CHAPTER 5
DISCUSSION AND CONCLUSIONS

5.1 Discussion

5.1.1 Prevalence of *Salmonella* in fresh minced pork

Pork and pork products are recognized as one of the major sources for human salmonellosis. It was estimated that approximately 15% (5–25%) of the human cases of salmonellosis were associated with the consumption of contaminated pork in the Netherlands (Berends *et al.*, 1998). The reported prevalence of *Salmonella* in retail meat varies widely in different countries. As a rule of thumb, *Salmonella* is found less frequently in retail meat in the developed countries, with prevalences in retail pork in the range of 1 to about 10%. For the United States, 0.8 to 10.4% were reported (Duffy *et al*., 2001), 1.8% in Austria (Paulsen *et al*., 2006), 2.6% in Ireland (Prendergast *et al*., 2009) and 0.3% to 4.3% in the Belgian pork meat chain (Delhalle *et al*., 2009), whereas Padungtod and Kaneene (2006) reported a prevalence of 29% in Thailand, 10.3% in India (Sharma *et al.*, 1987).

Still, these data must be interpreted with caution, as there are considerable differences between studies within one country and even within a region/district. For example, a Belgian survey from 2000 to 2003 indicated that the mean prevalence values of *Salmonella* in 25 g samples of pork cuts and minced meat were 17.3% (95% CI: 15.0–19.7%) and 11.1% (95% CI: 9.4–13.0%) (Ghafir *et al.*, 2005), respectively, whereas Delhalle *et al*., (2009) reported a much lower prevalence in minced pork at the retail level ranging from 0.3% to 4.3%. Similarly, Zhao *et al*. (2001) report a prevalence of 1 to 3.3% in pork chops, whereas White *et al*. (2001) detected *Salmonella* sp. in 16 % of ground pork of 3 retail supermarkets from the same region (Washington D.C. area).

Prevalence of *Salmonella* in the fresh pork samples in Vietnam was found to range from 14.1%, 41.7%, 64.0% to 69.9% (To, 1999; Le, 2003; Van *et al.*, 2007;

Phan *et al*., 2005). In this study *Salmonella* was detected in 36.6% of fresh minced pork samples from the retail markets in Hanoi, Vietnam.

In a survey of pork production chains in Hue, Vietnam, *Salmonella* spp. was detected in 32.8% of the retail pork (Takeshi *et al*, 2009).

There are many opportunities for cross-contamination with *Salmonella* spp. in butcher shops. The purchased primal cuts and carcasses are handled intensively during the processing into retail ready items. Primal cuts and finished items are often processed by the same persons with the same utensils, the same machinery and on the same cutting without any interim cleaning and disinfection (Berends *et al.*, 1998). The carcasses were shown to be the main source of contamination. If a slaughterhouse produces contaminated carcasses, all post-harvest stages will be contaminated (Delhalle *et al.*, 2009).

In order to reduce *Salmonella* contamination at post-harvest special attention must be paid to controlling carcass contamination at the slaughterhouse (Berends *et al.*, 1998; Swanenburg *et al*., 2001). After the slaughterhouse the most important parameters with regard to contamination, are handling, general hygiene and time and temperature at each stage (Lo Fo Wong *et al*., 2002). These parameters are important to avoid cross-contamination but also to maintain the level of contamination at levels as low as possible.

Processed meat products often contain a range of microorganisms that can cause infection or intoxication of humans. The skills of the slaughterhouse workers are an important means of preventing the contamination of muscles with gut contents and fecal matter. The input level of microorganisms to products can be minimized by good layout, hygienic operational practices and rapid chilling; it will be increased by poor hygiene and badly controlled storage conditions. If carcasses are cooled too slowly, stand for long periods, and microbiological contamination of deep muscle or bone has occurred, the risk of the growth of mesophilic bacteria, especially *Salmonella*, *Clostridia* and *bacilli*, leading to spoilage, increases (Brown, 2000).

