

Phenotypic and Genotypic Characterization of Paratyphoid Salmonellae isolated from Poultry in Delta Area- Egypt

Mohamed A. Lebdah¹, Amal A.M. Eid¹, Soad A. Nasef² and Enas M. Hamad^{3*}

¹Avian and Rabbit Medicine Department, Faculty of Veterinary Medicine, Zagazig University, 44511, Egypt

²Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Dokki, Giza, Egypt

³Animal Health Research Institute, Mansoura Provincial Lab, Egypt

Article History: Received: 8/5/2017 Received in revised form: 25/8/2017 Accepted: 8/9/2017

Abstract

The present work aimed to isolate and characterize Salmonellae from chickens, ducks, quails and turkeys in five Egyptian Governorates. Polymerase chain reaction (PCR) was used for the detection of common virulence genes. A total of 265 flock samples (150 chickens, 60 ducks, 30 quails and 25 turkeys) were collected from Dakahlia, Kafrelsheik, Damietta, Sharkia and Gharbia Governorates. Birds were subjected to either clinical and/or post-mortem examination, in addition to isolation and identification of salmonellae from internal organs including liver, lung, spleen, caecum and unabsorbed yolk sac. Biochemical and serological identification of the isolates was done. Twenty eight birds (10.6%) were found positive for Salmonella isolation. The number and percentage of positive chickens, ducks, quails and turkeys were 16 (10.7%), 7 (11.7%), 3 (10%) and 2 (8%), respectively. *Salmonella* Typhimurium, *S. Enteritidis*, *S. Kentucky*, *S. Paratyphi A*, *S. Molade*, *S. Heidelberg*, *S. Infantis* and *S. Apeyeme* were isolated from chickens. While *S. Enteritidis*, *S. Typhimurium*, *S. Paratyphi A*, *S. Kentucky*, *S. Inganda* and *S. Bargny* were isolated from ducks. While, *S. Virchow*, *S. Tamale* and *S. Typhimurium* were isolated from Quails and *S. Wingrove*, finally, *S. Kentucky* were isolated from turkeys. Molecular characterization of common virulence genes Salmonella outer proteins (*sopB*), Plasmid encoded virulence gene (*spvC*), salmonella enterotoxin (*stn*) and bacterial colonization factor (*bcfC*) showed the presence of *stn* and *bcfC* genes in all isolates, while, *sopB* and *Spv* genes were present in 64.3% and 10.7%, respectively. It is concluded that salmonellae with common virulence genes were widely spread among domestic birds in Delta areas, Egypt, resulting in economic and public health problems which require the application of strictly biosecurity measures in poultry rearing.

Keywords: *Salmonella* spp., Poultry, Delta, Virulence Genes

Introduction

Paratyphoid (PT) infection is an infectious disease of all domestic and wild birds. More than 2400 serotypes of salmonellae were recognized, and they can infect humans and different animals [1]. Paratyphoid infection is bacterial disease causing high economic losses among avian species. Paratyphoid salmonellae are Gram-negative; non-spore forming, non capsulating and motile by means of peritrichous flagellae [2]. The clinical findings of paratyphoid infection in all species of young birds are similar and include a progressive state of somnolence manifested by a tendency to keep head downward, close eyes with droopy wings, ruffled feathers, marked anorexia, increased water consumption, profuse watery

diarrhea with pasting of the vent and a tendency of birds to huddle together near to the source of heat [3-5]. The dead birds in acute state show septicemia, while, those dying later show necrotic foci in the liver and/or heart, enlargement of gall bladder and unabsorbed yolk sac [6]. However, in severe outbreaks of PT infection in newly hatched birds, rapidly developing septicemia can cause a high rate of mortality with few or no apparent lesions. When the course of the disease is longer, severe enteritis is often accompanied by focal necrotic lesions in the mucosa of the small intestine. Cheesy cecal cores are often observed, spleens and livers are commonly swollen and congested with hemorrhagic streaks or necrotic foci, while, kidneys may sometimes be enlarged and

*Corresponding author email: (enashamad77@gmail.com), Animal Health Research Institute, Mansoura Provincial Lab, Egypt.

congested [7]. Fibrinopurulent perihepatitis and pericarditis have been reported on numerous occasions, In addition, unabsorbed, coagulated yolk sac and other lesions occasionally are observed including hypopyon, panophthalmitis, purulent arthritis, serous typhilitis, air sacculitis and omphalitis [7].

