PREVALENCE AND VIRULENCE GENE OF *Salmonella* STRAINS ISOLATED FROM DUCKS IN NORTH VIETNAM

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Abstract

In order to determine the prevalence of *Salmonella* infection and Salmonellosis on ducks, feces and cloacal swap samples were collected from 3,700 ducks in Thai Nguyen, Bac Giang, Bac Ninh, Lang Son and Tuyen Quang provinces in 2016 and 2018. The results show that: Diseases caused by *Salmonella* in ducks were found in 100% of the farms and 11.51% of the flocks. Of all *Salmonella* bacteria studied, 6.63% belonged to serotype *Salmonella enteritidis*, 14.79% were *Salmonella typhimurium*, 11.73% were *Salmonella anatum*, 7.65% were *Salmonella montevideo*, 10.20% were *Salmonella heidelberg*, 8.67% were *Salmonella indiana*, 7.65% were *Salmonella orion*, 10.20% were *Salmonella senftenberg*, and *Salmonella wetevreden* accounted for 7.65%. DNA encoded genes enterotoxin (*Stn*), adherence (*fimA*), and invasion (*InvA*) were identified; *Salmonella enteritidis*, 92.30%, 69.23%, and 53.84%; *Salmonella typhimurium*, 89.65%, 72.41%, and 65.51%; *Salmonella anatum*, 82.60%, 69.56%, and 56.52%; *Salmonella montevideo*, 86.66%, 60.00%, and 46.66%; *Salmonella indiana*, 88.23%, 58.82%, and 35.29%; *Salmonella orion*, 80.00%, 53.33%, and 33.33%; *Salmonella senftenberg*, 95.00%, 60.00%, and 45.00%; and *Salmonella wetevreden*: 86.66%, 40.00%, and 26.66% respectively. *Salmonella* isolates were susceptible to nalidixic acid, ceftazidime, ciprofloxacin, rifampicin, spectinomycin, trimethoprim-sulfamethoxazole, and nitrofurantoin. Of which, the antibiotics with the strongest antimicrobial resistance from *Salmonella* isolates were kanamycin, colistin, and neomycin.

Keywords: **Duck, *Salmonella*, virulence gene, antibiotic**

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Introduction

Salmonellosis in ducks is an infected disease caused by *Salmonella* bacteria. Ducks of all age groups could be infected with the disease. Accute diseases are often found in those under three weeks of age, which can cause a high dead rate of up to 60% (Pan *et al*., 2010; Adzitey *et al*., 2012). Chronic diseases are often found in fully grown up ducks, bearing and discharging pathogens to the environment. Main *Salmonella* infection routes are through food, water, or from mother ducks to the eggs and thus the ducklings. The diseases caused decrease of hatching rate, slow down growth rate, while increase infection of other diseases. Therefore, ducks and duck eggs are causes of *Salmonella* infection in human, causing Diarrhea and food poisoning, which negatively affect the well being of those infected (Nor Faiza *et al*., 2013; Owen *et al*., 2016).

Epidemiological study on Salmonellosis and virulence of the isolated *Salmonella* in domesticated ducks in North Vietnam (Thai Nguyen, Bac Giang, Bac Ninh, Lang Son and Tuyen Quang provinces) would provide further information about epidemiological features, characteristics of the sources and pathogenicity of the disease. It is hoped that the information would pave the way for new researches on more effective dianosis and treatment of the disease, as well as effective prevention of *Salmonella* discharge to the environment, causing diseases to other animals and food poisons on human.

