CHAPTER 4

RESULTS

4.1 Detection and identification of *Salmonella* spp. in fresh minced pork

4.1.1 Overall prevalence of *Salmonella* in fresh minced pork

A total of 251 fresh minced pork samples from retail markets in Hanoi were collected for the *Salmonella* isolation. In 92 of these samples, *Salmonella* spp. was detected, giving an overall sample prevalence of 36.6% (95% CI: 30.68%- 42.94%), see Figure 6.

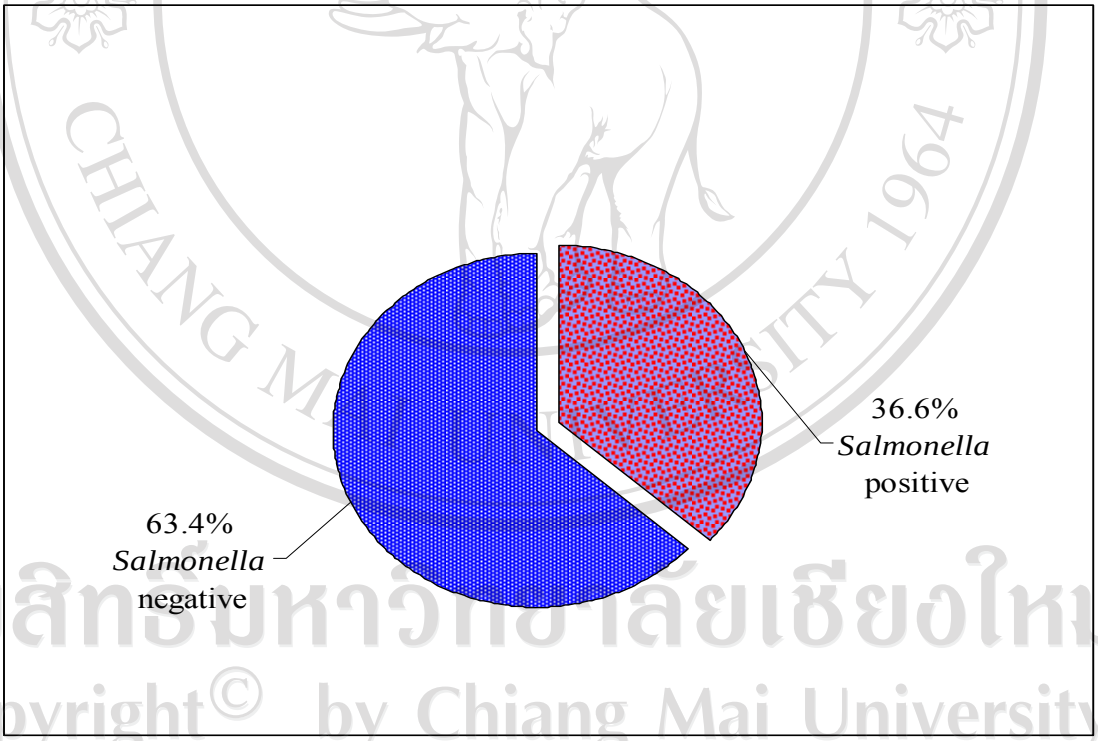

Figure 6: Prevalence of *Salmonella* spp. in fresh minced pork from the retail markets in Hanoi

4.1.2 Overall microbiological contamination analysis of fresh minced pork purchased in the Hanoi retail markets

4.1.2.1 Bacteriological profile

The overall bacteriological profile of fresh minced pork purchased describes the level of the microbiological contamination in terms of log CFU/g. Maximum APC and *E. coli* counts were 9.05 and 6.33, respectively. The minimum concentrations were 4.19 for APC and 1.16 for *E. coli*.

The mean of APC 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 (Table 13).