The gold standard method for *Salmonella* detection is bacteriological culture, while, serotyping can also be used [8]. *S. Enteritidis* and *S. Typhimurium* are the most common serotypes isolated from poultry [9]. Differences in virulence among *Salmonella* serovars and in the course of *Salmonella* infections in various host species have been attributed to the variable acquisition and evolution of virulence genes [10]. *Salmonella* species contain upwards of sixty virulence genes and have three antigenic (H, O, and Vi) determinants [11].

The fimbrial gene (*bcfC*) is located on a fimbrial structure and has a role in cell invasion [12]. Moreover, *Salmonella* outer proteins (*Sop*) has a role in the invasion of the bacteria through deformation of membranes and rearrangement of the host cells' cytoskeleton [13,14]. *Spv* is related to survival and growth of the bacterium in host cells [15]. The salmonella enterotoxin (*stn*) gene encodes Stn protein, causing gastroenteritis with symptoms that include nausea, vomiting, abdominal pain, fever, and diarrhea [16]. The present study aimed to isolate salmonellae from birds in Delta area of Egypt and to determine the occurrence of PT in different poultry species. Moreover, detection of common virulence genes among isolated salmonellae was carried out using PCR.

Material and Methods

Sample collection

A total of 265 flock samples of diseased and freshly dead chickens, ducks, quails and turkeys were collected from Dakahlia, Damietta, Gharbia, Kafrelsheik and Sharkia Governorates. Birds were subjected to either clinical and/or post-mortem examination for the isolation and identification of paratyphoid salmonellae from internal organs including liver, lung, spleen, caecum and unabsorbed yolk sac. The internal organs of 150 chickens, 60 ducks, 30 quails, 25 turkeys were collected under aseptic condition as possible to prevent cross contamination in ice box and were then transferred to the laboratory.

Isolation of *Salmonella* species

It was done according to ISO 6579 [17]. The tested sample was initially inoculated into a non-inhibitory liquid medium to favor the repair and growth of stressed or sublethally-injured salmonellae arising from exposure to heat, freezing, desiccation, high osmotic pressure or wide temperature fluctuations.

Samples were weighed and suspended in Buffered Peptone water as 1:10 dilution and then incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 18 ± 2 h. From the pre-enrichment culture, 0.1 mL of the broth was transferred to a tube containing 10 mL of the Rappaport Vassiliadis broth and then incubated at $41.5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 ± 2 h. From the enrichment culture, 10 μL were inoculated onto the surface of Xylose Lysine Deoxycholate (XLD), Hektoen Enteric (HE) and MacConkey's plates, separately, then incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$ for 24 ± 2 h. The plates were then checked for the growth of typical *Salmonella* colonies.

Biochemical identification

Hydrolysis of urea, H_2S production, TSI and Simmon's Citrate agar were done according to ISO 6579 [17]. Isolated strains were inoculated on to Christensen's urea agar slant and incubated at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$, then examined after four hours. If there was no change it was left for 24 h at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$. A Simmon's citrate agar slopes were inoculated as a single streak on the surface with the tested isolates and incubated at 37°C for 48 h.

Serological typing of paratyphoid salmonellae

The isolates that were identified biochemically as *Salmonella* were subjected to serological identification according to Kauffman-White Scheme [18] for determination of somatic (O) and flagellar (H) antigens [19].

Molecular identification of virulence genes

All the isolates were examined by PCR for the presence of four virulence associated genes [20]. The genes under investigation were *Salmonella* outer protein B (*sopB*), *Salmonella* plasmid virulence (*spvC*), *Salmonella* enterotoxin encoding gene (*stn*) and bacterial colonization factor encoding gene (*bcfC*). The primers sequences' and PCR product sizes are shown in (Table 1) [12,21].

