4. RESULTS

4.1 SAMPLE PREVALENCE OF *SALMONELLA*

Of the 181 lymph node samples examined, 116 were *Salmonella* positive, (64.1 %; 95% CI: 56.6 – 72.1%), while, 151 fecal samples were positive (83.4 %; 95% CI: 77.2 – 88.5%). As for carcass swabs, before use of chlorinated-water spray, 60 were *Salmonella* positive (33.1 %; 95% CI: 26.3 – 40.5%). Out of 180 carcass swabs sampled after chilling, 24 were *Salmonella* positive (13.3 %; 95% CI: 8.7 – 19.2%). Results of sample-specific prevalence of *Salmonella* are summarized in Table 8.

Table 8: Sample prevalence of *Salmonella* and 95% Confidence Intervals

Sample	Numbers of samples	*Salmonella* positive	% positive	95% CI
Mesenteric lymph nodes	181	116	64.1	56.6-71.1
Feces	181	151	83.4	77.2-88.5
Swabs before spray	181	60	33.1	26.3-40.5
Swabs after chilling	180	24	13.3	8.7-19.2

*CI = Confidence Intervals

4.2 *SALMONELLA* SEROTYPES

The distribution of *Salmonella* somatic serogroups is presented in Table 9. Of 351 *Salmonella* positive samples, 167 (47.6%) were *Salmonella* serogroup C, 117 (33.3%) serogroup B, 38 (10.8%) serogroup E, 14 (4.0%) serogroup D and 15 (4.3%) serogroup F-67.

Table 9: Distribution of *Salmonella* somatic serogroups

	Somatic serogroup (%)					**Sample Prevalence (%)**
Sample	**B**	**C**	**D**	**E**	**F-67**	
Mesenteric lymph nodes	30 (25.6%)	65 (38.9%)	6 (42.9%)	12 (31.6%)	3 (20.0%)	116 (64.1%)
Feces	52 (44.4%)	66 (39.5%)	6 (42.9%)	20 (52.6%)	7 (46.7%)	151 (83.4%)
Swabs before spray	21 (18.0%)	26 (15.6%)	2 (14.2%)	6 (15.8%)	5 (33.3%)	60 (33.1%)
Swabs after chilling	14 (12.0%)	10 (6.0%)	-	-	-	24 (13.3%)
Total (%)	**117 (100.0%)**	**167 (100.0%)**	**14 (100.0%)**	**38 (100.0%)**	**15 (100.0%)**	**351 (100.0%)**

The distribution of *Salmonella* somatic serogroups isolated from lymph node samples is given in Table 10. The distribution of serogroups varied from farm to farm with the most prevalent groups being as follows: C (65%), B (30%), E (10.3%), D (5.2%), and F-67 (2.6%).

Table 10: *Salmonella* somatic serogroup found in lymph nodes by farms

	Number of *Salmonella* somatic serogroup					**Grand**
Farm	**B**	**C**	**D**	**E**	**F-67**	**Total**
1		5		1		6
2	3	4		1		8
3	3	1	1	3		8
4	2	5			3	10
5	1		5			6
6	2	4		1		7
7		8				8
8		3				3
9	1	3		1		5
10	1	2				3
11		2				2
12	6	2				8
13	5			1		6
14	2	1		1		4
15		1				1
16	1	4				5
17		3				3
18	2	4				6
19		3		2		5
20		5				5
21	1	5		1		7
Grand Total	**30**	**65**	**6**	**12**	**3**	**116**
(%)	**(25.9%)**	**(56.0%)**	**(5.2%)**	**(10.3%)**	**(2.6%)**	**(100%)**

Fecal proportions of *Salmonella* somatic serogroups are shown in Table 11. The serogroup C was still the most prominent serogroup followed by B, E, F-67, and D.

