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Prevalence and Virulence of Salmonella Bacteria Causing Salmonellosis in Post Weaning Pigs at Swine Farms in Bac Giang Province, Vietnam

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Summary

The release of *Salmonella* of 256 breeder sows from 20 swine farms in Hiep Hoa district, Bac Giang province, Viet Nam; The medical waste samples were collected in order to determine the prevalence, serotype and virulence of 166 *Salmonella*bacteria strains isolated from medical waste of post weaning pigs with Salmonellosis. It was found that:

Out of the 166 strains studied, the number of serotypes identified included 2 *Salmonella* weltevreden, 3 *Salmonella*dublin, 5 *Salmonella* anatumand *Salmonella* senftenberg, 6 *Salmonella* Heidelberg, 9 *Salmonella* enteritidis, 30 *Salmonella* typhimurium, 41 *Salmonella* choleraesuis, and 10 unknown *Salmonella* serotype.

Salmonella weltevreden which bore the encoded gene Stnac counted for 50.0 %; Salmonella dublinand Salmonella heidelberg accounted for 66.6 %; Salmonella typhimuriumaccounted for 73.3 %;Salmonella senftenberg, Salmonellaanatum,and *Salmonella unknown* accounted for 80.0 %; Salmonella enteritidisaccounted for88.8 %; Salmonella choleraesuiscarrying the DNA bearing the gene producing Stnac counted for 92.6 %.

Salmonelladublinbearing the gene fim A accounted for 33.3 %; *Salmonella* weltevreden accounted for 50.0 %; *Salmonella unknown* accounted for 60.0 %; *Salmonella* typhimuriumand *Salmonella* heidelberg accounted for 66.6%; *Salmonella* senftenberg and *Salmonella* anatum accounted for 80.0 %;*Salmonella* enteritidisaccounted for 88.8 %; and *Salmonella* choleraesuis accounted for 92.6 %.

Salmonella typhimurium bearing the gene InvA accounted for 26.6 %; *Salmonella unknown* accounted for 30.0 %;*Salmonella* heidelberg and *Salmonella* dublin accounted for 33.3 %; *Salmonella* choleraesuis accounted for 39.0 %; *Salmonella* anatumand *Salmonella* senftenberg accounted for 40.0 %;*Salmonella* weltevreden accounted for 50.0 %; and *Salmonella* enteritidis accounted for 66.6 %.

Salmonella choleraesuis strains were resistant to nalidixic acid (2.4 %); ciprofloxacin, rifampicin, spectinomycin (7.3 %); ceftazidime, oxytetracycline (9.7 %); nitrofurantoin (12.1 %); trimethoprim-sulfamethoxazole (19.5 %); kanamycin (21.9 %).

Salmonella enteritidis strains were resistant to ciprofloxacin, rifampicin, ceftazidime, spectinomycin, nitrofurantoin (11.1 %); trimethoprim-sulfamethoxazole, kanamycin (22.2 %).

Salmonella typhimurium strains were resistant to nitrofurantoin, nalidixic acid and ceftazidime (3.3%); ciprofloxacin, spectinomycin and rifampicin (6.6 %); trimethoprim-sulfamethoxazole (16.6 %); kanamycin (20.0 %).

Keywords: Pig; *Salmonella*; Bacteria; Virulence

Introduction

Salmonella was first isolated from pigs by Salmon and Smith in 1886 [25]. The bacteria were found in the intestines of both warm and cold blood animals. With more than 2,400 serotypes, Salmonella has been identified as the cause for a number of diseases on human and animals. Having been identified for more than a hundred years, yet *Salmonella* is still the subject for numerous studies due to worldwide epiological problems on human and animals caused by the bacteria. It is estimated that every year, 155,000 people worldwide die of Salmonellosis and food poisoning [7, 8,9,10,19].

Of all the serotypes found, the ones which cause diseases on pigs are mainly *Salmonella* choleraesuis and *Salmonella* typhimurium. *Salmonella* derby, *Salmonella* Heidelberg, *Salmonella* Dublin and *Salmonella* enteritidis also cause diseases in pigs, with lower rates. *Salmonella* on pigs are found to cause diseases on pigs and food poisoning on human [14]. Pigs are the source of the diseases, bearing the bacteria and release highly virulent pathogens to the environment, causing diseases on cattles and poultry and diseases and food poisoning on human [19,25]. Food contaminated with *Salmonella* is the vector factor carrying pathogen, which causes food poisoning on human [33].

In December 2016, in Hiep Hoa district, Bac Giang, there were 97 swine farms and more than 220 small family farms for cattles and poultry. Of which, there were 25 swine farms with at least 50 sows for breeding and 150 small family farms with at least 100 market pigs (for meat). A number of families raising thousands of poultry for eggs or meat.

This study focus on identifying the prevalence of *Salmonella* on sows and post weaning pigs (healthy pigs and pigs with diarrhea), enriching scientific materials on the prevalence of the disease (etiology), which may lead to new studies on diagnosis and effective control methods for the prevalence and release of *Salmonella*which causes diseases on animals and food poisoning on human.

Materials and Methods

The study objectives were to investigate *Salmonella* prevalence; serotyping and virulence factors distribution of 256 breeder sows from 20 swine farms in Hiep Hoa district, Bac Giang province, Viet Nam; the disease samples from the post weaning Salmonellosis pigs were collected and cultured for *Salmonella* role; serotyping and virulence of 166 *Salmonella* bacteria strains isolates from disease samples of the post weaning diarrhea pigs was determined. Approximately, a total of 210 disease samples of post weaning Salmonellosis pigs will be collected from the swine farms. The samples were collected in sterilized polyethylene bags and transported to the Institute of Life Science, Thai Nguyen Agriculture and Forestry Universityin an icebox for further processing and microbiological analysis. All the samples collected are shown in Table 8.

Collect faces samples to determine number of bacteria; collect medical waste for isolation; examine biological and chemical characteristics; determine virulence, test for antibiotic and pharmachemical resistance of *Salmonella* according to Quinn P.J, et al.; Wallace H. Andrews, et al. [21,30]. Bacteriological Analytical Manual (Chapter 5,*Salmonella*).