Table 13: Bacteriological profile of fresh minced pork in the retail markets in Hanoi (data in log CFU/g)

Parameter	Number of samples examined	Mean	SD	Median	Min	Max
APC	98	**6.84**	1.14	7.05	4.19	9.05
E. coli	98	3.19	1.71	**3.39**	1.16	6.33

APC= Total Aerobic Plate Count

E. coli = Escherichia coli

4.1.2.2. Evaluation of fresh minced pork

The national standards on 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. In EU law, the surveillance of *Salmonella*, *Enterobacteriaceae* and APC on pig carcasses is used as process hygiene criteria. For minced meat the batch is qualified as acceptable if *E. coli* counts are below 1.70 log CFU/g. It is defined as being marginal if in not more than 2 out of 5 samples *E. coli* are between 1.70 (“m”) and 2.70 (“M”) log CFU/g; as unacceptable if in more than

two samples *E. coli* are in the range of 1.70 to 2.70 log CFU/g or if in one or more samples *E. coli* counts exceed "M" (i.e. 2.70 log CFU/g); Similarly, EU regulation defines APC limits, where "m" = 5.70 and "M" = 6.70 log CFU/g.

In our study, we used a national limit of 6.0 log CFU/g for APC and for *E. coli* the national limit of 2.7 log CFU/g.

The microbiological contamination of fresh minced pork represented by the bacteriological parameters resulted in a rather high rate. In fifty two (53.1%) of the 98 examined samples of fresh minced pork *E. coli* were detected at levels ≥1 log CFU/g. In 52 samples the limit of $5x10^2$ *E. coli*/g was exceeded (Table 14). There were twenty six samples met $\leq 10^6$ CFU/g for the APC. Out of the 98 samples 46 samples met $\leq 5x10^2$ *E. coli* counts.

Table 14: Evaluation of APC and *E coli* counts in fresh minced pork sold in the retail markets in Hanoi

Parameter	Number of samples examined	Number of samples met standards	Percentage	95% CI	
				Lower limit	Upper limit
APC	98	26	26.5	17.6	35.4
E.coli	98	46	46.9	36.9	56.9

The distribution of the results is displayed in Table 15 and Figure 7 for APC and in Table 16 and Fig. 8 for *E. coli*.

Table 15: Total Aerobic Count in 98 fresh minced pork samples collected from retail markets (data in log CFU/g).

Log CFU/g	Number of samples in range	Cumulative frequency (%)
4.0 – 5.5	10	10
>5.5 -6.0	16	27
>6.0 – 6.5	14	41
>6.5 – 7.0	7	48

Log CFU/g	Number of samples in range	Cumulative frequency (%)
>7.0 – 7.5	27	76
>7.5 – 8.0	7	83
> 8.0 – 8.5	14	97
>8.5 – 9.0	3	100
Total number of samples	98	

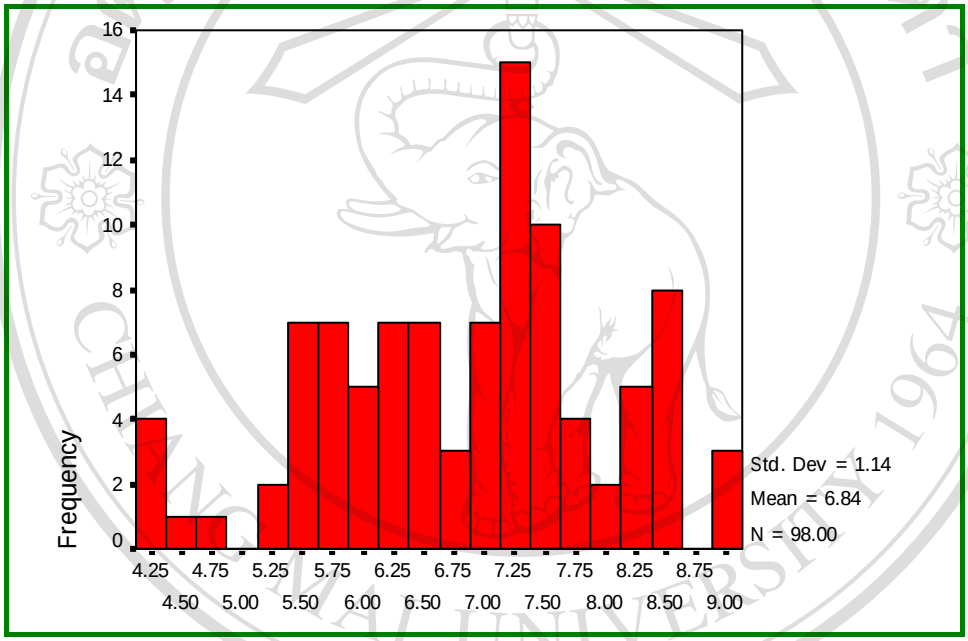

Figure 7: Frequency of the distribution of the Total Aerobic Count in fresh minced pork from retail markets in Hanoi

Table 16: *E. coli* counts in 98 fresh minced pork samples collected from retail markets in Hanoi (data in log CFU/g).

