting knives and dehairing tables of the unaccredited abattoir while it was also found in eviscerating tables of the accredited abattoir.

Salmonella serovars and serotypes present

This study identified *S.* enterica subsp. enterica ser. 4,5,12:i:- as the predominant *Salmonella* serotype in 21% (6/28) of isolates from environmental and carcass samples from slaughterhouses. This is followed *S.* Rissen and *S.* Weltevreden, each in 18% (5/28) of isolates. On the other hand, *S.* enterica subsp. enterica ser. 16:lv:, *S.* Kentucky and *S.* Newport were each identified in 3 out of 28 samples (11%). The serotypes with the least percentages were *S.* Derby (7% with 2/28 samples) and *S.* Typhimurium (3% with 2/28 samples). All the above-mentioned serotypes aside from *S.* Newport were detected in samples from different environmental sites along the slaughterline. Meanwhile, only *S.* Newport was detected in carcass samples.

Several similar studies have identified the common and frequently isolated *Salmonella* serotypes in primary and secondary swine production in countries in South East Asia. In a study in 2005, the most frequent serotype identified from 351 samples from slaughter pigs and carcasses was *S.* Rissen (45.9%), *S.* Stanley (11.7%) and *S.* Typhimurium (10.8%) (Chantong, 2005). Another study in the same year identified the five most prevalent serotypes isolated mostly from pork and also the slaughterhouse environment as the following: *S.* Rissen (45.3%), *S.* Typhimurium (16.3%), *S.* Krefeld (10.6%), *S.* Stanley (6.3%) and *S.* Lagos (6.0%). In addition, that study found that the most frequent serogroup in pork and environmental samples was serogroup C and suggested that the quality of carcasses coming to cutting affected slaughterhouse environment and affect the quality of pork in terms of bacterial contamination (Sanguankiat et al., 2010). In a study on pre-slaughter pigs in a production compartment of Northern Thailand, serotypes isolated were *S.* Rissen (49%) followed by *S.* Typhimurium (19%), *S.* Stanley (12%) and *S.* Weltevreden (4%) (Dorn-in et al., 2009). *S.* Derby was most common in occurrence (49.7%) followed by *S.* Typhimurium (37.6%), both comprising 87.3% of all *Salmonella* isolates in a study which found a significant difference between *Salmonella* prevalence in backyard and intensive farms in Vietnam (Thai, 2007). Meanwhile, in a study in Laos, the most predominant serotypes in cecal samples from slaughter pigs were *S.* Derby (51%) followed by *S.* Anatum (45%), *S.* Weltevreden (15%) and *S.* Stanley (5%) (Boonmar et al., 2008).

Among serotypes identified in this study, *S.* Kentucky and *S.* Newport are not commonly isolated in countries in South East Asia. *S.* Newport is reported as a top serovar in humans by the regions of Europe, North America and Latin America. Meanwhile, *S.* Kentucky is reported as the among the most common serovars occurring in cattle and bovine meat in the United States, Canada and Europe and the top ranked serovar associated with chicken or broiler meat for the same regions in 2007 (Hendriksen, 2007).

5.2 Conclusions of the Study

The number of hygiene indicator microorganisms in slaughterhouses in this study was determined and found to be within acceptable limits according to Philippine guidelines. Furthermore, this study found that despite observable differences in terms of infrastructure, equipment and facilities, results show TVC as well as number of *Enterobacteriaceae* in both types of slaughterhouses to be not statistically different in general.

The detection of *E. coli* and coliforms in water samples in high levels in the unaccredited slaughterhouse and the occurrence of contamination in the accredited facility, despite lower levels, indicate gaps in the management of water and monitoring of water quality in both slaughterhouses and thus emphasize a need for improvement.

Salmonella spp. was detected in 7.5% of all carcass samples collected in this study. All positive carcass samples were from the unaccredited facility. This recorded prevalence is not acceptable as Philippine guidelines require total absence of *Salmonella* spp. in a sample. A higher overall prevalence of *Salmonella* was determined in the unaccredited slaughterhouse compared to the accredited facility. A similar trend was observed in comparing prevalence in these facilities specifically for environmental samples and for carcass samples.

The predominant serotypes of *Salmonella* in this study were *S.* enterica subsp. enterica ser. 4,5,12:i:- (21%) followed *S.* Rissen and *S.* Weltevreden, each in 18% of isolates from environmental and carcass samples. Other identified serovars were *S.* enterica subsp. enterica ser. 16:1v:-, *S.* Kentucky, *S.* Newport, *S.* Derby and *S.* Typhimurium