Examine serotype *Salmonella* isolates using Test Kit O antigens, H (antigens phase 1, antigens phase 2) of Bio-Rad (Bacterial serotyping guide for *Salmonella*); Oxoid *Salmonella*Test Kit.

Antibiotic susceptibility testing was performed by the Kirby-Bauer disc-diffusion test, which conforms to the recommended standard as described by [21]. Briefly, an inoculum of each pure bacterial isolate was emulsified in 3 mL of sterile normal saline and the density adjusted to 0.5 McFarland standard. A sterile cotton swab was dipped into the standardized suspension of bacterial cultures and used to inoculate Mueller-Hinton agar (MHA) plates (Oxoid, England), and the plates were allowed to dry. Antibiotic discs with the following drug contents amoxicilline, nitrofurantoin, ciprofloxacin, bacitracin, erythromycin, oxytetracycline, ceftazidime, nalidixic acid, gentamycin, vancomycin, oxacillin, kanamycin, and rifampicin, (Antibiotic Becton, Dickson and Company, Sparks, USA; Le Pont de Claix, France) were placed onto MHA plates. The plates were incubated at 37°C for 24 hours. The zone diameter was measured and results were interpreted based on Quinn PJ, et al. [21]. The reference strains *Salmonella*were used to verify the quality and accuracy of the testing procedure.

Multiplex polymerase chain reaction analysis of the targeted genes of interest was performed using DreamTaq DNA polymerase (Thermo Scientific, USA). For the amplification, five microlitres of DNA was added to 20 µL of master mix containing 12.5 µL of DreamTaq DNA polymerase (2X DreamTaq Green Buffer, dATP, dCTP, dGTP, and dTTP, 0.4 mM each, and 4 mM $MgCl_2$) (Thermo Scientific, USA), 0.5 µL (0.2 µM) of respective oligonucleotide primers and the reaction volume was made up with nuclease free water. PCR was performed in a thermal cycler (Bio-Rad Laboratories, USA). The amplification cycles consisted of an initial DNA denaturation at 95°C for 15 min, followed by 35 cycles of denaturation at 94°C for 45 s, primer annealing at 55°C, for 45 s, extension at 68°C for 2 min, and a final single elongation at 72°C for 5 min. The primers used to amplify the targeted genes were as previously reported by Institute of Genome Research, Vietnam Academy of Science and Technology and are summarized in Table 1. Negative controls, substituting DNA template with ultrapure water (Sigma-Aldrich, UK), were included in all PCR runs. Amplified DNA was resolved by 2% agarose gel electrophoresis and visualised under UV transillumination

Biological statistic was processed with SPSS: Statistical analysis was performed using SPSS version 22.0. The chi-square test was used to compare rate of isolation of the various disease samples and the different study sites. Comparisons were also done among the farms. Differences were considered significant at $P<0.05$.

Table1: Primer pairs used for virulence characterization of Salmonella isolates

Primer pair target	Primer sequence (5'→3')	Annealing temp (°C)	Length (bp)	Reference
invA	F: GTG AAA TTA TCG CCA CGT TCG GGC AA R: TCA TCG CAC CGT CAA AGG AAC C	63	521	[12]
stn	F: CTT TGG TCG TAA AAT AAG GCG R: TGC CCA AAG CAG AGA GAT TC	55	260	[15]
fimA	F: CCT TTC TCC ATC GTC CTG AA R: TGG TGT TAT CTG CCT GAC CA	56	85	[16]

Results and Discussion

Release of *Salmonella* in sows and young pigs

Release of Salmonella in Sows

Faces samples from 265 sows in 20 swine farms in eight communes and one district town in Hiep Hoa district, Bac Giang province were collected to determine the prevalence and release of *Salmonella*. The results are presented in Table 2.

Table 2: Release of *Salmonella*in sows by place and individual

Research site	Release of *Salmonella* by place			Release of *Salmonella* by individual		
	No. of farms studied	No. of farms with sowsreleasing of *Salmonella*	Rate	No. of sows studied	No. of sowsreleasing of *Salmonella*	Rate
			(%)			(%)
TT Thang	2	2	100	31	12	38.7
Duc Thang	3	3	100	33	9	27.2
Ngoc Son	3	3	100	39	15	38.4
Danh Thang	2	2	100	30	11	36.6
Bac Ly	3	3	100	30	13	43.3
Dong Lo	2	2	100	32	12	37.5
Luong Phong	2	2	100	36	10	27.7
Mai Trung	3	3	100	34	9	26.4
Total	20	20	100	265	91	34.3

From Table 2, it can be seen that: The release of *Salmonella* occurred in all the sow farms (accounting for100%). The highest rate of *Salmonella* release was 26.4 % (Mai Trung), and the lowest rate was 43.3 % (Bac Ly). The average rate of *Salmonella* release from sows to the farm environment was 34.3 %.With P>0.05 it can be said that the difference on the rates of *Salmonella* release from sows between the farms are not significant. The results were totally in line with those of Lo FO Wong , et al. on release of *Salmonella* from pigs [14]. In an earlier study, Jerome C. Nietfeld, et al. also found the rate of *Salmonella* isolates from pig rectal swabs was 46.6 %.

Release of *Salmonella* in Sows by Parity

Faces samples were collected from 240 local breeder sows in Hiep Hoa district, Bac Giang by parities at different time frames in order to determine the level of *Salmonella* release. The results are presented in Table 3.

Table 3: Release of Salmonellain sows by parity

Sample collection time	Parity 1 sows (Primiparous)			Sows in parities 2-5 (Pluriparous)			Higher than 5 parities sows (Aged sows)			Total
	No. of sows studied	No. of sows realeasing bacteria	Rate (%)	No. of sows studied	No. of sows realeasing bacteria	Rate (%)	No. of sows studied	No. of sows realeasing bacteria	Rate (%)	
Two weeks before farrowing	12	3	25	28	14	50	24	4	16.6	21/64 (32.8%)
One week after farrowing (7 to 14 days)	13	5	38.4	31	16	51.6	25	5	20	26/69 (37.6%)
Two weeks after farrowing (14 to 21 days)	11	2	18.1	29	18	62	22	5	22.7	25/62 (40.3%)
One week after weaning (21 to 28 days)	14	2	14.2	35	12	34.2	21	5	23.8	19/70 (27.1%)

Table 3 shows that Release of *Salmonella*in sows according to the stages of two weeks before farrowing, one week after farrowing, (piglets of 14 to 21 days), one week after weaning (piglets of21 to 28 days). The details are as follows:

Two weeks before farrowing, the release of *Salmonella* was 25.0 %in one-parity sows; 50.0 %in sows in parities 2-5; and 16.6 % in higher-than-five-parity sows (In total in one week before farrowing sows the release of *Salmonella* accounted for32.8 %).

