

4. RESULTS

4.1. Results of *Salmonella* Isolation and Serotyping

4.1.1. Results of *Salmonella* Isolation

Table 9 shows the distribution of farm faecal prevalence of *Salmonella*, which ranged from 30-88% with an average of 62.9% (95% CI: 56%-70%). In the open farms, a prevalence ranging from 38% to 88% with an average of 65.3% (95% CI: 57%-73%) was obtained, while in the closed farms, prevalence ranged from 30% to 80% with an average of 56.0% (95% CI: 41%-70%). These two averages were not significantly ($p=0.308$) different.

Table 10 shows a total of 415 samples from 22 farms examined for *Salmonella*. Overall *Salmonella* was isolated in 71.3% (296/415). Specifically, *Salmonella* was isolated in 62.9% (122/194) of the faecal samples, 94.8% (147/155) of the floor swab samples and 40.9% (27/66) of the water samples. Farm 11 had the highest proportion of the isolates, the lowest number of isolates were received from farm 18. The proportion of *Salmonella* isolates from closed farms and open farms of 69.0% and 72.1% was not significantly ($p = 0.643$) different.

Table 11 shows the percentage of samples tested positive for *Salmonella* for the three water types. In general, the same source of water was used for drinking and cleaning the pens. However, drinking water samples were collected from the nipples, while cleaning water samples were collected from the pipe at the front or beside the pig house. The drinking water and cleaning water had similar results of 13.6% positivity while waste water had 95.5% positive result.

Table 9: Distributions of farm faecal prevalence of *Salmonella* obtained from pigs in Chiang Mai province, Thailand

Farm	Faecal Sample	No. Positive	% Proportion (95% CI)
Open Farms			
1	10	8	80 (44-97)
2	10	7	70 (35-93)
4	10	7	70 (35-93)
5	10	7	70 (35-93)
7	8	7	88 (47-100)
8	8	7	88 (47-100)
9	8	5	63 (24-91)
10	8	4	50 (16-84)
11	8	7	88 (47-100)
12	8	5	63 (24-91)
14	8	4	50 (16-84)
17	8	5	63 (24-91)
18	8	3	38 (9-76)
19	8	4	50 (16-84)
20	8	4	50 (16-84)
21	8	5	63 (24-91)
22	8	5	63 (24-91)
Closed Farms			
3	10	6	60 (26-88)
6	10	8	80 (44-97)
13	10	6	60 (26-88)
15	10	5	50 (19-81)
16	10	3	30 (7-65)
Total Open Farm	144	94	65.3 (57-73)
Total Closed Farm	50	28	56.0 (41-70)
Overall Total	194	122	62.9 (56-70)

Table 10: Proportion of *Salmonella* isolates from various samples in the farms

Farm	Total isolated sample				Total positive (%)			
	Feces	Floor swab	Water	Total	Feces	Floor swab	Water	Overall
Open Farms								
1	10	8	3	21	8 (80)	8 (100)	1 (33)	17 (81)
2	10	7	3	20	7 (70)	6 (86)	1 (33)	14 (70)
4	10	7	3	20	7 (70)	7 (100)	1 (33)	15 (75)
5	10	7	3	20	7 (70)	7 (100)	2 (67)	16 (80)
7	8	7	3	18	7 (88)	7 (100)	1 (33)	15 (83.3)
8	8	7	3	18	7 (88)	7 (100)	1 (33)	15 (83.3)
9	8	7	3	18	5 (63)	6 (86)	1 (33)	12 (66.7)
10	8	7	3	18	4 (50)	7 (100)	1 (33)	12 (66.7)
11	8	7	3	18	7 (88)	7 (100)	2 (67)	16 (88.9)
12	8	7	3	18	5 (88)	7 (100)	1 (33)	13 (72.2)
14	8	7	3	18	4 (50)	7 (100)	0 (0)	10 (55.6)
17	8	7	3	18	5 (63)	7 (100)	1 (33)	13 (72.2)
18	8	7	3	18	3 (38)	5 (71)	1 (33)	9 (50)
19	8	7	3	18	4 (50)	7 (100)	1 (33)	12 (66.7)
20	8	7	3	18	4 (50)	7 (100)	1 (33)	12 (66.7)
21	8	7	3	18	5 (63)	5 (71)	1 (33)	11 (61.1)
22	8	7	3	18	5 (63)	7 (100)	3 (100)	15 (83.3)
Closed Farms								
3	10	7	3	20	6 (60)	6 (86)	3 (100)	15 (75)
6	10	7	3	20	8 (70)	7 (100)	1 (33)	16 (80)
13	10	7	3	20	6 (60)	7 (100)	1 (33)	14 (70)
15	10	7	3	20	5 (50)	7 (100)	1 (33)	13 (65)
16	10	7	3	20	3 (30)	6 (86)	1 (33)	11 (55)
Total Open Farm	144	120	51	315	94 (65.3)	113 (94.2)	20 (39.2)	227 (69.0)
Total Closed Farm	50	35	15	100	28 (56.0)	34 (97.1)	7 (46.7)	69 (72.1)
Overall Total	194	155	66	415	122 (62.9)	147 (94.8)	27 (40.9)	296 (71.3)