5.1.2 Seasonal variation

In the northern part of Vietnam there are four seasons; winter, spring, autunm, summer; however the sampling periods were winter and spring. In the cold months the average temperature was at 9 – 14^{O}C. The temperature was cold from December to February. In the warm spring months the average temperature in 2008 ranged from 20 – 28^{O}C with high humidity, south wind, a drizzling rain from March to middle of April. The average temperature is of different levels from 10 to 14 ^{O}C between winter and spring time. In this study the contamination of *Salmonella* spp. in fresh minced pork was a statistically significant difference between warm months and cold months.

In general, the extent of contamination can also be related to the climate and the temperature of the storage of fresh minced pork. As shown the overall occurrence of *Salmonella* is influenced by the season. The higher contamination level observed in the warm months may partly be explained by an increased *Salmonella* excretion of infected pigs and partly by an increased proliferation of bacteria in the environment (Hald *et al*., 2003). The seasonal variation in the prevalence of *Salmonella* spp. results in positive meat and meat products in butcher shops with the highest prevalence in summer when the *Salmonella* spp. will be able to proliferate on the machinery and chopping blocks to such an extent that more meat becomes cross-contaminated. Increased ambient temperature could lead to a potential multiplication of the pathogen in the environment which in turn eventually contributes to an increased infection of pigs as well as a contamination of carcasses (Lo Fo Wong *et al*., 2002).

The Centers for Disease Control and the Food borne Diseases Active Surveillance Network (FoodNet) data indicate that outbreaks and clusters of food-borne infections peak during the warmest months of the year (CDC, 2001). The reasons for this seasonal pattern are not fully known. But they may include increased prevalence of the pathogens in livestock or vehicles of transmission during the summer, greater human exposure to contaminated foods during improper handling (e.g. temperature abuse) or incomplete cooking of products during warm months.

Although the retail levels of pork production depend on the quality of the raw materials and the products that are received, they too bear a responsibility for the quality of the end product and for the prevention of contaminated products reaching the consumer. The three main factors which influence the microbiological quality of the meat are handling, time and temperature (Lo Fo Wong *et al*., 2002). Proper and sensible handling of raw materials is vital to avoid cross-contamination between the products. *Salmonella* and *E. coli* start growing at a temperature of around 7^{O}C (ICMSF, 1996). Time and temperature abuse create situtations that support the survival and propagation of micro-organism that are present in the food. Moreover, retail display is possibly the weakest link in the commercial cold chain (James *et al.,* 1990) adding to the concern that *Salmonella* may proliferate to hazardous numbers during periods of temperature abuse in display case (Lo Fo Vong, *et al.,* 2002).

At the retail markets of Hanoi the cutting of meat or the preparation of meat for a consumer takes place in this environment. The scales are used without any paper or the like between the meat. This means that liquid from one piece of meat is transferred to another piece one after the other. Storing pork in an environment of up to 28ºC or more without any protection can pose a greater human health hazard from *Salmonella* spp. and *E. coli*. Wooden, rather than easily disinfected polythene plastic, meat cutting blocks are still used, although some traders have used ceramic, stainless steel plates on table surfaces.

5.1.3 Microbiological contamination in terms of APC, *E. coli counts*

Indicator organisms are larger groups of bacteria, including certain pathogenic bacteria, which are relatively easy to measure as a group and whose presence is likely to indicate the presence of pathogenic bacteria ("index bacteria" in the sense of Mossel, 1982). The APC is a general measure of the microbiological status of meat (Loncaric *et al.*, 2009) but the APC results and the number of the present pathogens may not always be related. Testing for *E. coli* count, a group of indicator organisms that live in the intestines of animals and the environment will give a better indication of the likelihood of the pathogenic organisms being present (Whyte *et al.,* 2002). The further processing of meat into minced meat and meat products provides an opportunity for any dangerous bacteria on the surface of the carcass meat to be spread throughout the product and also for new bacteria to be introduced from the environment, handling and processing.