One week after farrowing, the release of *Salmonella* was 38.4%in one-parity sows; 51.6 %in sows in parities 2-5; 20.0 %in higher-than-five-parity sows (In total, in one week after farrowing sows the release of *Salmonella* accounted for 37.6 %).

Two weeks after farrowing, the release of *Salmonella* was18.1%in one-parity sows; 62.0 %in sows in parities 2-5;22.7 %in higher-than-five-parity sows(In total, in two weeks after farrowing sows, the release of *Salmonella* accounted for40.3 %).

One week after weaning, the release of *Salmonella* was14,2 % in one-parity sows; 34.2 % in sows in parities 2-5;23.8 % in higher-than-five-parity sows(In total, in one week after weaning sows, the release of *Salmonella* accounted for27.1 %).

With P<0.05, it can be said that the differences between the stages of sows in the rates of Salmonella release are statistically significant. The results are similar to those of Tran TP, et al. on the release of Salmonella from pigs, chicken and ducks in a study in Mekong delta, Vietnam; and Chiara F. Magistrali et al., 2011 on the release of *Salmonella* from groups of sows in Italy, which showed that *Salmonella* release accounted for 33.3 %of one-parity sows(in primiparous), 28.8 % of sows in parities 2-5 (in pluriparous), and 4.6 % of higher-than-five-parity sows(aged sows) [5,27].

Release of *Salmonella*in Sows by Season

Due to the climate characteristics of Hiep Hoa district, Bac Giang, which belongs to northern mountaineous region of Vietnam, with four distinct seasons, faces samples were collected from sows in eight communes and district towns, including Thang district town (T) and the communes of Duc Thang (DT), Ngoc Son (NS), Danh Thang (DT), Bac Ly (BL), Dong Lo (DL), Luong Phong (LP) and Mai Trung (MT) in order to determine the release of *Salmonella* by season. The results are presented in Table 4.

Table 4: Release of *Salmonella*in sows by season

Study site	Spring (Feb-Apr)			Summer (May-Jul)			Thu (Aug-Oct)			Winter (Nov-Jan)		
	Sows examined	Sows releasing the bacteria	Rate (%)	Sows examined	Sows releasing the bacteria	Rate (%)	Sows examined	Sows releasing the bacteria	Rate (%)	Sows examined	Sows releasing the bacteria	Rate (%)
T	8	5	62.5	6	3	50	9	3	33.3	8	1	12.5
DT	9	4	44.4	9	3	33.3	8	2	25	7	0	0
NS	11	6	54.5	9	4	44.4	10	4	40	9	1	11.1
DT	8	5	62.5	6	2	33.3	8	2	25	8	2	25
BL	7	5	71.4	7	4	57.1	8	3	37.5	8	1	12.5
DL	8	5	62.5	6	3	50	9	2	22.2	9	2	22.2
LP	10	4	40	9	3	33.3	8	2	25	9	1	11.1
MT	8	3	37.5	8	3	37.5	9	3	33.3	9	0	0

Table 4 shows that there were differences in the release of *Salmonella*in sowsby seasons: spring (Feb-Apr); summer (May-Jul); autumn (Aug-Oct); winter (Nov-Jan) with higher rates in spring and summer, and lower rates in autumn and winter. The details are as follows:

In spring, the release of *Salmonella* from sows waslo west with 37.5 % (MT), and the highest rate was 71.4 % (BL); in summer, the release of *Salmonella* showed some signs of decreasing yet not clear (P>0.05), The lowest release rate was 33.3% (LP, DT, DT), and the highest rate was 57.1 % (BL); autumn rate of release of *Salmonella*in sows decreased remarkably (P<0.05), The lowest rate of release was 22.5 % (DL), the highest rate of release was 40.0 % (NS); in winter Release of *Salmonella* continued to decrease, similarly, autumn (P<0.05), The lowest rate of release was 0 % (MT, DT), the highest rate of release was 25.0 % (DT).

The results were in line with those of Wendy Wilkins, et al. in Alberta and Saskatchewan, Canada on pig samples found positive with *Salmonella*: the ones found positive with *Salmonella* accounted for 36 %, of which *Salmonella* isolates from sows accounted for 43 %, from weaners accounted for 29 %; in finishing pigs, the rate of *Salmonella* release accounted for 28 %; the rates of *Salmonella* isolated from farm environment ranged from 1% to 79 % [32].

Release of Salmonella in Small Pigs

Faces samples were collected from post weaning pigs to determine the release of *Salmonella* in the faces before and after diarrhea suspected of contracting Salmonellosis. The results are presented in Table 5.

Table 5 shows that there are significant differences (P < 0.05) in the release of *Salmonella* in healthy pigs and pigs with diarrhea suspected of contracting Salmonellosisin both before and after weaning. The details are as follows:

Before weaning (7 to 14 days): The lowest rate of release of *Salmonella*in healthy pigs (with no symptom of diarrhea) were from 2.0 % (LP) to the highest rate of 3.9 % (DT). Three communes were found with no positive sample to *Salmonella* (MT, NS and DL). The total rate of release of *Salmonella* in healthy pigs in this stage was 6/357, accounting for 1.68 %; The lowest rate of positive samples to *Salmonella* in pigs with diarrhea was from 25.0 % (Pigs with diarrhea in DL) to 33.3 % (Pigs with diarrhea in DT), Pigs with diarrhea in the remaining communes were found negative to *Salmonella* (MT, LP, BL, DT, NS, T). The total rate of release of *Salmonella* in this stage in pigs with diarrhea was 2/22, accounting for 9.0 %. The isolation of *Salmonella* from pigs with diarrhea symptoms in this stage was in line with the