Log CFU/g	Number of samples in range	Cumulative frequency (%)
0.0 – 0.0	5	5
>1.16 -1.38	13	18
>1.5 - 2.0	11	30
>2.0 – 2.5	14	44

Log CFU/g	Number of samples in range	Cumulative frequency (%)
>2.5 – 3.0	5	49
>3.0 – 3.5	2	51
>3.5 – 4.0	11	62
> 4.0 – 4.5	8	70
>4.5 – 5.0	10	81
> 5.0 – 5.5	11	92
>5.5 – 6.0	6	98
>6.0 – 6.5	2	100
Total number of samples	98	

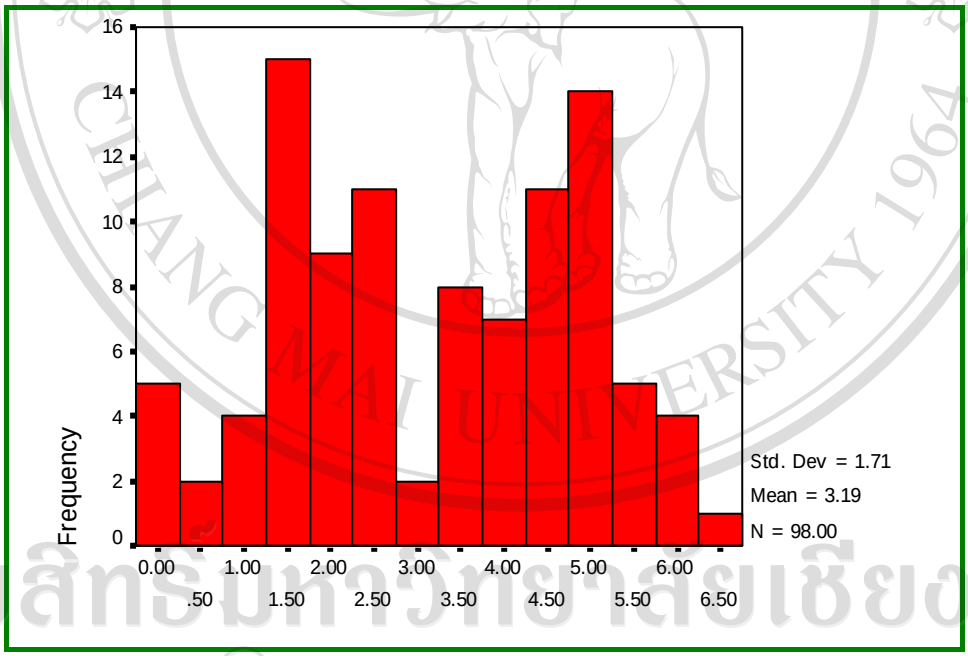

Figure 8: Frequency of the distribution of *E. coli* counts in fresh minced pork from the retail markets in Hanoi

4.2 Results of the questionnaire surveys

The questionnaire survey was conducted at 251 different butcher shops in retail markets to assess the relations between the routine practices of fresh minced pork handling and the microbiological contamination.

The results of the questionnaire survey are shown in Table 17. These results refer to the distribution of selected factors related to meat selling practices, as shown in Appendix 2.

The results of evaluation of the education attainment of the retailers (the butcher shop owners) indicated that none of them had taken part in a training course on food safety and food hygiene practice in the meat business. The retailers often listened to broadcastings about food safety and hygiene which the Food Safety and Hygiene Department (a branch of the Ministry of Health) had launched an "***Action Month***" for quality of food safety and hygiene.