Table 11: Type of water samples and percentage of *Salmonella* positive

Type of water samples	Total samples	No positive (%)
Drinking water	22	3 (13.6)
Cleaning water	22	3 (13.6)
Waste water	22	21 (95.5)

4.1.2. Results of *Salmonella* Serotyping

Table 12 shows the most frequently found serogroups of *Salmonella*. A total of 295 isolates was tested. The serogroup with the highest proportion was *Salmonella* group C (47.1%), followed by group B (32.5%), group E (14.6%), group D (2.0%) and group F-67 (3.7%). The serogroups found in both open and closed farms were basically the same.

Faecal samples and floor swab samples was found to be contaminated with *Salmonella* group C in the highest frequency (54.5% and 43.5%), but in water samples *Salmonella* group B was the most frequently found (37.0%) (Table 13).

From Table 14, there was one 1 farm contaminated with *Salmonella* serogroup C only, 19 farms contaminated with *Salmonella* serogroup B and C, 8 farms contaminated with *Salmonella* serogroup B, C and E and 2 farms contaminated with *Salmonella* serogroup D.

Table 12: Distribution of *Salmonella* serogroups in the farms

Farm	Number of samples in each group					Total
	B	C	D	E	F-67	
Open farms						
1	2	5	-	10	-	17
2	3	10	-	1	-	14
4	3	3	-	-	9	15
5	3	7	4	2	-	16
7	2	13	-	-	-	15
8	2	13	-	-	-	15
9	5	2	-	4	1	12
10	7	5	-	-	-	12
11	9	7	-	-	-	16
12	5	8	-	-	-	13
14	5	1	-	4	-	10
17	9	4	-	-	-	13
18	6	2	-	-	-	8
19	4	8	-	-	-	12
20	2	7	-	3	-	12
21	5	6	-	-	-	11
22	-	6	-	9	-	15
Closed farms						
3	-	5	2	7	1	15
6	-	16	-	-	-	16
13	8	6	-	-	-	14
15	9	2	-	2	-	13
16	7	3	-	1	-	11
Total Open Farm (%)	72 (31.9)	107 (47.3)	4 (1.8)	33 (14.6)	10 (4.4)	226
Total Closed Farm (%)	24 (34.8)	32 (46.4)	2 (2.9)	10 (14.5)	1 (1.4)	69
Overall Total (%)	96 (32.5)	139 (47.1)	6 (2.0)	43 (14.6)	11 (3.7)	295

Table 13: Distribution of *Salmonella* serogroups in each type of samples

Serogrouping	Number and % positive		
	Faecal sample	Water sample	Floor swab sample
B	34 (28.1)	10 (37.0)	52 (35.4)
C	66 (54.5)	9 (33.3)	64 (43.5)
D	3 (2.5)	1 (3.7)	2 (1.4)
E	14 (11.6)	5 (18.5)	24 (16.3)
F-67	4 (3.3)	2 (7.4)	5 (3.4)
Total	121	27	147