Table5: Release of *Salmonella*in before and post weaning pigs

Study site	Before weaning (7-14 days)						After weaning (21-35 days)					
	Healthy pigs			Pigs with diarrhea			Healthy pigs			Pigs with diarrhea		
	Sows examined	Sows releasing the bacteria	Rate (%)	Sows examined	Sows releasing the bacteria	Rate (%)	Sows examined	Sows releasing the bacteria	Rate (%)	Sows examined	Sows releasing the bacteria	Rate (%)
T	42	1	2.3	2	0	0	42	5	11.9	3	3	100
DT	51	2	3.9	3	1	33.3	51	3	5.8	3	3	100
NS	39	0	0	2	0	0	39	4	10.2	2	2	100
DT	36	1	2.7	3	0	0	36	5	13.8	4	4	100
BL	44	1	2.2	3	0	0	44	4	9	3	3	100
DL	49	0	0	4	1	25	49	4	8.1	4	4	100
LP	50	1	2	2	0	0	50	6	12	3	3	100
MT	46	0	0	3	0	0	46	5	10.8	3	3	100

characteristics of Salmonellosis in pigs, which usually only occur in post weaning pigs, and with the characteristics of Colibacillosis in pigs before weaning, which are usually caused by E. coli [11,25].

Post weaning pigs (21 to 35 days): the lowest rate of release of *Salmonella* in healthy pigs was 5.8 % (DT), and the highest rate was 13.8 % (DT). The total rate of release of *Salmonella* in healthy pigs in this stage was 36/357, accounting for 10.0 % ; the rate of release of *Salmonella* in small pigs with diarrhea was 100 %. Thus, the results were in line with those of Wendy Wilkins, et al.; Tran TP, et al.; Chiara F. Magistrali, et al.; and Pires AF, et al.; Li Bai, et al. on the release of *Salmonella* from pigs in several farms all over the world [5,12,13,20,27,32].

Differences in the Total Count of *Salmonella* released in The Faces in Post Weaning Pigs

Table 5 shows that face samples were collected from post weaning pigs, both healthy ones and ones with diarrhea due to Salmonellosis to determine the differences in the total count of *Salmonella* pathogens released in one gram of faces. This is to determine the total count of *Salmonella* released and to determine the role of *Salmonella* in the causing Salmonellosisin post weaning pigs in Hiep Hoa district, Bac Giang province. The results are presented in Table 6.

Table 6: The differences in total count of Salmonella released from post weaning pigs

Study site	Healthy pigs			Pigs with diarrhea (Salmonellosis)		
	Samples examined	Positive samples	Salmonella in 1 gr of faces	Samples examined	Positive samples	Salmonella in 1 gr of faces
T	42	5	0.32×10^6	3	3	0.65×10^9
DT	51	3	0.93×1^{06}	3	3	0.29×10^9
NS	39	4	0.28×10^6	2	2	0.34×10^9
DT	36	5	0.51×10^6	4	4	0.46×10^9
BL	44	4	0.83×10^6	3	3	0.18×10^9
DL	49	4	0.68×10^6	4	4	0.23×10^9
LP	50	6	0.39×10^6	3	3	0.56×10^9
MT	46	5	0.62×10^6	3	3	0.75×10^9

Table 6 shows that: The number of *Salmonella* released from pigs with Salmonellosis was from 0.18 x 109 CFU/Gram of faces to 0.75 x 109 CFU/Gram of faces, higher than the number of *Salmonella* released from healthy pigs, which was from 0.28 x 106 CFU/Gram of faces to 0.93 x 106 CFU/Gram of faces ($P < 0.05$). Thus, the number of *Salmonella* released from pigs with diarrhea was higher than that from healthy pigs(from 0.28 x 106CFU/Gr to 0.93 x 106) and pigs with diarrhea(from 0.23 x 109CFU/Gr to 0.75 x 109CFU/Gr), which was similar to the results of the experiment by Nicole C. Burdick Sanchez et al., 2017 on determining the release of *Salmonella* in the faces 24h, 48h, and 72h after the pigs were infected with *Salmonella* (from 3.9 x 109CFU/Grto 4.1 x 109CFU/Gr), and similar to the results by Pires AF, et al., 2013 on the release of *Salmonella* in finishing pigs [17,20].

In addition, it can be seen that the numbers of *Salmonella* released in the faces of post weaning pigs with diarrhea in our research are nearly similar to those used for infecting post

weaning pigs of Walsh. MC , et al.;Nicole C. Burdick Sanchez, et al. when post weaning pigs were infected by drinking soups with the bacteria with the doses of 1010 CFU/pig and 4.7x109 CFU/pig with the strain of *Salmonella* typhimurium [17,31].

Prevalence of Salmonellosisin post weaning pigs

Epidemic Characteristics of Salmonellosis in Post Weaning Pigs

A survey was conducted to determine the prevalence of Salmonellosisin pigs from post weaning (21 days) to finishing (4 months) stages. The results are presented in Table 7. From Table 7, it can be seen that post weaning pigs with Salmonellosis accounting for from 5.1 % (Ngoc Son) to11.1 %(Danh Thang); Mortality rate were from 25.0 %(Danh Thang) to 33.3 %(Luong Phong and Thang district town), in other communes (Duc Thang, Ngoc Son, Bac Ly, Dong Lo, Mai Trung) the mortality rate of pigs with Salmonellosis was 0%. The results were similar to those of Steven A. Carlson, et al. on diseases caused by *Salmonella* on post weaning pigs [25].

Table 7: Prevalence of Salmonellosis in post weaning pigs

Study site	No. of pigs studied	No. of pigs with Salmonellosis	Rate of pigs with Salmonellosis (%)	No. of dead pigs	Mortality rate (%)
TT Thang	42	3	7.1	1	33.3
Duc Thang	51	3	5.8	0	0
Ngoc Son	39	2	5.1	0	0
Danh Thang	36	4	11.1	1	25
Bac Ly	44	3	6.8	0	0
Dong Lo	49	4	8.1	0	0
Luong Phong	50	3	6	1	33.3
Mai Trung	46	3	6.5	0	0

Isolation of *Salmonella* from Medical Waste of Post Weaning Pigs with Diarrhea

Medical waste samples were collected from post weaning pigs with Salmonellosis (liver, kidney, heart blood, intestine nodes, small and large intestine fluid, with 30 samples each) to isolate *Salmonella*. The results are presented in Table 8.