None of the butcher shops were equipped with cooling facilities for storage of fresh minced pork. Hundred percent (100%) of the fresh minced pork was stored in natural condition. It was also observed that fresh pork was openly displayed on tables and exposed to the sun. With regard to the covering of meat during the transportation: 74.5% was not covered, 25.5% of pork meat was covered. The materials used for covering meat were often fine cloth or sack cloth. According to the respondents such cloth is frequently washed with water and soap powder and dried in the sun before being reused for the next time.

In relation to the origin of the fresh pork supply, the meat of 143 out of 251 butcher shops or 56.9% of fresh pork were self-traded by the butchers. The remaining 43.1% of the butcher shops obtained fresh pork by reliable suppliers to establish a direct contract with the butcher shop owners regarding a constant supply.

Hundred percent (100%) of the butcher shops sold only fresh minced pork without mixing internal white organs. The daily average amount of sold meat was in the range of 50 kg/day up to 150 – 200 kg/day (approximately 2 – 2.5 pig carcasses).

The table surfaces in butcher shops were made of wood, ceramic and stainless steel in 54.2%, 24.3% and 21.5% of the shops, respectively.

The type of vehicles for transportation of the pork was mainly motorbike 96.8%. Merely 8 out of 251, or 3.2%, used trucks.

None of the butcher shops owners used protective gloves during the handling of the meat.

Inspection: There is no real in-depth inspection of the quality of the meat. The Hanoi Sub- Department of Animal Health inspectors sometimes take samples for further examination in laboratories. These samples are brought to the laboratory in chilled boxes. Most of the retail markets need stronger meat safety and hygiene enforcement in order to prevent food-borne diseases. However, rules on food hygiene and safety for all kinds of food businesses have already been issued by the Ministry of Health. Thus, as they do not comply with the rules recent food poisoning incidents particularly in small families, schools, factories, wedding parties and the like had been the result.

Water source: All shops reported to use tap water from the public (i.e. potable water).

The cleaning of tables, equipments and utensils was done by using detergents.

Remain: In most shops all meat was sold within one day or processed into other food. The remaining meat was sold cheaper in the afternoon (12 am – 1 pm).

Slaughter place: 86 out of 251 butcher shops or 34.3% of meat in all shops was supplied from the concentrative slaughterhouse places of Hanoi. The rest 65.7% of the shops was supplied from the slaughter points.

Table 17: Descriptive factors related to the meat selling practice taken from a questionnaire surveys in the retail markets in Hanoi.

Factors	**Level**	**Number of meat retailers selected**	**% meat retailers selected**	**95% CI**
Training	Yes	0		
	No	251	100	
Season	Winter	159	63.4	57.3 – 69.3
	Spring	92	36.6	30.6 – 42.6

Factors	Level	Number of meat retailers selected	% meat retailers selected	95% CI
Hygiene/ Use of gloves	Yes	0		
	No	251	100	
Shop/Surface	Wood	136	54.2	47.9 – 60.4
	Ceramic	61	24.3	18.9 – 29.6
	Stainless steel	54	21.5	16.4 – 26.6
Cleaning (by detergent)	Yes	251	100	
	No	0	0	
Water/source	Tap	251	100	
	Well	0		
Transport/ vehicles	Truck	8	3.2	1.0 – 5.4
	Motor bicycle	243	96.8	94.6 – 99.0
Storage/Freezer	Yes	0		
	No	251	100	
Covering of meat	Yes	64	25.5	20.1 – 30.9
	No	187	74.5	69.1 – 79.9
Inspection	Yes	251	100	
	No	0		
Mix internal white organ	Yes	0	0	
	No	251	36.6	30.6 – 42.7
Supply	Self trade	143	56.9	50.8 – 63.1
	Contract	108	43.1	36.9 – 49.2
Remains/within a day	Yes	251	100	
	No	0		

Factors	Level	Number of meat retailers selected	% meat retailers selected	95% CI
Remain treating meat	Lower price	233	92.8	89.6 – 96.0
	Process other food	18	7.2	3.9 – 10.4
Slaughter place	Slaughterhouse	86	34.3	28.4 – 40.2
	Slaughter point	165	65.7	59.8 – 71.6

4.3 The results of the questionnaire survey of selected factors related to the microbiological condition of fresh minced pork selling practice.

4.3.1 *Salmonella* positive samples in fresh minced pork.