Materials and Methods

Materials

Common and screening media for culturing, islolating, selecting, and examining *Salmonella* bacterial. The suspected *Salmonella* samples were placed on Mueller Hinton Agar (Oxoid) and incubated at 37°C for 18-24h. The colonies were subjected to biochemical reactions using Enteric API 20E according to manufactures' instructions (BioMerieux, France) for further confirmation. Serotyping was done with the 113 of *Salmonella* strains were found from the clinical samples of infected ducks, 44 *Salmonella* strains were found from the samples of ducks which showed no symptoms (using cloacal swaps and feacal samples), and 19 *Salmonella* strains were found from duck egg samples by using slide agglutination with *Salmonella* polyvalent A, B, C, T, Vi antisera (Bio-Rad Laboratories, Inc. Oxoid *Salmonella* Test Kit) according to the Kauffmann-White classification scheme.

Anti-biotic resistance kit was used for determine the serotype of *Salmonella* isolates. All the isolates were also tested for susceptibility to 11 different antimicrobial agents using the disk diffusion method on Mueller Hinton agar following the European Committee on Antimicrobial Susceptibility Instructions (EUCAST). All the strains were subjected to a set of 11 antibiotics to study their antibiogram by using Bauer–Kirby disk diffusion method.

Laboratory machinary and equipment, including a PCR System 9700 (Applied Biosystem, the USA), a Powerpac 300 (Bio-Rad, the USA), a DNA Mini transilluminator (Bio-Rad, the USA), a camera (Amersham Pharmacia Biotech, Swidden), a Vortex (Mimishaker, IKA, Germany), a Speed - Vac 110A (Savant, the USA), a centrifuge, a surge generator (Gen Pulser), and other equipment.

Methods

Collect feacal samples and cloacal swap and egg tampons as described by Adzitey *et al*. (2013) and Owen *et al*. (2016); Identifiy *Salmonella* bacteria according to ISO 6579 (2017); Part 1: Detection of *Salmonella* spp.; Operate on infected ducks to collect clinical samples for isolation; Examine biological and chemical characteristics; Conduct tests on levels of virulence and anti-biotic and chemical resistance of *Salmonella* bacteria as described by Quinn *et al*. (2002); Wallace *et al*. (2016) - Bacteriological Analytical Manual (Chapter 5, *Salmonella*).

Use PCR to determine the encoded genes of *stn* (encodes *Salmonella* enterotoxin and is specific for *Salmonella* enterica), and

invA (invasion protein, for simultaneous identification of *Salmonella* at the genus level) as described by Lee *et al*. (2009); determine the *fimA* virulence encoded gene (major subunit of type 1 fimbriae) of *Salmonella* bacteria isolated as described by Chaudhary *et al*. (2015). SAS 9.3.1 statistical software was used for bioligical statistics processing.

Results and Discussion

Diseases Caused by *Salmonella* in Ducks

Table 1 presents the results of the survey on prevalence of diseases caused by *Salmonella* in ducks raised at sixteen (16) farms in Thai Nguyen (4 farms), Bac Giang (3 farms), Bac Ninh (3 farms), Lang Son (3 farms) and Tuyen Quang (3 farms).

The results show that diseases caused by *Salmonella* were found in all of the farms studied (16/16 farms, or 100%). The average rate of infected ducks in the flocks was 11.51%, ranging from 10.33% (Lang Son province) to 13.77% (Bac Giang province). The results were higher than those in the study by Tsai and Hsiang (2005) in Taiwan, with 4.6% (91/2,000 ducks) by flocks and 20% by farms, and lower than those in the studies by Nor Faiza *et al*. (2013) in Malaysia, with the total infection rate of 16.00%, and by Dey *et al*. (2016) in Bangladesh with 38.1% of the ducks studied found infected with diseases caused by Samonella.

Salmonella Prevalence in Ducks

Table 2 presents the results of Samonella prevalence study on samples of feacal and cloacal swap of 560 healthy ducks (which showed no symptoms of diseases) belonging to four varieties; Co ducks, Beijing ducks, Laying ducks, and Muscovy ducks.