Table 8 : Isolation of Salmonella from medical waste of post weaning pigs with Salmonellosis

Types of sample	Number	No. of positive samples	Rate (%)
Liver	30	16	53.3
Kidney	30	18	60
Heart blood	30	22	73.3
Intestine nodes	30	20	66.6
Small intestine fluid	30	30	100
Large intestine fluid	30	30	100
Diarrhea faces	30	30	100
Total	**210**	**166**	**79**

Table 8 shows that the rate of *Salmonella* isolated was from 53.3 % (liver samples) to 60.0 % (kidney samples), 66.6% (intestine nodes), and 73.3 % (heart blood samples). From the samples of small and large intestines, and diarrhea faces, the rate of *Salmonella* isolates was 100 %. Similar to Table 6, the results were similar to those of Steven A. Carlson, et al. on infection ability of *Salmonella*, and of Nicole C. Burdick Sanchez, et al. on the rate of Salmonella isolates in medical waste samples of liver, spleen, kidney, large intestine, intestine nodes, and faces after the pigs were infected with the disease [16,17,18,25].

Testing for Biological and Chemical Characteristics of Salmonella Isolates

A test for biological and chemical characteristics was performed on the *Salmonella* strains isolated (91 strains). The results are presented in Table 9.

Table9: Biological and chemical characteristicsof Salmonella isolates

Tests onBiological and chemical characteristics	Results		
	No. of strains tested	Positive	Rate (%)
Grow and multiply inRappaport-Vassiliadis environment at 42oC	166	166	100
Gram negative stain	166	166	100
Mobility	166	58	34.9
Hemolysis on blood agar	166	0	0
Lactose fermentation	166	0	0
Production of H_2S	166	166	100

Table 9 shows that *Salmonella* isolates processed typical biological and chemical characteristics of the genus; 100 % of the strains isolated grew and multiply well in Rappaport-Vassiliadis environment at 42^0c; Gram-negative stain (stained of safranin red of Gram dye); 34.9 % have mobility; did not cause hemolysis in blood agar environment; there were no lactose fermentation;

100 % of the strains tested produced H_2S. The results were in line with those of Quinn P J, et al.; Steven A. Carlson, et al. on biological characteristics of *Salmonella* causing disease on pigs and food poisoning on human [21,22,23,24,25].

Virulence of *Salmonella* Isolates

Virulence of *Salmonella* isolates was tested on healthy tested mice (Specific Pathogen Free). The results are presented in Table10.

Table10: Virulence of Salmonella isolates

Sources	Strains	Mice tested	Dose of abnormal injection	No. of dead mice after infection (a)					Dead rate (%)
			(ml/mouse)	8 hours	24 hours	32 hours	48 hours	6 days	
Liver	16	32	0.2	18	23	32		32	100
Kidney	18	36	0.2	12	26	36		36	100
Heart blood	22	44	0.2	19	28	35	43	43	97.7
Intestine nodes	20	40	0.2	19	28	40		40	100
Small intestine fluid	30	60	0.2	36	48	55	56	56	93.3
Large intestine fluid	30	60	0.2	34	43	51	53	53	88.3
Diarhea faces	30	60	0.2	38	53	56	51	51	85

From Table 10, it can be seen that *Salmonella* isolates were highly virulent on tested mice. Sau 48 hours after being infected with the bacteria, the tested mice died of the strains isolated from diarrhea faces; large intestine fluid, small intestine fluid, heart blood, and from intestine nodes, kidney and liver were 85.0 %, 88.3 %, 93.3 %, 97.7 %, and 100 % respectively. From the dead mice, *Salmonella* was again isolated from the similar sources of medical waste samples.

Determination of the serotypes of *Salmonella* isolates

Determination of the serotypes of 166 *Salmonella* strain isolates was performed with quick agglutination reactionon glass slides using O, H antigen kit (H anstigens phase 1, H anstigens phase 2) from Bio-Rad Laboratories, Inc. The results are presented in Table11.

Table 11: Serotype of Salmonella isolates from medical waste of pigs with Salmonellosis

Serotype	Sources of Salmonellaisolation							Total
	Liver	Kidney	Heart blood	Intestine nodes	Small intestine fluid	Large intestine fluid	Diarrhea faces	
Salmonellaanatum	1	-	-	2	-	-	2	5
Salmonella choleraesuis	3	2	1	9	11	9	6	41
Salmonella enteritidis	1	-	-	-	3	2	3	9
Salmonelladublin	-	2	1	-	-	-	-	3
Salmonellaheidelberg	-	-	-	2	1	1	2	6
Salmonella typhimurium	2	1	3	5	8	6	5	30
Salmonellasenftenberg	1	-	-	2	-	-	2	5
Salmonella weltevreden	1	1	-	-	-	-	-	2
Salmonellaunknown	2	1	1	-	2	2	2	10
Total	**11**	**7**	**6**	**20**	**25**	**20**	**22**	**111**

From Table 11 it can be seen that there were 2 strains found positive with serotype *Salmonella* weltevreden; 3 strains with*Salmonella* dublin; 5 strains with serotype *Salmonella*anatum and *Salmonella* senftenberg; 6 strains with *Salmonella* heidelberg; 9 strains with *Salmonella* enteritidis; the highest number belonged to 2 serotypes of *Salmonella* typhimurium (30 strains), and*Salmonella* choleraesuis (41 strains); and for the unknown *Salmonella* group, there were 10 strains.

Our results were similar to those of Tran TP, et al. on the rate of serotype *Salmonella* weltevreden determined yet different in the number of serotype *Salmonella* heidelberg (6/166); similar to the results of Patchanee P, et al. on serotype *Salmonella* heidelberg, Wendy Wilkins, et al. on the rate of serotype *Salmonella* typhimurium, Uzzau S, et al. Sylvie Côté, et al. and Steven A. Carlson, et al. on the rates of the serotypes *Salmonella* dublin, *Salmonella* choleraesuis, *Salmonella* enteritidis, *Salmonella*dublin, *Salmonella*anatum, and several unknown*Salmonella* strains of the serotypes [19,25,26,27,28, 29,32].