Table 1: Sequences of the used oligonucleotide primers for identification of virulence genes among *Salmonellae* in poultry

Genes	Specificity/ location	Sequence of nucleotides (5'-3')	Amplified product (bp)
<i>Stn</i>	Enterotoxin/ chromosomal	F- TTGTGTCGCTATCACTGGCAACC R-ATTCGTAACCCGCTCTCGTCC	617
<i>sopB</i>	Effector protein/ SPI-5	F-TCAGAAGTCGTCTAACCCTC R-TACCGTCCTCATGCACACTC	517
<i>befC</i>	Fimbrial usher protein/ chromosome	F-ACCAGAGACATTGCCTTCC R-TTCTGCTCGCCGCTATTCC	467
<i>spvC</i>	Plasmid encoded virulence gene/ plasmid	F-ACCAGAGACATTGCCTTCC R-TTCTGATCGCCGCTATTCC	467

Results

The clinical signs of the examined birds were retarded growth, depression, lameness, ruffled feathers, chicks huddling together, respiratory troubles, whitish watery diarrhea and accumulation of faecal matter around the vent (Figure 1: A, B). The postmortem examination of both freshly dead and

sacrificed birds revealed gross lesion in the form of septicemia, bronze discoloration enlarged liver with necrotic foci, splenomegaly with necrotic foci, pericarditis, enlarged heart, peritonitis, congested kidneys, inflammation of intestine and caecum and unabsorbed yolk sac in young birds (Figure 1: C,D,E,F,G,H).

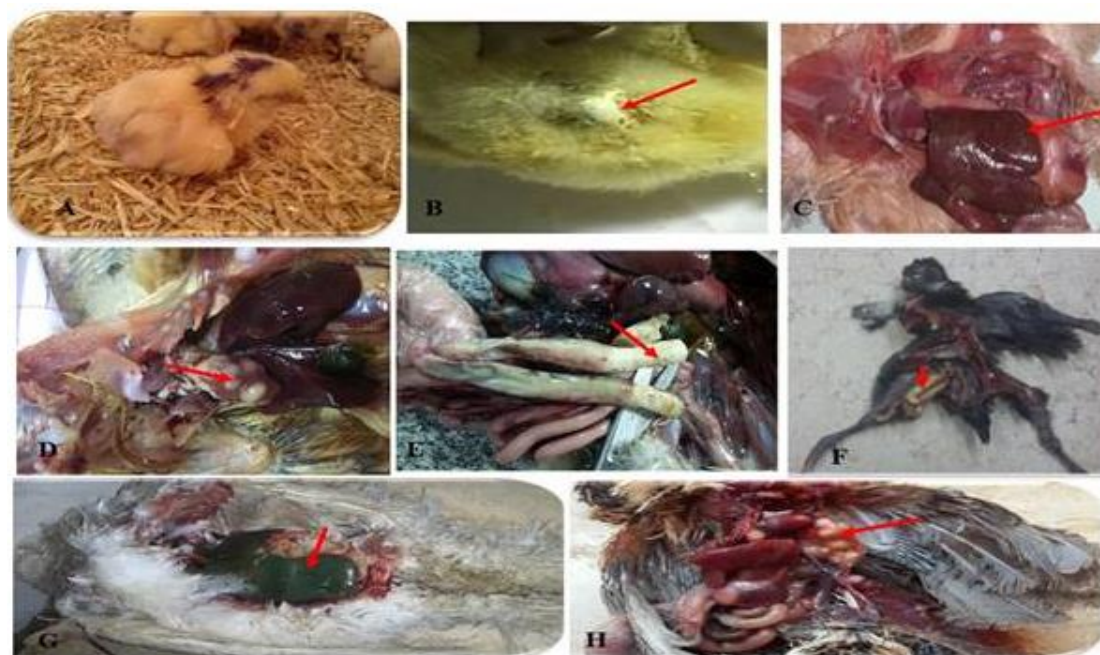


Figure 1: Signs and PM lesions of examined birds. A: Three days-old chick was sleepy with droopy wings. B: Diseased birds with pasty vent and whitish diarrhea. C: Twelve days-old Saso chicken died showing necrotic foci in the congested liver. D: 16 days-old chick showing several nodules on the heart and congested liver. E: 30 days-old chick showing Cecal cores, enteritis and septicemia. F: Ten days-old turkey with septicemia and cecal cores. G: Twenty days-old duckling died showing bronzy liver. H: Twenty five days-old quail died showing septicemia and nodules in the lungs and the heart.