Table 11: Fecal proportions of *Salmonella* somatic serogroups distributed by farms

Farm	Number of *Salmonella* somatic serogroup					Grand Total
	B	C	D	E	F-67	
1	1	3		2		6
2	2	5		2		9
3	4	2	2	2		10
4	1	3			6	10
5	4	5	1			10
6	3	5				8
7		8				8
8	2	1		1		4
9	3	2		3		8
10	2	5				7
11	1	6				7
12	6	1				7
13	5	1		1		7
14		1	3	3	1	8
15	7			1		8
16		3				3
17		4				4
18	3	3				6
19	4			4		8
20	4	2				6
21		6		1		7
Grand Total	**52**	**66**	**6**	**20**	**7**	**151**
(%)	**(34.4%)**	**(43.7%)**	**(4.0%)**	**(13.2%)**	**(4.6%)**	**(100%)**

Salmonella of all somatic serogroups were found in carcass swabs prior to chlorinated-water spray (Table 12). The serogroup C was the prominent one (43.3%) among the carcass swab-1 samples. This was followed by serogroup B (35.0%), E (10.0%), F-67 (8.3%), and D (3.3%).

Table 12: Distribution of swab-1 prevalence of *Salmonella* somatic serogroups before use of chlorinated-water spray by farms

	Number of *Salmonella* somatic serogroup					**Grand**
Farm	**B**	**C**	**D**	**E**	**F-67**	**Total**
1		3		3		6
2		2	1			3
3	1			1		2
4		3			5	8
5	2	2	1			5
6	3					3
7		2				2
8		3				3
9		1		1		2
10		1				1
12	6	1				7
13	1					1
14	1					1
15	5					5
16	1					1
17		1				1
18	1					1
19		1				1
20		5				5
21		1		1		2
Grand Total	**21**	**26**	**2**	**6**	**5**	**60**
(%)	**(35.0%)**	**(43.3%)**	**(3.3%)**	**(10.0%)**	**(8.3%)**	**(100%)**

Table 13 presents the sample prevalences of *Salmonella* somatic serogroups isolated from carcasses after an overnight chilling. Only two serogroups (B and C) were found. From 24 *Salmonella* positive samples, 14 (58.3%) isolates were serogroup B and 10 (41.7%) were serogroup C.

Table 13: Distribution of *Salmonella* serogroups isolated from carcasses after overnight chilling classified by farms

Farm	Number of *Salmonella* somatic serogroup		Grand Total
	B	C	
3		2	2
4		1	1
5		1	1
6	1		1
9	2		2
12	3		3
14	2		2
15	3		3
17	1		1
18		3	3
19		1	1
20	1	2	3
21	1		1
Grand Total (%)	**14 (58.3%)**	**10 (41.7%)**	**24 (100%)**

From all 21 farms, one farm had only one *Salmonella* serogroup and another had all *Salmonella* serogroups, B, C, D, E, and F-67. The other farms had groups in different frequencies. The graphical proportion of all serogroups found in 21 farms is shown in Figure 11.