Determination of the Virulenceof *Salmonella* Isolates

PCR reaction was perfomed in order to determine the DNA with theencoded geneproducing virulence factors including Stnenterotoxin, fimA adherence, InvA invasion of 111 *Salmonella* strain isolatesfrom medical waste ofpost weaning pigswith Salmonellosis. The results are presented in Table 12.

Table 12: Frequency of occurence of the encoded gene producing Stn, fimA, InvA pathogens ofSalmonella Causing Salmonellosisin post weaning pigs

Serotype Salmonella isolates	Strains tested	Frequecy occurance of virulence gene					
		Stn		fimA		InvA	
		Strainsbearing encoded gene	Rate %	Strains bearing encoded gene	Rate %	Strains bearing encoded gene	Rate %
Salmonellaanatum	5	4	80	4	80	2	40
Salmonella choleraesuis	41	38	92.6	38	92.6	16	39
Salmonella enteritidis	9	8	88.8	8	88.8	6	66.6
Salmonelladublin	3	2	66.6	1	33.3	1	33.3
Salmonellaheidelberg	6	4	66.6	4	66.6	2	33.3
Salmonella typhimurium	30	22	73.3	20	66.6	8	26.6
Salmonella senftenberg	5	4	80	4	80	2	40
Salmonella weltevreden	2	1	50	1	50	1	50
Salmonellaunknown	10	8	80	6	60	3	30
Total	**111**	**91**	**81.9**	**86**	**77.4**	**41**	**36,9**

Table 12 shows that the DNA bearingencoded geneStn enterotoxin, fimA adherence, andInvA invasionwere found in all the serotype Salmonellaisolates. The details are as follows:

Stn enterotoxin: *Salmonella* weltevredenwith the DNA bearing the gene producing Stn accounted for 50.0 %; *Salmonella* dublin and *Salmonella* heidelberg accounted for 66.6 %; *Salmonella* typhimuriumaccounted for 73.3 %; *Salmonella* senftenberg, *Salmonella* anatum, *Salmonella unknown* accounted for 80.0 %; serotype *Salmonella* enteritidis accounted for 88.8 %; and serotype *Salmonella* choleraesuis with the DNA bearing the gene producing Stn accounted for 92.6 %.In total, the rate of *Salmonella* with the encoded gene of Stn enterotoxin accounted for 81.9 % (Figure 1).

Fim A adherence: *Salmonella*dublinbearing the encoded gene of fim A adherence accounted for the lowest rate of 33.3 %; *Salmonella* weltevreden accounted for 50.0 %; Unknown *Salmonella* accounted for 60.0 %; *Salmonella* typhimurium and *Salmonella* Heidelberg accounted for 66.6 %; *Salmonella* senftenbergand*Salmonella*anatumaccounted for 80.0 %;*Salmonella* enteritidisaccounted for 88.8 %; and serotype

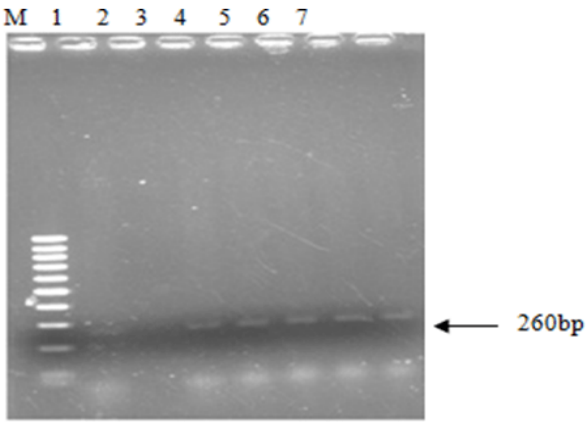

Figure 1: Agarose gel electrophoresis of PCR amplification products using specific Stnenterotoxingene (Stn primers) of Salmonella spp. sisolated. Lane M: 100 bp ladder as molecular DNA marker (M: Marker 100 bp, Fermentas, USA), Lane 1: Control positive, Lane 2: Negative Salmonella spp.for Stn production; Lane 3, Lane 4, Lane 5, Lane 6, and Lane 7: Positive Salmonella spp. for Stn production.

Salmonella choleraesuiscarrying the DNA with the encoded gene of fim A adherence accounted for 92.6 %. In total, the rate of the *Salmonella* strains carrying the encoded gene of fim A adherence accounted for 77.4 %.

InvA invasion: *Salmonella* typhimuriumbearing the encoded gene of Inv A invasion accounted for 26.6 %; *Salmonella* unknown accounted for 30.0 %; *Salmonella* heidelberg and *Salmonella* dublinaccounted for 33.3 %; *Salmonella* choleraesuisaccounted for 39.0 %; Salmonella anatum and Salmonella senftenberg accounted for 40.0 %;Salmonella weltevredenaccounted for 50.0 %; and serotype Salmonella enteritidis with the DNA bearing the encoded gene of Inv A invasion accounted for 66.6 %.

Thus, in comparison with the reports of Chaudhary. J. H,et al. on the ability of producing Stn, fim A, InvA of Salmonella typhimurium and Salmonella enteritidis isolated from pork and slaughterhouses inAhmedabad, Gujarat, our results were similar qualitively yet lower in term of the rate of occurrences of the DNA bearing the encoded gene (our rates are presented above, compated to all the rates of 100 % from Chadhary's report); and our results were also lower than those found by Arunava Das et al., 2012 in a study on the virulence of *Salmonella* isolated from port, beef, and poultry meat in Tamil Nadu, India (in Arunava Das's report, the rates found were: Stn (100 %), and InvA (100 %), yet there were similarity in the rate of adherence production (plasmid encoded fimbriae pefA, accounted for 51.42 %) [1,2,3] (Figure 2).