Out of 265 flock samples (150 chickens, 60 ducks, 30 quails and 25 turkeys), twenty eight birds (10.6%) were positive for Salmonella isolation. The number and percentage of positive chickens, ducks, quails and turkeys were 16 (10.7%), 7 (11.7%), 3 (10%) and 2 (8%), respectively (Table 2). Salmonella isolates were serotyped using poly and monovalent “O” and “H” antisera and the results revealed that 16 strains isolated from chickens from different Governorates comprised of 4 (25%) *S. Typhimurium*, 3 (18.8%) *S. Enteritidis*, 3 (18.8%) *S. Kentucky*, 2 (12.5%) *S. Paratyphi A*, 1 (6.25%) *S. Molade*, 1 (6.25%) *S. Heidelberg*, 1 (6.25%) *S. Infantis* and 1 (6.25%) *S. Apeyene*. Results of serotyping of 7 different salmonella strains from ducks showed 6 different serogroups

identified as *S. Enteritidis* (28.6%), *S. Typhimurium* (14.3%), *S. Paratyphi A* (14.3%), *S. Kentucky* (14.3%), *S. Inganda* (14.3%) and *S. Bargny* (14.3%). Serotyping of 3 different salmonellae from quails showed that 3 different serogroups were identified as *S. Virchow* (33.3%), *S. Tamale* (33.3%) and *S. Typhimurium* (33.3%). While, serotyping of 2 different salmonellae from turkeys showed 2 different serogroups identified as *S. wingrove* (50%) and *S. Kentucky* (50%).

Molecular characterization using PCR revealed *bcfC* and *stn* genes in 100% of Salmonella isolates while, *sopB* gene was detected in 18 (64.3%) isolates and *spvC* gene was detected in 3 isolates (10.7%) (Table 3 and Figure 2).

Table 2: The isolation rates of *Salmonella* serotypes from poultry flocks

Types of flocks	No of examined samples	Prevalence of <i>Salmonella</i> isolation				Isolated serotypes	
		Positive samples		Negative samples		serotype	Number (%)
		No	%	No	%		
Chickens	150	16	10.7	134	89.3	<i>S. Typhimurium</i>	4(25%)
						<i>S. Enteritidis</i>	3(18.8%)
						<i>S. Kentucky</i>	3(18.8%)
						<i>S. Paratyphi A</i>	2(12.5%)
						<i>S. Molade</i>	1(6.25%)
						<i>S. Heidelberg</i>	1(6.25%)
						<i>S. Infantis</i>	1(6.25%)
						<i>S. Apeyeme</i>	1(6.25%)
Ducks	60	7	11.7	53	88.3	<i>S. Enteritidis</i>	2(28.6%)
						<i>S. Typhimurium</i>	1(14.3%)
						<i>S. Paratyphi A</i>	1(14.3%)
						<i>S. Kentucky</i>	1(14.3%)
						<i>S. Inganda</i>	1(14.3%)
						<i>S. Bargny</i>	1(14.3%)
						<i>S. Virchow</i>	1(33.3%)
Quails	30	3	10	27	90	<i>S. Tamale</i>	1(33.3%)
						<i>S. Typhimurium</i>	1(33.3%)
						<i>S. Wingrove</i>	1(50%)
Turkeys	25	2	8	23	92	<i>S. Kentucky</i>	1(50%)
						Total n. of isolates	28
Total*	265	28	10.6	237	89.4		

* The percentage was calculated according to the total number of examined samples.