The number of *Salmonella* positive samples in each level of risk factors is shown in Table 18. There were 4 out of 15 factors with a statistically significant difference ($p<0.05$) associated with the sample proportion in the univariate analysis.

Table 18: Summary results of potential factors and the levels for *Salmonella* contamination in fresh minced pork (n=251)

Factors	Level	Number of samples tested	Number of samples positive	% positive	95% CI	P- value
Training	Yes	0	0			
	No	251	92	36.6	30.6 - 42.6	
Season	Winter	159	34	21.4	15.3 - 28.6	0.0001
	Spring	92	58	63.0	52.3 - 72.9	
Hygiene/ Use of gloves	Yes	0	0			
	No	251	92	36.6	30.6 - 42.6	

Factors	Level	Number of samples tested	Number of samples positive	% positive	95% CI	P- value
Shop/ Surface	Wood Ceramic Stainless steel	136 61 54	46 21 25	33.8 34.4 46.3	25.9 - 42.4 27.7 - 47.6 32.6 - 60.4	0.263
Clean table	Yes No	251 0	92 0	36.6 -	30.6 - 42.6	
Water/ source	Tap Well	251 0	92 0	36.6	30.6 - 42.6	
Transport / vehicles	Truck Motor bicycle	8 243	0 92	0.0 37.9	31.7 - 44.2	
Storage/ Freezer	Yes No	251 0	92 0.00	36.6	30.6 - 42.6	
Covering of meat	Yes No	64 187	15 77	23.4 41.2	13.8 - 35.6 34.1 - 48.6	0.011
Inspection	Yes No	251 0	92 0	36.6	30.6 - 42.6	
Mix organ	Yes No	0 251	0 92	36.6	30.6 - 42.6	
Supply	Self trade Contract	143 108	70 22	48.9 20.4	40.5 - 57.4 12.2 - 29.2	0.0001
Remains/ day	Yes No	251 0	92 0	36.6	30.6 - 42.6	

Factors	Level	Number of samples tested	Number of samples positive	% positive	95% CI	P- value
Remain meat treating	Lower price	233	82	35.2	29.1- 41.7	0.0847
	Other food	18	10	55.6	30.8 - 78.5	
Slaughter place	Slaughterhouse	86	24	27.9	18.8 - 38.6	0.0383
	Slaughter point	165	68	41.2	33.6 - 49.1	

The four factors found significantly associated with the sample contamination of *Salmonella* were "season", "origin of fresh pork supply", "covering of meat" and "slaughter place" (Table 18).

Seasonally, 21.4% of the samples gathered during winter time were positive while 63.0% of the spring samples were positive for *Salmonella* spp. Those two seasonal proportions were significantly different ($p<0.0001$). An odds ratio (OR) of 6.2 (95% CI, 3.55-11.07) implies that in spring it was 6.2 times more likely to recover *Salmonella* spp. from minced pork than in winter (Table 19).

The odds ratios of "season", "origin of fresh pork supply", "covering of meat" and "slaughter place" were >1 indicating some association between these factors and the contamination of *Salmonella* in fresh minced pork in Hanoi's urban retail markets. Nevertheless, the factors "type of table surface", "remaining meat per day" were not statistical significantly associated ($p>0.05$).

Table 19: Logistic regression of risk factors associated with sample prevalence of *Salmonella* spp. in fresh minced pork (n=251)

Factors	Level	OR	P-value	95%CI
Season	Winter	1	-	0
	Spring	6.2	0.0001	3.55 - 11.07
Supply	Contract	1	-	0
	Self trade	3.7	0.0001	2.08 - 7.14
Covering of meat	Yes	1	-	0
	No	2.3	0.011	1.19 - 4.35
Slaughter place	Slaughter house	1	-	0
	Slaughter point	1.8	0.0383	1.02 - 3.20

4.3.2 Risk factors and the level of the Aerobic Plate Count (APC) in fresh minced pork from retail markets in Hanoi

The level of *E. coli,* APC contamination in fresh minced pork was statistically significant ($p<0.001$) lower in *cold* winter *months* than in the *warm* spring *months* (2.19 log CFU/g; 6.38 log CFU/g vs. 4.99 log CFU/g; 7.74 log CFU/g), respectively.