The results show that there were differences between ducks and local musk ducks in *Salmonella* infection rate by variety ($P<0.05$). However, the difference of infection rates between feacal and cloacal swap samples are not clear ($P>0.05$). Follows are the rates of *Salmonella* infection in ducks by variety and types of samples: Co ducks: 10.71% (cloacal

Table 1. Diseases caused by *Samonella* in ducks by individual and flocks

Place of study	Farms			Flocks		
	No. of flocks	No. of flocks with infected ducks	Rate (%)	No. of ducks	No. of ducks infected with the diseases	Rate (%)
Thai Nguyen	4	4	100	800	95	11.87
Bac Giang	3	3	100	900	124	13.77
Bac Ninh	3	3	100	800	89	11.12
Lang Son	3	3	100	600	62	10.33
Tuyen Quang	3	3	100	500	56	11.20
Total	**16**	**16**	**100**	**3700**	**426**	**11.51**

Table 2. *Samonella* prevalence in ducks

Variety	No. of ducks	No. of duck feacal samples		No. of cloacal swab samples	
		No. of positive samples	Rate (%)	No. of positive samples	Rate (%)
Co ducks	140	18	12.85	15	10.71
Beijing ducks	140	29	20.71	25	17.85
Laying ducks	140	30	21.42	26	18.57
Muscovy ducks	140	6	4.28	2	1.42
Total	**560**	**83**	**14.82**	**74**	**13.21**

Table 3. *Salmonella* prevalence in duck eggs

Variety	No. of samples	No. of samples infected with *Salmonella*	Infection rate (%)
Co ducks	120	9	7.50
Beijing ducks	120	15	12.50
Laying ducks	120	11	9.16
Muscovy ducks	120	0	0
Total	**480**	**39**	**8.12**

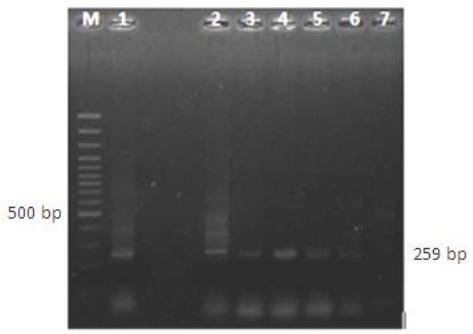

Figure 1. Agarose gel electrophoresis of PCR amplification products using specific *Stn* enterotoxin gene (*Stn* primers) of *Salmonella* spp. isolated. Lane M: 100 bp ladder as molecular DNA marker (M: Marker 100 bp, Fermentas, USA), Lane 1: Control positive, Lane 7: Negative *Salmonella* spp. for Stn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

swap samples) and 12.85% (feacal samples); Beijing ducks: 17.85% (cloacal swap samples) and 20.71 (feacal samples); Laying ducks: 18.57% (cloacal swap samples) and 21.42% (feacal samples); and Muscovy ducks: 4.28% (feacal samples), and 1.42% from cloacal swap samples.

Salmonella Prevalence of Duck Eggs

Table 3 presents the results of *Salmonella* prevalence study in the duck eggs with composite samples of six egg-tampon each as described by Owen *et al*. (2016).

The results show that the eggs produced by Co ducks, Beijing ducks, and Laying ducks are infected with *Salmonella* with different levels of infection. The rates of Salmonella infection in the eggs studied, ranging from highest to lowest level of infection, were as follows: Beijing ducks: 12.50%, Laying ducks: 9.16%, and Co ducks: 7.50%. None of the 120 samples of the eggs produced by Muscovy ducks was found possitive with *Salmonella* bacteria.

The rates of *Salmonella* infection in eggs produced by Co ducks, Beijing ducks, and Laying ducks in our study were higher than the figure of 1.4% infection in a similar study by Owen *et al*. (2016) in England. Nor Faiza *et al*. (2013) in Malaysia stated that although *Salmonella* infection rate in ducks raised in industrial farms was as high as 16%, non of the egg samples was found possitive with *Salmonella*, which is the same as our results on local musk duck eggs in this study.