Table 14: General distribution of *Salmonella* serogroup

<i>Salmonella</i> Serogroup	Frequency (farm)	Percentage
C	1	4.54
C, E	1	4.54
C, D, E, F-67	1	4.54
B, C	10	45.54
B, C, F-67	1	4.54
B, C, E	6	27.27
B, C, E, F-67	1	4.54
B, C, D, E	1	4.54
Total	22	1.00

Table 15 shows *Salmonella* serotypes isolated from each type of samples. Of the total 295 isolated samples, 19 serotypes were isolated. Of overall samples, *S. Rissen* was the most frequently serotype isolated (45.4% of all isolates), followed by *S. Typhimurium* (18.3%), *S. Stanley* (11.5%), *S. Weltevreden* (4.1%), *S. Krefeld* (3.1%) and *S. Anatum* (2.0%).

From faecal isolation, 10 serotypes were isolated in the open farm and 5 serotypes were isolated in the closed farms. The most frequently serotypes found were was *S. Rissen* (53.7%), followed by *S. Stanley* (15.7%) and *Typhimurium* (9.9%).

From floor swab isolation, 13 serotypes were isolated in the open farm and 8 serotypes were isolated in closed farms. The most frequently serotypes found were *S. Rissen* (41.5%), followed by *S. Typhimurium* (23.8%) and *S. Stanley* (8.8%).

The serotypes often found contaminated in water sample were *S. Rissen* and *S. Typhimurium*; which were found 29.6% for each serotypes.

Table 15: *Salmonella* serotypes of isolates in each type of samples and compare between open farms and closed farms

<i>Salmonella</i>		Number of isolates in each type of samples								
Sero-group	Serotypes	Faeces		Floor swab		Type of water			Total	
		Open Farm	Closed Farm	Open Farm	Closed Farm	Drinking Water	Cleaning Water	Waste Water	Total Number	%
B	Typhimurium	8	4	26	8	-	1	7	54	18.3
	Stanley	16	3	10	4	-	-	1	34	11.5
	Agona	2	-	1	-	-	-	1	4	1.4
	Hato	-	-	-	1	-	-	-	1	0.3
	Derby	-	-	1	-	-	-	-	1	0.3
C	Rissen	49	16	47	14	-	-	8	134	45.4
	Afula	-	-	2	1	-	-	1	4	1.4
D	Panama	1	2	2	-	-	-	-	5	1.7
	Israel	-	-	-	-	1	-	-	1	0.3
E	Weltevreden	3	-	7	-	1	1	-	12	4.1
	Krefeld	4	-	5	-	-	-	-	9	3.1
	Anatum	1	3	2	-	-	-	-	6	2.0
	Regent	2	-	1	1	-	-	1	5	1.7
	O3,15:f,g,r:	-	-	3	-	-	-	-	3	1.0
	O3,10:e,h:	-	-	-	3	-	-	-	3	1.0
	Alfort	-	-	-	1	-	1	-	2	0.7
	Langensalza	-	-	1	-	-	-	-	1	0.3
	Rideau	1	-	-	-	-	-	-	1	0.3
	O3,15:f,g:	-	-	-	-	-	-	1	1	0.3
Others	6	-	5	1	1	-	1	13	4.7	
Total		93	28	113	34	3	3	21	295	100.0

4.2. Results of *Salmonella* Antibody Testing from Serum Samples

A total of 428 serum samples from 22 farms was analyzed using the SALMOTYPE® Pig LPS ELISA (Labor Diagnostik Leipzig, Germany) (cut-off value of OD%>40). The results in Table 16 show the distribution of sero-prevalence of *Salmonella*, ranging from 25-95% with an average of 64.4% (95% CI: 60%-69%). Specifically, in the open farms, the sero-prevalence ranged from 30% to 95% with an average of 67.6% (95% CI: 62%-73%), while in the closed farms, the sero-prevalence ranged from 25% to 70% with an average of 54.0% (95%CI: 44%-64%) were obtained. These results were significantly ($p=0.0168$) different.