Discussion

The clinical signs of the examined birds were retarded growth, depression, lameness, ruffled feathers, chicks huddling together, respiratory troubles, whitish watery diarrhea and accumulation of faecal matter around the vent. The postmortem examination of both freshly dead and sacrificed birds revealed gross lesion in the form of septicemia, bronze

discolouration enlarged liver with necrotic foci, splenomegaly with necrotic foci, pericarditis, enlarged heart, peritonitis, congested kidneys, inflammation of intestine and caecum and unabsorbed yolk sac in young birds. Similar signs obtained by Gast and Beard [3] Shivaprasad *et al.* [4] and Gast and Beard [5] and similar postmortem lesions obtained by Hoop and Posuschil [7] Shalaby

and Abdel-Hamid [22] and Abd El-Nasser *et al.* [23]. The occurrence of *Salmonella* species was 10.6% from different poultry species. This is nearly similar to Taha [24] who isolated salmonellae from chicken with a percentage of 10% in Egypt, and Roy *et al.* [25] who isolated 11.99% *Salmonella* spp.) from poultry and poultry products. While, higher isolation rates were reported by Osman [26] who reported the isolation of *Salmonella* spp. (30%) from poultry

dropping from different broiler farms in Egypt. However, El-Zeedy *et al.* [27] reported lower isolation rate of *Salmonella* spp. from different poultry samples (4.1%) in Egypt. Such variation could be attributed to differences in environmental contamination, health control programs, management systems and/or the sensitivity of the procedure used in examination.

Table 3: Distribution of some virulence genes in the examined 28 *Salmonella* isolates among different poultry species

Code	Serovars	Source	<i>sopB</i>	<i>bcfC</i>	<i>spvC</i>	<i>stn</i>
1	<i>S. Kentucky</i>	chickens	+	+	-	+
2	<i>S. Molade</i>	chickens	+	+	-	+
3	<i>S. Typhimurium</i>	chickens	+	+	-	+
4	<i>S. Kentucky</i>	chickens	+	+	-	+
5	<i>S. Heidelberg</i>	chickens	+	+	-	+
6	<i>S. Enteritidis</i>	chickens	+	+	-	+
7	<i>S. Paratyphi A</i>	chickens	+	+	-	+
8	<i>S. Typhimurium</i>	chickens	+	+	-	+
9	<i>S. Typhimurium</i>	chickens	+	+	-	+
10	<i>S. Infantis</i>	chickens	+	+	-	+
11	<i>S. Enteritidis</i>	Chickens	+	+	-	+
12	<i>S. Typhimurium</i>	Chickens	+	+	-	+
13	<i>S. Kentucky</i>	Chickens	+	+	-	+
14	<i>S. Paratyphi A</i>	Chickens	+	+	-	+
15	<i>S. Apoyeme</i>	Chickens	+	+	-	+
16	<i>S. Enteritidis</i>	Chickens	+	+	-	+
17	<i>S. Typhimurium</i>	Ducks	+	+	-	+
18	<i>S. Paratyphi A</i>	Ducks	+	+	-	+
19	<i>S. Enteritidis</i>	Ducks	-	+	-	+
20	<i>S. Kentucky</i>	Ducks	-	+	-	+
21	<i>S. Inganda</i>	Ducks	-	+	-	+
22	<i>S. Bargny</i>	Ducks	-	+	+	+
23	<i>S. Enteritidis</i>	Ducks	-	+	-	+
24	<i>S. Virchow</i>	Quails	-	+	-	+
25	<i>S. Tamale</i>	Quails	-	+	+	+
26	<i>S. Typhimurium</i>	Quails	-	+	+	+
27	<i>S. Wingrove</i>	Turkeys	-	+	-	+
28	<i>S. Kentucky</i>	Turkeys	-	+	-	+
Total	28		18 (64.3%)	28 (100%)	3 (10.7%)*	28 (100%)

* The percentage was calculated according to the total number of identified serovars.

The number and percentages of positive chickens, ducks, quails and turkeys were 16 (10.7%), 7 (11.7%), 3 (10%) and 2 (8%), respectively. The highest percentage of *Salmonella* isolation was from ducks while the lowest percentage was from turkeys. The results of salmonella isolation from chickens (10.7%) in this study coordinated with El-Azzouny [28] who recorded a percentage of 10% in broilers and Rehan [29] who isolated

Salmonella spp. from 12% of broiler chickens. Lower percentages were previously reported by Sadoma [30] and Mohamed *et al.* [31] who isolated *Salmonella* from chicken farms in Gharbia and Kafr-Elsheikh with an overall prevalence of 2% and 2.5%, respectively. However, higher percentage was recorded by Osman [26] who collected 150 random samples from different broiler farms and isolated 45 *Salmonella* strains with the

percentage of 30%. The variation in the percentage of *Salmonella* detection among poultry could be attributed to different factors including management, biosecurity, as well as, prophylactic antibiotics used in each circumstance [19].