Determination of the Serotype of *Salmonella* Isolates

Table 4 presents the results of determination of the serotype of *Salmonella* isolates with rapid agglutination reaction on glass plates, using the O and H standard antigen Kit (H antigens phase 1, and H antigens phase 2) of Bio-Rad Laboratories, Inc (Oxoid *Salmonella* Test Kit). The number of Salmonella strains found were: 113 from the clinical samples of infected ducks, 44 from the samples of ducks (cloacal swaps and feacal samples), and 19 from duck egg samples.

The results show that nine subspecies and one group of unknown serotypes of *Salmonella* were identified, with the following number of strains from the total of 196 found, and the corresponding rate; *Salmonella enteritidis* 13 (6.63%), *Salmonella typhimurium*;

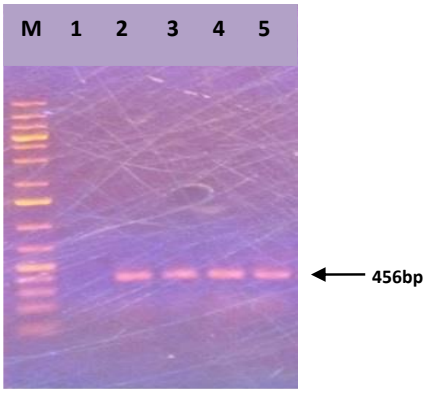

Figure 2. Agarose gel electrophoresis of PCR amplification products using specific invasion gene (*invA* primers) of *Salmonella* isolated. Lane M: 100 bp ladder as molecular DNA marker, Lane 1: Negative Salmonella for *invA* production, Lane 2: Control positive, Lane 3, Lane 4 and lane 5: Positive *Salmonella* for *invA* production

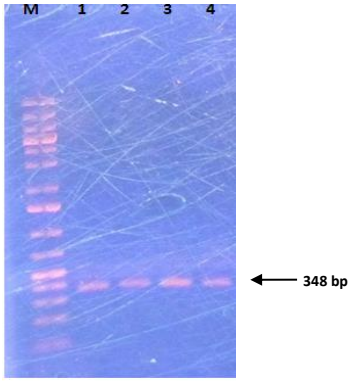

Figure 3. Agarose gel electrophoresis of PCR amplification products using specific fimbriae gene (*fimA* primers) of *Salmonella* isolated. Lane M: 100 bp ladder as molecular DNA marker, Lane 1: Control positive, Lane 2, Lane 3 and Lane 4: Positive *Salmonella* for fimbriae production

Table 4. Serotype of *Salmonella* isolates

Species	Salmonella isolates from clinical samples of infected ducks	Salmonella in ducks with no symptoms			Total
		Eggs	Cloacal swaps	Feacal	
Salmonella enteritidis	9	1	0	3	13
Salmonella typhimurium	18	3	2	6	29
Salmonella anatum	15	3	1	4	23
Salmonella montevideo	10	0	2	3	15
Salmonella heidelberg	14	2	1	3	20
Salmonella indiana	12	2	0	3	17
Salmonella orion	10	2	0	3	15
Salmonella senftenberg	12	3	1	4	20
Salmonella wetevreden	8	2	1	4	15
Unknown Salmonella	5	1	1	2	9
Total	**113**	**19**	**9**	**35**	**176**

29 (14.79%), *Salmonella anatum*; 23 (11.73%), *Salmonella montevideo*; 15 (7.65%), *Salmonella heidelberg*; 20 (10.20%), *Salmonella indiana*; 17 (8.67%), *Salmonella orion*; 15 (7.65%), *Salmonella senftenberg*; 20 (10.20%), *Salmonella wetevreden*; 15 (7.65%), and the unknown *Salmonella*; 9 (4.59%).