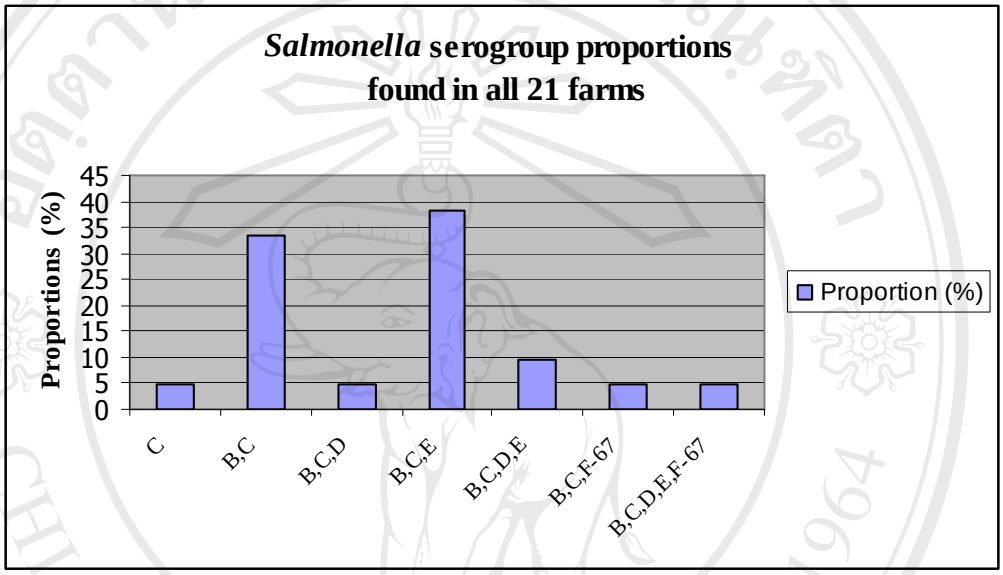

Figure 11: *Salmonella* serogroup proportions found in all 21 farms

4.3 DISTRIBUTION OF *SALMONELLA* SEROTYPES

In all the 723 samples, 351 (48.5%) were *Salmonella*-positive. Definitive *Salmonella* serotyping was conducted from these 351 samples. The three most prevalent serotypes were *S.* Rissen (161 isolates), *S.* Stanley (41 isolates), and *S.* Typhimurium (38 isolates), which were 45.9%, 11.7%, and 10.8%, respectively. Other *Salmonella* serotypes were also found in this study. Overall, the summary distributions of all serotypes obtained are given in Table 14.

Table 14: *Salmonella* serotypes isolated from the slaughter pigs and carcasses

Serotype	No. of identified isolates (%)	Main somatic group (O)	Somatic Sub-group O	Flagella Phase 1	Flagella Phase 2
1. Rissen	161 (45.9%)	C	O6, 7	f, g	-
2. Stanley	41 (11.7%)	B	O4, [5], 12	d	1, 2
3. Typhimurium	38 (10.8%)	B	O4, [5], 12	i	1, 2
4. Glaucester	17 (4.8%)	B	O4, [5], 12	i	l, w
5. Anatum	16 (4.6%)	E	O3, 10	e, h	1,6
6. Panama	14 (4.0%)	D	O9, 12	l, v	1, 5
7. Krefeld	12 (3.4%)	E	O1, 3, 19	y	l, w
8. Weltevredren	10 (2.8%)	E	O3, 10 [15]	r	z6

Continued

Table 14: Continued

Serotype	No. of identified isolates (%)	Main somatic group (O)	Somatic Sub-group O	Flagella Phase 1	Flagella Phase 2
9. Lagos	9 (2.6%)	B	O4, [5], 12	i	1, 5
10. Tsevie	8 (2.3%)	B	O4, [5], 12	i	e, n, z15
11. Saintpoul	1 (0.3%)	B	O4, [5], 12	e,h	1, 2
12. Eppendof	1 (0.3%)	B	O4, [5], 12	d	1, 5
13. Group II (F-67)	15 (4.3%)				
Other*	**8 (2.3%)**				
Total (%)	**351 (100.0%)**				

* Other =Self-agglutination (6 isolates) and unidentified (2 isolates)

The *Salmonella* serotypes identified in various samples are given in Tables 15. The most frequent serotype found in this study was *S*. Rissen, which was mainly found in mesenteric lymph nodes (63 of 116, 54.3%) and feces (63 of 116, 41.7%). *S*. Stanley, Typhimurium, and other strains were generally distributed in all the samples.