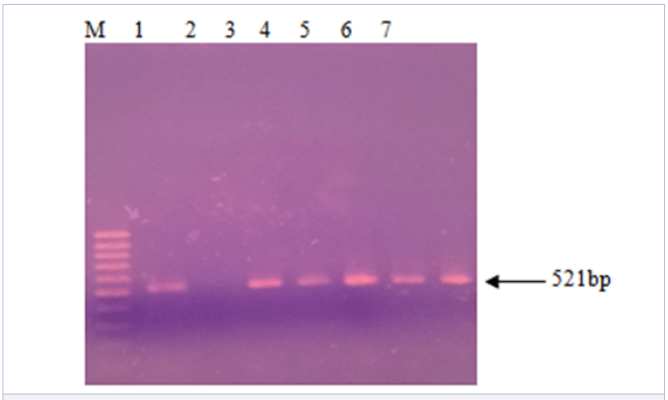

Figure 2: Agarose gel electrophoresis of PCR amplification products using specific Invasion gene (InvA primers) of Salmonella spp. isolated. Lane M: 100 bp ladder as molecular DNA marker (M: Marker 100 bp, Fermentas, USA), Lane 1: Control positive, Lane 2: Negative Salmonella spp.for InvA production; Lane 3, Lane 4, Lane 5, Lane 6, and Lane 7: Positive Salmonella spp. for InvA production.

Table 13 shows that *Salmonella* isolates from post weaning pigs with Salmonellosis had symptoms of resistance to common antibiotics with different scales by types of antibiotics. The results were similar to those of Quinn P J, et al.; Cheng-Hsun Chiu, et al.; Patchanee P, et al.; Steven A. Carlson, et al. and several other authors [4,19,21,25]. The details are as follows:

Serotype *Salmonella* choleraesuis was found with 1/41strains resisting to nalidixic acid (2.4 %); 3/41 strains resisting to ciprofloxacin, rifampicin, spectinomycin (7.3 %); 4/41 strains resisting toceftazidime, oxytetracycline (9.7 %); 5/41 strains resisting to nitrofurantoin (12.1%); 8/41 strains resisting totrimethoprim-sulfamethoxazole (19.5 %); and 9/41 strains resisting to kanamycin (21.9 %).

Table 13: Antibiotic resistance of Salmonella isolates

Antibiotics used	Salmonella choleraesuis			Salmonella enteritidis			Salmonella typhimurium			Salmonella heidelberg		
	No. of strains tested	No. of strains resisted	Rate (%)	No. of strains tested	No. of strains resisted	Rate (%)	No. of strains tested	No. of strains resisted	Rate (%)	No. of strains tested	No. of strains resisted	Rate (%)
Nitrofurantoin	41	5	12.1	9	1	11.1	30	1	3.3	6	0	0
Trimethoprim-sulfamethoxazole	41	8	19.5	9	2	22.2	30	5	16.6	6	1	16.6
Ciprofloxacin	41	3	7.3	9	1	11.1	30	2	6.6	6	0	0
Ceftazidime	41	4	9.7	9	1	11.1	30	1	3.3	6	0	0
Kanamycin	41	9	21.9	9	2	22.2	30	6	20	6	2	33.3
Rifampicin	41	3	7.3	9	1	11.1	30	2	6.6	6	0	0
Nalidixic acid	41	1	2.4	9	0	0	30	1	3.3	6	0	0
Oxytetracycline	41	4	9.7	9	0	0	30	0	0	6	0	0
Spectinomycin	41	3	7.3	9	1	11.1	30	2	6.6	6	1	16.6

Serotype *Salmonella* enteritidis was found with no strains resisting to strains resisting to nalidixic acid and oxytetracycline (0 %); 1/9 strains resisting to ciprofloxacin, rifampicin, ceftazidime, spectinomycinandnitrofurantoin (11.1%); 2 and/9 strains resisting to trimethoprim-sulfamethoxazole and kanamycin (22.2 %).

Serotype *Salmonella* typhimurium was found with no strains resisting to strains resisting to oxytetracycline (0 %); 1/30 strains resisting to nitrofurantoin, nalidixic acid andceftazidime (3.3 %); 2/30 strains resisting to ciprofloxacin, spectinomycin and rifampicin (6.6 %); 5/30 strains resisting totrimethoprim-sulfamethoxazole (16.6 %); and6/30 strains resisting to kanamycin (20.0 %).

Serotype *Salmonella* heidelberg was found with no strains resisting to strains resisting to rifampicin, nalidixic acid, oxytetracycline, ceftazidime, ciprofloxacin and nitrofurantoin (0 %); 2/6 strains resisting to kanamycin (33.3 %); 1/6 strains resisting totrimethoprim-sulfamethoxazole, spectinomycin (16.6 %).

Thus, our results were similar to those of Li Bai, et al. in a recent study on antibiotic resistance of the serotypes of *Salmonella* typhimurium, *Salmonella* derby and *Salmonella* enteritidis isolated from chicken and pigs in slaughterhouses, with the rate of strains resisting to antibiotics as follows: ciprofloxacin from 8.6 % to 10.0 %, cefotaxime from 5.5 % to 8.6 % [13]. Similarly, David M Onyango, et al. also revealed that the serotype *Salmonella* choleraesuis strains isolated from pigs resisted to kanamycin (80.0 %), spectinomycin (31.6 %), sulfamethoxazole-trimethoprim (32.6 %), cephalothin (7.4 %), ofloxacin (24.2 %), and ciprofloxacin and norfloxacin (21.1 %) [6].

Conclusion

The release of *Salmonella*in breeder sows occurred in all the studied breeder sow farms in Hiep Hoa district, Bac Giang, Việt Nam (100 %); with the lowest rate of 26.4 %, and the highest rate of 43.3 %. The average rate of the release of *Salmonella* from breeder sows to the environment was 34.3 %.

The release of *Salmonella* from breeder sows varied by time and parities:

At the time of two weeks before farrowing, the release of *Salmonella* was 25.0 %in one-parity sows; 50.0 % in sows in parities 2-5and16.6 % in higher-than-five-parity sows(In total, in breeder sows two weeks before farrowing the release of *Salmonella* accounted for 32.8 %).

One week after farrowing (piglets from7 to 14 days old), the release of *Salmonella* was 38.4 %in one-parity sows; 51.6 % in sows in parities 2-5 and 20.0 % in higher-than-five-parity sows(In total, in breeder sows one week after farrowing the release rate of *Salmonella* was 37.6 %).