Thus, in comparison with other studies, our results are similar to those on *Salmonella* disease causing in ducks. For example, in a study by Tsai and Hsiang (2005) in Taiwan, the proportions of Salmonella strains found were as follow; *Salmonella indiana*, 14.3%; *Salmonella typhimurium*, 7.7%; and *Salmonella montevideo*, 2.2%. In a study by Adzitey *et al*. (2012) in Penang (Malaysia), the rates of *Salmonella* serotype found in ducks were: *Salmonella* typhimirium, 29.6%; and *Salmonella*

Table 5. Primer pairs used for virulence characterization of *Salmonella* isolates

Genes	Function of gene	Sequence of nucleotides	Size (bp)	Annealing temp (°C)	References
stn	Encodes *Salmonella* enterotoxin and is specific for *Salmonella* enterica	F-CGATCCCTTTCCCGCTATC R-GGCGAATGAGACGCTTAAG	259	55	[1, 2, 4, 11, 12]
invA	invasion protein, for simultaneous identification of *Salmonella* at the genus level	F-ACAGTGCTCGTTTACGACCTGAAT R-AGACGACTGGTACTGATCGATAAT	456	63	[1, 2, 4, 11, 12]
fimA	major subunit of type 1 fimbriae	F-CCTTTCTCCATCGTCCTGAA R-TGGTGTTATCTGCCTGACCA	348	56	[1, 2, 4, 11, 12]

Table 6. Frequency of the encoded genes producing *Stn*, *fimA*, and *InvA* pathogens of *Salmonella* isolates

Serotype Salmonella isolates	No. of strains	Frequency of virulence genes					
		Stn		*fimA*		*InvA*	
		No. of strains with the encoded genes	Rate (%)	No. of strains with the encoded genes	Rate (%)	No. of strains with the encoded genes	Rate (%)
S. enteritidis	13	12	92.30	9	69.23	7	53.84
S. typhimurium	29	26	89.65	21	72.41	19	65.51
S. anatum	23	19	82.60	16	69.56	13	56.52
S. montevideo	15	13	86.66	9	60.00	7	46.66
S. heidelberg	20	18	90.00	11	55.00	7	35.00
S. indiana	17	15	88.23	10	58.82	6	35.29
S. orion	15	12	80.00	8	53.33	5	33.33
S. senftenberg	20	19	95.00	12	60.00	9	45.00
S. wetevreden	15	13	86.66	6	40.00	4	26.66

enteritidis, 12.0%; In a study by Flament *et al*. (2012) in ducks in Belgium, 11 serotypes of Salmonella were identified, of which, Salmonella *indiana* accounted for 42.1%, *Salmonella typhimurium* and *Salmonella enteritidis* accounted for 1.1%; Cha *et al*. (2013) discovered that serotype *Salmonella typhimurium* rate was 45.88% (39/85), *Salmonella enteritidis* rate was 51.76% (44/85), and serotype Salmonella london rate was 2.35% (2/85); In Vietnam, a study on duck in the Mekong River Delta in the South of Vietnam by Tran *et al*. (2004) aslo identified serotypes *Salmonella* javiana and *Salmonella wetevreden*.

Determination of Virulence Gene of Salmonella Isolates

The primer pairs described in Table 5 (Primer pairs used for virulence characterization of *Salmonella* isolates) were used to determine virulence gene of *Salmonella* isolates. The results were presented in Table 6.

Table 6 presents the results of determination of the DNA bearing the encoded genes which produce pathogens including enterotoxin *Stn*, adherence *fimA*, and invasion *InvA*, of *Salmonella* isolates, using PCR reaction.

The results show that *Salmonella* isolates from clinical, egg, feacal, and cloacal swap samples from the ducks and local musk ducks bore the virulence encoded gene (enterotoxin *Stn*) and non-toxic factors (adherence gene *fimA*, and invasion gene *InvA*). The proportion of genes bearing enterotoxin gene *Stn*, adherence gene *fimA*, and invasion gene *InvA* of each type of *Salmonella* isolates were; *Salmonella enteritidis*, 92.30%, 69.23%, and 53.84%; *Salmonella typhimurium*, 89.65%, 72.41%, and 65.51%; *Salmonella anatum*, 82.60%, 69.56%, and 56.52%; *Salmonella*

montevideo, 86.66%, 60.00%, and 46.66%; *Salmonella heidelberg*, 90.00%, 55.00%, and 35.00%; *Salmonella indiana*, 88.23%, 58.82%, and 35.29%; *Salmonella orion*, 80.00%, 53.33%, and 33.33%; *Salmonella senftenberg*, 95.00%, 60.00%, and 45.00%; and *Salmonella wetevreden*: 86.66%, 40.00%, and 26.66% respectively.