Table 16: Results of *Salmonella* antibody testing from serum samples in each farm, using ELISA test with a cut-off value at 40 OD%

Farm	Serum Sample	No Positive	% Proportion (95% CI)
Open Farms			
1	8	4	50 (16-84)
2	20	9	45 (23-68)
4	20	9	45 (23-68)
5	20	18	90 (68-99)
7	20	17	85 (62-97)
8	20	18	90 (68-99)
9	20	11	55 (32-77)
10	20	19	95 (75-100)
11	20	13	65 (41-85)
12	20	6	30 (12-54)
14	20	18	90 (68-99)
17	20	11	55 (32-77)
18	19	8	42 (20-67)
19	20	11	55 (32-77)
20	20	18	90 (68-99)
21	20	16	80 (56-94)
22	20	15	75 (51-91)
Closed Farms			
3	20	13	65 (41-85)
6	20	11	55 (32-77)
13	20	11	55 (32-77)
15	20	5	25 (9-49)
16	20	14	70 (46-88)
Total Open Farm	327	221	67.6 (62-73)
Total Closed Farm	100	54	54.0 (44-64)
Overall Total	427	275	64.4 (60-69)

4.3. Correlation between a number of *Salmonella* Isolation and ELISA Results

Blood serum and faecal samples were taken from 189 pigs. Table 17 shows the relationship between antibody detection in the serum and *Salmonella* presence throughout the faeces. The antibody detection method used was ELISA with a cut-off value of 40 OD%. 60.8% (115/189) of pigs were ELISA positive and 62.4 (118/189) were isolation positive. 74 pigs were found *Salmonella* positive in both faeces and serum. 30 pigs were negative in both. 44 pigs were found *Salmonella* positive in the faeces but not in the serum. 41 pigs were found negative in faeces but positive in serum. The total number of pigs with the same result (both tests were positive or negative) was 104 pigs. From this result, the correlation between the two methods of examination was found to be very low ($\kappa = 0.0492$, $OR=1.23$, $p=0.5399$).

Table 17: Correlation of *Salmonella* isolation results and serological results obtained from ELISA (cut-off value at 40 OD%)

Test		ELISA		Total
		Positive	Negative	
Faecal Isolation	Positive	74	44	118
	Negative	41	30	71
Total		115	74	189

4.4. Farm Management Characteristics and *Salmonella* Isolation

All the farms included in the survey had similar management because of the regulations given by the particular slaughterhouse the animals were shipped to. The most obvious differences among farms were the type of farm (closed/open house farm), DLD (Department of Livestock Development) certification, the source of water used in farms, waste management, herd size, loss rate and the drinking containing probiotics (EM; Effective Microorganisms).

Results from the questionnaires: the percentage of loss (mortality and culling) in the 22 farms ranged from 1.7% to 14.4% (mean = 4.25%, median = 3.45%). The standard loss rate set by the company was 3%, only 7 farms (31.8%) had a loss < 3%. The number of pigs per pen ranged from 20 to 32 pigs per pen (with mean, median and modes = 25 pigs per pen).

4.4.1. Results from Univariate Analysis

Table 18.1 shows the relationship between the particular management characteristics and the percentage of positive *Salmonella* faecal samples (univariate analysis, Chi-square tests). Among the factors, the type of waste management was the only significant characteristic associated with *Salmonella* isolation: pigs raised in farms with a slurry waste management system had higher *Salmonella* infection than pigs raised in farms with a biogas waste management system (69.2% and 52.7%, OR=2.01, p=0.023).

Table 18.1: Relationship between farm management characteristics and *Salmonella* detection in faecal samples (univariate analysis)

Factor	Status	No. of samples	% Positive	OR (95% CI)	p-value
Herd Size	< 400	42	73.8	2.01 * (0.93, 4.35)	0.1531
	401 - 800	132	58.3		
	>800	20	70.0		
DLD certified	Certified	72	56.9	1.58 * (0.79, 3.15)	0.3994
	Applying	68	67.6		
	Non-certified	54	64.8		
Housing system	Open house	144	65.3	1.48 (0.77, 2.84)	0.3079 **
	Closed house	50	56.0		
Water Source	Tab water	10	60.0	1.22 * (0.32, 4.61)	0.8862
	Underground water	96	64.6		
	Surface water	88	61.4		
Probiotic (EM)	Used	126	64.3	1.19 (0.65, 2.18)	0.6411 **
	Not Used	68	60.3		
Lime Ash	Not used	82	64.6	1.14 (0.63, 2.06)	0.7637 **
	Used	112	61.6		
Waste management	Slurry	120	69.2	2.01 (1.11, 3.66)	0.0228 **
	Biogas	74	52.7		