With respect to the APC, 73.5% of the samples had counts above the national limit of 6.0 log CFU/g. However, to interpret this result correctly, the mode of production has to be considered. A German study from 2001 (Hildebrand *et al.,* 2001) demonstrated, that in small - scale production (butchers, supermarket outlets) the APC of minced meat was significantly higher compared to that produced in large meat plants, with mean APC counts of 6.09 and 6.64 log CFU/g vs. 4.73 CFU/g. Similar results for industrially produced minced meat were reported by Schalch *et al*. (1996). Interestingly, the local production of minced pork in markets in Hanoi, which is suffering from several hygiene deficiences, yielded quite similar results (mean APC of 6.84 log CFU/g). Highest APC concentrations >8 log CFU/g were found in 17% of the samples, which is a high percentage. Still, a few decades ago, similar results (about 7% of minced meat showed APC >8 log CFU/g) were not uncommon in Germany (Schellhaas, 1982).

A group of bacteria lives in the intestines and is shed in the feces of man and of food producing animals. The presence of *E. coli* is an indicator of fecal contamination. The test procedure does not specifically recover *E. coli* 0157: H7 but does indicate the risk of contamination and other dangerous fecally derived bacteria.

In other developed countries such as Switzerland the proportion of *E. coli* contamination was generally much lower because *Enterobacteriaceae* were detected in only 20.2% of 650 pig carcasses collected by swab sampling from 5 abattoirs (Zweifel *et al*., 2005). In a prospective study with 648 food items from 10 retail markets in the Minneapolis-St.Paul area during 2001 – 2003 the prevalence of *E. coli* contamination in pork was 69% (Johnson *et al*, 2005).

A Belgian pork meat chain survey indicated that the *E. coli* counts in meat cuts and minced meat ranged from 0.21 ± 0.50 to 1.23 ± 0.89 log CFU/g and 1.33 ± 0.58 to 2.78 ± 0.43 log CFU/g, respectively, at the meat-mincing plant. The *E. coli* counts contamination ranged from 0.75 - 0.83 log CFU/g to 1.12 - 0.36 log CFU/g at the retail level (Delhalle, *et al*., 2009). In a German survey on minced meat, *E. coli* was detected in 21%, 30% and 36% in minced meat produced in large meat plants, butchers´ shops and supermarket outlets, respectively (Hildebrand *et al.,* 2001). *E. coli* counts did not exceed 3 log CFU/g, with median values <2 log CFU/g. Again, the small-production structures were associated with a higher frequency of *E. coli.*

Many factors can affect the contamination with *E. coli* in fresh minced pork during slaughter, storage, distribution, processing and handling meat for sale. In this connection slaughtering is a potential stage for bacterial contamination of meat (Warrier *et al*., 2002; Pearce *et al*., 2006; Namvar and Warriner, 2006). Wegener (1999), a consultant on the hygiene inspection system of the veterinary services in Vietnam, indicated that at slaughter points or slaughterhouses almost every part of the carcass comes into contact with the dirty floor or other contaminated structures. Intestinal tracts and bones were all lying on the floor. Therefore, most fresh meat was contaminated with micro-organisms, especially, intestinal bacteria. Besides, the practice in meat selling also contributes to the contamination with micro-organisms (Hanashiro *et al*., 2005). The results of the questionnaire survey of selected factors related to the routine practices of fresh minced pork handling and the microbiological contamination in retail fresh minced meat revealed that fresh meat was preserved and transported under poor sanitary conditions. As mentioned above *E. coli* can be transferred to the meat surface from many sources and proliferate there. As a result of that, the median of *E. coli* counts in fresh minced pork samples was 3.39 log CFU/g. Fifty two (53.1%) of the 98 examined samples of fresh minced pork in Hanoi retail markets had *E. coli* contamination. In an earlier study the proportion of *E. coli* positives in Hanoi retail fresh pork was 82.4% (Chu, 2007).

National standards in Food Quality, Hygiene and Safety No 46/2007/Decision of the Ministry of Health Vietnam issued on 19th December 2007 and the European regulation (EC) No 2073/2005 (Anonymous, 2005) set down microbiological criteria for foodstuffs. The surveillance of *Salmonella*, *Enterobacteriaceae* and APC on pig carcasses is used as process hygiene criteria. *Salmonella* in minced meat and prepared meat is a food safety criterion. The product is considered as unsafe if the presence of the bacterium is detected in 10 or 25 g of any of the 5 examined sample units. *E. coli* counts are used as hygiene criteria in meat-mincing plants.