Table 15: Distribution of *Salmonella* serotypes identified from various types of samples

Serotype	Distribution of serotypes by samples				All isolated serotypes (%)
	MLN* (%)	Feces (%)	SW1** (%)	SW2*** (%)	
1. Rissen	63 (54.3%)	63 (41.7%)	26 (43.3%)	9 (37.5%)	161 (45.9%)
2. Stanley	10 (8.6%)	25 (16.6%)	3 (5.0%)	3 (12.5%)	41 (11.7%)
3. Typimurium	11 (9.5%)	14 (9.3%)	9 (15.0%)	4 (16.7%)	38 (10.8%)
4. Glaucester	6 (5.2%)	5 (3.3%)	5 (8.3%)	1 (4.2%)	17 (4.8%)
5. Anatum	7 (6.0%)	8 (5.3%)	1 (1.7%)	0	16 (4.6%)
6. Panama	6 (5.2%)	6 (4.0%)	2 (3.3%)	0	14 (4.0%)
7. Krefeld	2 (1.7%)	5 (3.3%)	5 (8.3%)	0	12 (3.4%)
8. Weltevreden	3 (2.6%)	7 (4.6%)	0	0	10 (2.8%)
9. Lagos	2 (1.7%)	3 (2.0%)	2 (3.3%)	2 (8.3%)	9 (2.6%)
10. Tsevie	0	3 (2.0%)	2 (3.3%)	3 (12.5%)	8 (2.3%)

Continued

Table 15: Continued

Serotype	Distribution of serotypes by samples				All isolated serotypes (%)
	MLN* (%)	Feces (%)	SW1** (%)	SW2*** (%)	
11. Saintpoul	1 (0.7%)	0	0	0	1 (0.3%)
12. Eppendof	0	1 (0.7%)	0	0	1 (0.3%)
13. Group F-67	3 (2.6%)	7 (4.6%)	5 (8.3%)	0	15 (4.3%)
Other	2 (1.7%)	4 (2.6%)	0	2 (8.3%)	8 (2.3%)
Total (%)	**116 (100.0%)**	**151 (100.0%)**	**60 (100.0%)**	**24 (100.0%)**	**351 (100.0%)**

- * MLN = Mesenteric Lymph Nodes
- ** SW1 = Carcass swabs before chlorinated-water spray
- ** SW2 = carcass swabs after overnight chilling

From 21 farms of origin, *Salmonella* serotypes were classified as presented in Table 16. *Salmonella* serotype Rissen was generally distributed in all farms, while *Salmonella* Typhimurium was mostly found in farm 12 and *Salmonella* group II (F-76) mostly found in farm 4. Other serotypes were commonly dispersed. The meaning of serotype abbreviations is described as followed;

Stnly = Stanley TM = Typhimurium Glcstr = Glaucester
Anat = Anatum Krfld = Krefeld Wetvd = Weltevedren
St.pl = Saintpoul Eppdf. = Eppendorf Gr.II = Group II (F-67)

Table 16: *Salmonella* serotypes classified by farms of origin

	Numbers of *Salmonella* serotypes														
Farm	**Rissen**	**Stnly**	**TM**	**Glcstr**	**Anat**	**Panama**	**Krfld**	**Wetvd**	**Lagos**	**Tsevie**	**St.pl**	**Eppdf**	**Gr. II**	**Other**	**Total**
1	11	1					6								18 (5.1%
2	11	1	1	2	5	1		3	1						25 (7.1%
3	5	2	5	1		3	1								17 (4.8%
4	12		1	1					1				14		29 (8.3%
5	8	2	2			7			1	1	1				22 (6.3%
6	9	4	2					1	1	2					19 (5.4%
7	18														18 (5.1%
8	7	1	1		1									2	12 (3.4%
9	6	1		2			5					1			15 (4.3%
10	8		3												11 (3.1%
11	8	1													9 (2.6%
12	4	2	19												25 (7.1%
13	1	10	1		1			1							14 (4.0%
14	2	3	1	1	2	3		2					1		15 (4.3%
15	1	1	1	7				1	3	3					17 (4.8%
16	7			2											9 (2.6%
17	5								1					3	9 (2.6%
18	9	5	1											1	16 (4.6%
19	4	2		1	6				1					1	15 (4.3%
20	13	3								2					18 (5.1%
21	12	2			1			2						1	18 (5.1%
	161	41	38	17	16	14	12	10	9	8	1	1	15	8	351
Total	(45.9%)	(11.7%)	(10.8%)	(4.8%)	(4.6%)	(4.0%)	(3.4%)	(2.8%)	(2.6%)	(2.3%)	(0.3%)	(0.3%)	(4.3%)	(2.3%)	(100%)