Remark * Highest OR obtained from 2*2 table of the factors
 ** p-value from Fisher's Exact

4.4.2. Results from Multivariable Analysis

Table 18.2 shows the relationship between particular management characteristics and positive results of *Salmonella* in faecal samples (multivariable risk factors analysis, SAS statistic program). All relevant factors (Table 17.2) were included in this calculation. Without the interaction of other farm characteristics, the significant characteristic associated with *Salmonella* isolation was the housing system: the open house system had a significantly higher *Salmonella* isolation than the closed house system (OR=1.59, p=0.0496). Herd size was also a significant characteristic associated with *Salmonella* isolation: a smaller herd size (< 800 pigs/herd) tended to

have lower *Salmonella* isolation than the larger herd size (> 800 pigs/herd) (OR=0.18, $p \leq 0.0002$). The lower number of pigs per pen was also significantly associated with lower *Salmonella* infection (OR=0.91, $p < 0.0001$).

Table 18.2: Relationship between all farms management characteristics and *Salmonella* detection in faecal samples (multivariable analysis)

Factor	Status	OR	p-value
Herd size	< 400	0.21	0.0002
	401 - 800	0.18	< 0.0001
	>800	1.00	.
DLD certified	Certified	0.68	0.0412
	Applying	1.72	0.033
	Non-certified	1.00	.
Housing system	Open house	1.59	0.0496
	Closed house	1.00	.
Water source	Tab water	0.95	0.9524
	Underground water	1.76	0.014
	Surface water	1.00	.
Probiotic (EM)	Not Used	0.56	< 0.0001
	Used	1.00	.
Lime ash	Not used	1.03	0.9314
	Used	1.00	.
Waste management	Slurry	1.50	0.1168
	Biogas	1.00	.
No. of pigs/pen	20 to 32 pigs per pen	0.91	< 0.0001
% loss	1.7% to 14.4%	0.98	0.7063

Pigs not fed probiotics (EM) appeared to have a significantly lower risk of harboring *Salmonella* than pigs fed probiotics (EM) (OR=0.56, $p < 0.0001$). There was a higher *Salmonella* isolation rate in farms using underground water than farms using surface water (OR=1.76, $p = 0.014$). Farms certified by DLD had significantly lower *Salmonella* isolation than farms non-certified by DLD (OR=0.68, $p = 0.0412$) while farms in the process of applying DLD certification appeared to have a significantly higher risk of getting *Salmonella* than non-certified farms (OR=1.72, $p = 0.033$). Waste management systems, using lime ash and the percentage of losses had no association with *Salmonella* isolation.

4.5. Farm Management Characteristics and *Salmonella* Antibody Testing

4.5.1. Results from Univariate Analysis

Table 19.1 shows the relationship between particular management characteristics and the percentage of positive *Salmonella* antibody detection in serum samples (univariate analysis of risk factors, Chi-square tests). Among those characteristics herd size, housing system (open/closed farms), water source, probiotic (EM) feed and waste management affected the sero-prevalence of *Salmonella*.

A herd size with more than 800 pigs per herd had a lower positive percentage of sero-prevalence (60.0%), herds lower than 400 pigs per herd had the highest sero-prevalence (78.4%). This difference was significant (OR=2.42, $p=0.0087$). Farms with the open house system had a higher sero-positive percentage (67.6%) than farms with the closed house system (54.0%); this was significantly different (OR=1.78, $p=0.0168$).

Farms that used underground water had a higher sero-positive percentage (74.0%) than farms using tap water (65.0%) or farms using surface water (54.3%). This was also significantly different (OR=2.40, $p=0.0002$).

Farms that did not feed pigs with probiotics (EM) had higher *Salmonella* sero-positive percentages compared to farms that fed probiotics (EM) (77.5% and 56.6%). These results were significantly different (OR=2.65, $p=0.00001$).