The microbiological contamination (APC) in fresh minced pork and some risk factors are shown in Table 20. Statistically significant potential factors to the level ($p<0.05$) are "season", "origin of fresh pork supply" and "slaughter place". The degree of contamination was higher in spring time, in self traded meat by the retailer, at the slaughter points, respectively.

Table 20: Summary results of potential factors and the levels for the Aerobic Plate Count (APC) (log CFU/g) in fresh minced pork (n=98)

Factors	**Level**	**Number of samples examined**	**% samples tested**	**Mean**	**P-value**
Season	Winter	65	66.3	6.38	0.0001*
	Spring	33	33.7	7.74	
Training	Yes	0	0		
	No	98	100	6.84	
Hygiene/ Use of gloves	Yes	0	0		
	No	98	100	6.84	
Shop/ Surface	Wood	49	50.0	6.92	0.139
	Ceramic	21	21.4	6.41	
	Stainless steel	28	28.6	7.02	
Clean	Yes	98	100	6.84	
	No	0	0		
Disinfectant (*Detergent*)	Yes	98	100	6.84	
	No	0	0		
Water/ source	Tap	98	100	6.84	
	Well	0	0		
Transport/ vehicles	Truck	5	5.1	6.05	0.1103
	Motor bicycle	93	94.9	6.88	
Covering of meat	Yes	32	32.6	6.93	0.56
	No	66	67.4	6.79	
Inspection	Yes	98	100	6.84	
	No	0	0		
Supply	Self trade	53	54.1	7.15	0.003*
	Contract	45	45.9	6.48	

Factors	Level	Number of samples examined	% samples tested	Mean	P-value
Remains/ day	Yes	98	100	6.84	
	No	0	0		
Remain meat treating	Lower price	87	88.8	6.81	0.431
	Other food	11	11.2	7.10	
Slaughter place	Slaughter house	38	38.8	6.49	0.017*
	Slaughter point	60	61.2	7.06	

4.3.3. Risk factors and the level of *E. coli* in fresh minced pork from the retail markets in Hanoi

The level of *E. coli* contamination in fresh minced pork sold at 9 urban districts in Hanoi and the most likely risk factors are shown in Table 21. The results of the univariate analysis identify some of the risk factors which are statistically significant ($p<0.05$). The examined fresh minced pork samples which have *E. coli* contamination have been associated with season (cold months and warm months), origin of supply meat and slaughter place. The level of *E. coli* contamination is higher in the warm months, in self traded meat of butcher shops and at the slaughter points.

Table 21: Summary results of potential factors and the levels for *E. coli* (log CFU/g) in fresh minced pork (n=98)

Factors	Level	Number of samples examined	% samples tested	Median	P- value
Season	Winter	65	66.3	2.19	0.0001*
	Spring	33	33.7	4.99	
Training	Yes	0	0		
	No	98	100	3.39	

Factors	Level	Number of samples examined	% samples tested	Median	P- value
Hygiene/ Use of gloves	Yes	0	0		
	No	98	100	3.39	
Shop/Surface	Wood	49	50.0	4.03	0.138
	Ceramic	21	21.4	2.27	
	Stainless steel	28	28.6	2.81	
Clean	Yes	98	100	3.39	
	No	0	0		
Disinfectant	Yes	98	100	3.39	
	No	0	0		
Water/source	Tap	98	100	3.39	
	Well	0	0		
Transport/ vehicles	Truck	5	5.1	1.57	0.066
	Motor bicycle	93	94.9	3.53	
Covering of meat	Yes	32	32.6	3.09	0.658
	No	66	67.4	3.69	
Inspection	Yes	98	100	3.39	
	No	0	0		
Supply	Self trade	53	54.1	4.44	0.0001*
	Contract	45	45.9	2.19	
Remains/day	Yes	98	100	3.39	
	No	0	0		
Remain meat treating	Lower price	87	88.8	3.58	0.311
	Other food	11	11.2	2.27	

Factors	Level	Number of samples examined	% samples tested	Median	P- value
Slaughter place	Slaughter House	38	38.8	2.14	0.0005*
	Slaughter point	60	61.2	3.97	