4.4 SEROLOGICAL RESULTS

One hundred and eighty one meat juice samples tested by SALMOTYPE® Pig LPS ELISA are presented in Table 17. Of those, one sample was negative for anti-*Salmonella* antibodies (cut-off O.D. % < 10), 49 samples were doubtful (cut-off O.D. % 10 - < 20), and 59 samples were weakly positive (cut-off O.D. % 20 - < 40). Positive ELISA results (cut-off O.D. % ≥ 40) were found in 72 samples (39.8%).

Table 17: Summary of serological results of all 181 meat juice samples

	Cut-off O.D. %*			
Meat juice samples (%)	**< 10 (%)**	**10 - < 20 (%)**	**20 - < 40 (%)**	**≥ 40 (%)**
181 (100%)	1 (5.5%)	49 (27.1%)	59 (32.6%)	72 (39.8%)

* Cut-Off values for meat juice samples (recommended by manufacturer's instructions);

≥ 40 OD%	positive
20 OD% - < 40 OD%	weak positive
10 OD% - < 20 OD%	doubtful (positive)
< 10 OD%	negative

At the individual pig level, serological ELISA results were compared with the results of conventional *Salmonella* culture (positive lymph node- and fecal culture, Tables 18 and 19). Using the lymph node culture results as the gold standard, sensitivity and specificity of the test were calculated using Win Episcope 2.0. Kappa statistics (Epi Info, version 2.0) was used to assess an agreement between those two different methods. Calculated kappa values were 0.122 and 0.057 compared to lymph node culture and fecal culture results, respectively.

Table 18: Comparison of lymph node culture and the ELISA results of *Salmonella* at the individual pig level

ELISA	**Lymph node culture**		
Result*	**+**	**-**	**Grand Total**
+	52	20	72
-	64	45	109
Grand Total	116	65	181

*ELISA Result (OD%≥ 40 = positive, OD%< 40 =negative)

	%	Lower limit	Upper limit
Sensitivity of ELISA test	44.8	35.8	53.9
Specificity of ELISA test	69.2	58.0	80.5

Kappa statistics** (Epi Info 2002)

Observed proportion of agreement = 0.536

Expected proportion of agreement = 0.471

Observed minus chance agreement = 0.065

Max possible agreement beyond chance = 0.529

Kappa = 0.122

95% Confidence Intervals = -0.050 – 0.295

**Kappa value (Dahoo *et al*., 2003);

<0.2:	slight agreement
0.2- 0.4:	fair agreement
0.4-0.6:	moderate agreement
0.6-0.8:	substantial agreement
>0.8:	almost perfect agreement

Table 19: Comparison of fecal culture and the ELISA results of *Salmonella* at the individual pig level

ELISA	**Fecal culture**		
Result*	**+**	**-**	**Grand Total**
+	63	9	72
-	88	21	109
Grand Total	151	30	181

*ELISA Result (OD%≥ 40 = positive, OD%< 40 =negative)

	%	Lower limit	Upper limit
Sensitivity of ELISA test	41.7	33.9	49.6
Specificity of ELISA test	70.0	53.6	86.4

Kappa statistics (Epi Info 2002)

Observed proportion of agreement = 0.364

Expected proportion of agreement = 0.464

Observed minus chance agreement = 0.032

Max possible agreement beyond chance = 0.568

Kappa = 0.057

95% Confidence Intervals = -0.036 – 0.150

Based on the results of kappa statistics and its 95% Confidence Intervals, the agreement between ELISA test results and bacteriological assays (lymph node- and fecal culture results) was slight (both Kappa values < 0.2). Thus, it was neither statistically significant between both different methods nor better than an expected proportion of agreement (expected proportion = 0.471 and 0.464) due to chance.