Pigs raised in farms with a slurry waste management system had higher *Salmonella* infection than pigs raised in farms with a biogas waste management system (67.9% and 58.5%, OR=1.50, $p=0.0597$).

The *Salmonella* sero-positive percentage in farms certified by DLD was not different from that of non-certified farms and farms that were in the process of

applying (62.8%, 68.1% and 61.1% respectively, OR=1.36, p=0.4414). Using lime ash in the cleaning and disinfection steps before receiving the new pigs was not different in *Salmonella* sero-prevalence from those farms not using lime ash (64.5% and 64.3%, OR=1.01, p=1.0000).

Table 19.1: Relationship between farm management characteristics and *Salmonella* detection from serum samples using ELISA test with a cut-off value at 40 OD% (univariate analysis)

Factor	Status	No sample	% positive	OR (95% CI)	p-value
Herd Size	< 400	88	78.4	2.42 * (1.08, 5.45)	0.0087
	401 - 800	299	60.9		
	>800	40	60.0		
DLD certified	Certified	159	62.9	1.36 * (0.82, 2.27)	0.4414
	Applying	160	68.1		
	Non-certified	108	61.1		
Housing system	Open house	327	67.6	1.78 (1.13, 2.80)	0.0168 **
	Closed house	100	54.0		
Water Source	Tab water	20	65.0	2.40 * (1.58, 3.65)	0.0002
	Underground water	208	74.0		
	Surface water	199	54.3		
Probiotic (EM)	Not Used	160	77.5	2.65 (1.70, 4.12)	0.00001
	Used	267	56.6		
Lime Ash	Not used	168	64.3	1.01 (0.67, 1.51)	1.0000 **
	Used	259	64.5		
Waste management	Slurry	268	67.9	1.50 (1.00, 2.26)	0.0597 **
	Biogas	159	58.5		

Remark * Highest OR obtained from 2*2 table of the factors

** p-value from Fisher's Exact

4.5.2. Results from Multivariable Risk Factor Analysis

Table 19.2 shows the relationship between particular management characteristics and positive results of *Salmonella* antibody detection in serum samples (multivariable risk factors analysis, SAS statistic program). All relevant factors (Table 18.2) were included in the calculation.

Without the interaction of other farm characteristics, the characteristics significantly associated with *Salmonella* isolation were the housing system in which the open house had a significant higher *Salmonella* isolation than closed housing system (OR=2.84, p=0.0496). The lower number of pigs per pen was also associated with higher *Salmonella* isolation (OR=1.16, p<0.0121).

DLD certified farms had significantly higher results of *Salmonella* infection than non-certified farms (OR=2.76, p=0.0525). Herd size of 400- 800 pigs/farms had lower *Salmonella* infection than farms which more than 800 pigs/farm (OR=0.25, p=0.0252).

Farms not using probiotic (EM) tended to have higher *Salmonella* infection than farms using probiotic (EM) (OR=2.49, p=0.0605).

Waste management, using lime ash, water source and percentage of loss had no association with *Salmonella* antibody detection result.

Table 19.2: Relationship between all farms management characteristics and *Salmonella* detection from serum samples using ELISA test with a cut-off value at 40 OD% (multivariable analysis)

Factor	Status	OR	p-value
Herd size	< 400	0.55	0.4733
	401 - 800	0.25	0.0252
	>800	1.00	.
DLD certified	Certified	2.76	0.0525
	Applying	2.15	0.1848
	Non-certified	1.00	.
Housing system	Open house	2.84	0.0475
	Closed house	1.00	.
Water source	Tab water	1.27	0.8351
	Underground water	2.23	0.1044
	Surface water	1.00	.
Probiotic (EM)	Not Used	2.49	0.0605
	Used	1.00	.
Lime ash	Not used	1.33	0.4035
	Used	1.00	.
Waste management	Slurry	0.55	0.2148
	Biogas	1.00	.
No. of pigs/pen	20 to 32 pigs per pen	1.16	0.0121
% loss	1.7% to 14.4%	0.93	0.4539