Thus, this finding was similar to that of Arunava Das *et al*. (2012); Chaudhary *et al*. (2015); Aviv *et al*. (2017); El-Sebay *et al*. (2017); Singh *et al*. (2017), and in comparison with the reports of Chaudhary *et al*. (2015) on the ability of producing *Stn*, *fimA*, *InvA* of isolated from pork and duck, our results were similar qualitively yet lower in term of the rate of occurance of the DNA bearing the encoded gene (our rates are presented above, compated to all the rates of 100% from Chaudhary's report); and our results were also lower than those found by Arunava Das *et al*. (2012) in a study (from Arunava Das's report) 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%).

Determination of Resistance of *Salmonella* Isolates to Selected Antibiotics and Chemicals

Table 7 presents the results of determnation of susceptibility to selected antibiotics of selected serotype *Salmonella* isolates, including *Salmonella anatum*: 23 strains; *Salmonella enteritidis*: 13 strains; and *Salmonella typhimurium*: 29 strains. The results show the number of strains of *Salmonella* isolates resistant to antibiotics as follows:

Of the 23 strains of *Salmonella anatum* examined: 1 was resistant to oxytetracycline, ceftazidime, and trimethoprim-sulfamethoxazole (4.34%); 2 were resistant to nitrofurantoin, ciprofloxacin, spectinomycin, and rifampicin (8.68%); 3 were resistant to neomycin, and colistin (13.04%); 4 were resistant to kanamycin (17.39%); and none was found resistant to nalidixic acid.

Of the 13 strains of *Salmonella enteritidis* examined: 1 were resistant to trimethoprim-sulfamethoxazole, ceftazidime, and rifampicin (7.69%); 2 were resistant to nitrofurantoin, ciprofloxacin, rifampicin, and spectinomycin (15.38%); 3 were resistant to kanamycin, and neomycin (23.07%); and none was found resistant to nalidixic acid.

Of the 29 strains of *Salmonella typhimurium* examined: 2 were resistant to ciprofloxacin, ceftazidime, and oxytetracycline (6.89%); 3 were resistant to nitrofurantoin, trimethoprim-sulfamethoxazole, rifampicin, and spectinomycin (10.34%); 4 were resistant to colistin (13.79%); 5 were resistant to neomycin (17.24%); 6 were resistant to kanamycin (20.68 %); and none was found resistant to nalidixic acid.

Thus, the results on antibiotic resistance of serotype *Salmonella* above were line with

Table 7. Resistance of Salmonella isolates to selected antibiotics and chemicals

Antibiotics	*Salmonella anatum*			*Salmonella enteritidis*			*Salmonella typhimurium*		
	No. of strains tested	No. of strains resistant to antibiotics	Rate (%)	No. of strains tested	No. of strains resistant to antibiotics	Rate (%)	No. of strains tested	No. of strains resistant to antibiotics	R (
Nitrofurantoin	23	2	8.68	13	2	15.38	29	3	1
Trimethoprim-sulfamethoxazole	23	1	4.34	13	1	7.69	29	3	1
Neomycin	23	3	13.04	13	3	23.07	29	5	1
Colistin	23	3	13.04	13	2	15.38	29	4	1
Ciprofloxacin	23	2	8.68	13	2	15.38	29	2	6
Ceftazidime	23	1	4.34	13	1	7.69	29	2	6
Kanamycin	23	4	17.39	13	3	23.07	29	6	2
Rifampicin	23	2	8.68	13	1	7.69	29	3	1
Nalidixic acid	23	0	0	13	0	0	29	0	
Oxytetracycline	23	1	4.34	13	2	15.38	29	2	6
Spectinomycin	23	2	8.68	13	2	15.38	29	3	1

those claimed by Dey *et al*. (2016); Lâm *et al*. (2000); and Patchanee *et al*. (2008) on susceptibility and antibiotic resistance of *Salmonella* causing diseases or infected in ducks in several regions and countries all over the world.