The median of *E. coli* counts in fresh minced pork from the Hanoi retail markets was found to be at a level considered to be unacceptable according to the requirements of the National standard regulation No 46/2007/Decision of the Ministry of Health.

Carcass delivery by the slaughterhouse is the main source of *E. coli* and some stages during the slaughtering process need to be well controlled in order to avoid contamination of fecal origin. Good hygiene and proper handling practices for raw materials at post processing are imperative to avoid cross contamination between the products. *E. coli* counts are an alternative way to obtain a quantitative measure of the contamination of the meat by the fecal route. They can provide information regarding the efficiency of slaughterhouse procedures in order to ensure the avoidance of fecal contamination which in turn limits pathogen contamination of the meat (Ghafir *et al*., 2007).

Developing countries tend to have weaknesses in their governmental public health systems that fail to ensure adequate consumer protection and also weaken their trading abilities for exporting food (FAO, 2002). Such weaknesses include:

- ♦ outdated food laws, standards and regulations and sometimes overregulation;
- ♦ no centralized approach, or even coordination among departments and agencies, to food control with jurisdictional confusion and overlap;
- ♦ lack of adequately trained personnel to carry out compliance activities, including food inspection;
- ♦ where food control laboratories exist, they have limited capacity in terms of physical structure, equipment, supplies and technical personnel;
- ♦ while food industries (preharvest, processing, retail, foodservice) are familiar with terms like good hygienic practices, good manufacturing practices and the hazard analysis critical control point (HACCP) systems, they do not have the technical ability or will to consistently follow through with these.

5.2 Conclusions

1) The overall prevalence of *Salmonella* spp. in fresh minced pork was 36.6% (95% CI: 36.68 – 42.94).
2) The prevalence of *Salmonella* in winter time and spring time (OR 6.2) (95% CI: 3.55-11.07) was 21.4% (95% CI: 15.29 -28.58) and 63.0% (95% CI: 52.34

-72.88), respectively, and the difference of contamination proportion of *Salmonella* spp. in fresh minced pork was statistically significant (p<0.001).

3) The mean of APC count and the median of *E. coli* count in fresh minced pork samples was 6.84 log CFU/g ± 1.14 and 3.39 log CFU/g, respectively.
4) The level of *E. coli* and APC contamination were statistically significantly (p<0.001) lower during the cold winter months than the warm spring months (2.19 log CFU/g; 6.38 log CFU/g versus 4.99 log CFU/g; 7.74 log CFU/g), respectively. Fifty two (53.1%) of the 98 examined samples of fresh minced pork had *E coli* contamination.
5) Among the risk factors examined, "season", "origin of fresh pork supply" and "slaughter place" were significantly (p<0.05) associated with contamination of *Salmonella* spp., APC and *E. coli* count in fresh minced pork. These results suggest that higher ambient or product temperatures were associated not only with a higher prevalence of *Salmonella* but also with APC and *E. coli* counts.

Although food hygienists stress the necessity of proper cooling of fresh meat this is not always practiced in Vietnam. Fresh meat is difficult to handle as there are many factors both before and after slaughter that can affect its quality and safety. To improve the safety of fresh meat along the entire production chain, training and education of butchers, food handlers and consumers will be emphasized and food safety and hygiene at retail markets in cities and provinces will be improved according to the Government Orientation (8^{th} June 2009, Food Safety and Hygiene Department – Ministry of Health Vietnam).

In summary, this study reflects cross-contamination from multiple sources at the slaughterhouse, poor hygiene during fresh pork meat mincing and handling at the butcher shops in retail markets. This will effect microbial contamination with both pathogenic bacteria and microorganisms indicating deficiencies in the processing chain. The need for low temperatures along the minced meat production chain is emphasized.