Two weeks after farrowing (pigsfrom14 đến 21 days old), the release of *Salmonella* was 18.1 % in one-parity sows; 62.0 % in sows in parities 2-5 and 22.7 % in higher-than-five-parity sows(In total, in breeder sows two weeks after farrowing the release rate of *Salmonella* accounted for 40.3 %).

One week after weaning (pigs from 21 to 28 days old), the release of *Salmonella* was 14.2 % in one-parity sows; 34.2 % in sows in parities 2-5; 23.8 % in higher-than-five-parity sows (In total, breeder sows one week after weaning the release rate of *Salmonella* was 27.1 %).

In spring, the release of *Salmonella* from sows was from 37.5 % to 71.4 %; in summer from 33.3 % to57.1 %; in autumn from22.5 % to 40.0 %; in winter from 0 %, and the highest rate of release was 25.0 %.

In before weaning piglets, for those without diarrhea, the rate of *Salmonella* occurred in the faces was from2.0 % to3.9 %, and the average rate was 1.68 %; for those with diarrhea, the rate of *Salmonella* release found was from 25.0 % to 33.3 %, and the average rate was 9.0 %.

In post weaning pigs, for the healthy ones, the rate of *Salmonella* release was from 5.8 % to13.8 %, and the average rate was 10.0 %; the rate of *Salmonella* release found in those with diarrhea was 100 %.

In pigs with Salmonellosis, the number of *Salmonella* release increased from 0.18 x 109 CFU/Gram of faces to 0.75 x 109 CFU/Gram of faces, higher than the rate of *Salmonella* released from healthy pigs (which showed no symptom of the disease), which was only from 0.28 x 106 CFU/Gram of faces to 0.93 x 106 CFU/Gram of faces.

Post weaning pigs found with Salmonellosis accounted for from 5.1 % to11.1 %; and the mortality rate were from 25.0 % to33.3 %.

The rates of *Salmonella* isolates were 53.3 % (liver samples), 60.0 % (kidney samples), 66.6 % (intestine nodes), 73.3 % (heart blood samples), and in the samples of small intestine fluid, large intestine fluid, and diarrhea faces the rates of *Salmonella* isolates were 100 %. *Salmonella* isolatesbore the typical biological and chemical characteristics of their genus; 100 % of the strains isolatedgrew well in Rappaport-Vassiliadis environment at 42^0C; were Gram negative stained; 34.9 % had mobility; did not cause hemolysis in blood agar environment; had no lactose fermentation; and 100 % of the tested strains produced H_2S.

Salmonella isolates were highly virulent on tested mice. At the time of 48 hours after being infected, 85.0 % of the tested mice died of the strains isolated from diarrhea faces; 88.3 % died of the *Salmonella* strains isolated from large intestine fluid; 93.3 % died of *Salmonella* strains isolated from small intestine fluid; 97.7 % died of *Salmonella* strain isolated from heart blood; and 100% died *Salmonella* strain isolated from intestine nodes, kidney, and liver.

Out of the 166 *Salmonella* bacteria strains isolates, the number of serotypes identified included 2 *Salmonella* weltevreden, 3 *Salmonella* Dublin, 5 *Salmonella* anatumand Salmonella senftenberg, 6 Salmonellaheidelberg, 9 *Salmonella* enteritidis, 30 Salmonella typhimurium, 41 *Salmonella* choleraesuis, and 10 unknown *Salmonella* serotype.

Salmonella weltevredenwhich bore the encoded gene Stn accounted for 50.0 %; SalmonelladublinandSalmonella heidelberg accounted for 66.6 %; Salmonella typhimuriumaccounted for 73.3 %;Salmonella senftenberg, Salmonella anatum,and *Salmonella* unknown accounted for 80.0 %; *Salmonella* enteritidisaccounted for 88.8 %; *Salmonella* choleraesuiscarrying the DNA bearing the gene producing Stn accounted for 92.6 %. In total, the rate of *Salmonella* strains carrying the encoded gene Stn enterotoxin accounted for 81.9 %. *Salmonella* Dublin bearing the gene fim A accounted for 33.3 %; *Salmonella* weltevreden accounted for 50.0 %; *Salmonella* unknown accounted for 60.0 %; *Salmonella* typhimurium and *salmonella* Heidelberg accounted for 66.6 %; Salmonella senftenberg and Salmonella anatum accounted for 80.0

%; Salmonella enteritidis accounted for 88.8 %; and Salmonella choleraesuis accounted for 92.6 %. Salmonella typhimurium bearing the gene InvA accounted for 26.6 %; Salmonella unknown accounted for 30.0 %; Salmonella heidelberg and Salmonella Dublin accounted for 33.3% ; Salmonella choleraesuis accounted for 39.0 %; *Salmonella* anatum and *Salmonella* senftenberg accounted for 40.0 %; *Salmonella* weltevreden accounted for 50.0 % ;and Salmonella enteritidis accounted for 66.6 %.Out of the 41 strains tested, *Salmonella* choleraesuis had 1 strain resisting to nalidixicacid (2.4 %); 3strains resisting to ciprofloxacin, rifampicin, and spectinomycin (7. 3 %); 4strains resisting toceftazidime and oxytetracycline (9.7 %); 5strains resisting to nitrofurantoin (12.1 %); 8strains resisting totrimethoprim-sulfamethoxazole (19.5 %); and9strains resisting to kanamycin (21.9 %).

Out of the 9 strains tested, *Salmonella* enteritidis was found with no strains resisting to nalidixic acid and oxytetracycline; 1 strain resisting to ciprofloxacin, rifampicin, ceftazidime, spectinomycinandnitrofurantoin (11.1%); and 2 strains resisting totrimethoprim-sulfamethoxazole and kanamycin (22.2 %).

Out of the 30 strains tested, serotype *Salmonella* typhimurium was found with no strains resisting to oxytetracycline; 1strain is resisting to nitrofurantoin, nalidixic acid and ceftazidime (3.3 %); 2strains resisting to ciprofloxacin, spectinomycin and rifampicin (6.6 %); 5strains resisting totrimethoprim-sulfamethoxazole (16.6 %); and6strains resisting to kanamycin (20.0 %).

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