Conclusions

Diseases caused by *Salmonella* in ducks (ducks and Muscovy ducks) were found in 100% of the farms and in 11.51% of the flocks.

Salmonella infection rates in feacal samples (14.82%) and cloacal swap samples (13.21%), and there were differences between ducks and local musk ducks in *Salmonella* infection rate by variety ($P<0.05$), but the difference of infection rates between feacal and cloacal swap samples are not clear ($P>0.05$); living on land (Muscovy duck) infection rate of *Salmonella* is lower (from 1.42% to 4.28%) than that of duck in water (Co ducks, Beijing ducks, and Laying ducks), from 10.71% to 21.42%.

Salmonella infection rates in eggs were 12.50% with Beijing ducks, 9.16% with Laying ducks, and 7.50% with Co ducks; and eggs of Muscovy ducks was negative with *Salmonella* bacteria.

Nine subspecies of *Salmonella* were identified; serotype *Salmonella enteritidis* accounted for 6.63%; *Salmonella typhimurium* (14.79%); *Salmonella anatum* (11.73%); *Salmonella montevideo* (7.65%); *Salmonella heidelberg* (10.20%); *Salmonella indiana* (8.67%); *Salmonella orion* (7.65%); *Salmonella senftenberg* (10.20%); and *Salmonella wetevreden* (7.65%).

The proportion of genes bearing enterotoxin gene *Stn*, adherence gene *fimA*, and invasion gene *InvA* of each type of *Salmonella* isolates were; *Salmonella enteritidis*, 92.30%, 69.23%, and 53.84%; *Salmonella typhimurium*, 89.65%, 72.41%, and 65.51%; *Salmonella anatum*, 82.60%, 69.56%, and 56.52%; *Salmonella montevideo*, 86.66%, 60.00%, and 46.66%; *Salmonella heidelberg*, 90.00%, 55.00%, and 35.00%; *Salmonella indiana*, 88.23%, 58.82%, and 35.29%; *Salmonella orion*, 80.00%, 53.33%, and 33.33%; *Salmonella senftenberg*, 95.00%, 60.00%, and 45.00%; and *Salmonella wetevreden*: 86.66%, 40.00%, and 26.66% respectively.

Salmonella isolales were highly susceptive to nalidixic acid, ceftazidime, ciprofloxacin, rifampicin, spectinomycin, trimethoprim-sulfamethoxazole, and nitrofurantoin; resitant to these antibiotics at different levels, with the highest level of resistance to kanamycin, colistin, and neomycin.

Due to the differences in behaviors and living conditions, the ratio of *Salmonella* infection on Muscovy ducks (living on land) is lower than that of ducks living in water such as Co ducks, Beijing ducks, and Laying ducks ($P<0.05$).

Salmonella isolated from cloacal swab, feces, and eggs samples with possiblilities of bearing disease causing factors such as virulence gene *Stn*, *fimA* adherence, and invasion protein *InvA*; 4 serotypes *Salmonella anatum*, *Salmonella enteritidis*, and *Salmonella typhimurium* which were highly resistant to common anitbiotics were shown to have disease causing ability. This may cause food born diseases to both humans and animals.

Treatment to Animals

In this study, the experiments related to animals strictly followed the Laws of Veterinary Medicine of Vietnam issued in 2015 (Article 21, Treatment to animals), which are in line with international standards on Animal welfare.

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