The results of salmonella isolation from ducks (11.7%) in this study coordinated with Abd El-Tawab *et al.* [32] who isolated *Salmonella* from ducks with the percentage of 9.6% and Hoszowski and Wasyl [33] who detected salmonella in ducks with percentage of 14.3%. Higher isolation rates were previously recorded by Osman *et al.* [26] who reported an isolation rate of *Salmonella* spp. from 18.5% of ducks. In addition, Ismail [34] reported the percentage of isolation from ducks was 27.02% .

The obtained results were nearly similar to those obtained by Abd El- Tawab *et al.* [32] who isolated *Salmonella* spp. from quails with the percentage of 10%, also, Palanisamy and Bamaiyi [35] reported *Salmonella* isolation from 11.11% of quails. However, the results were different than those reported in Iran, where, *Salmonella* isolation rate reached 40% as reported by Jalali *et al.* [36] and in Brazil reached 75% as reported by Neto *et al.* [37].

In the present study *Salmonella* spp. were isolated from turkeys with a percentage of 8%. This was nearly similar to 9.7% reported by Tel *et al.* [38] in fecal specimens and Alatifehy [39] who reported that *Salmonella* isolation rate was 6.25% in turkeys.

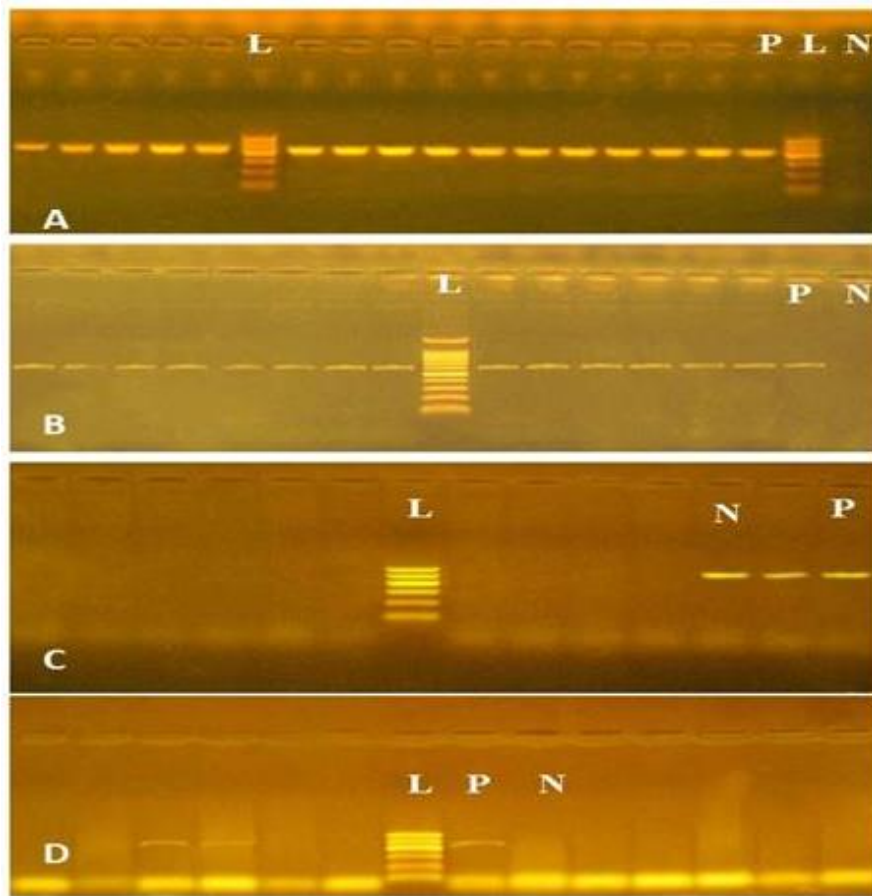


Figure 2: Agarose gel electrophoresis for amplified products of some virulence genes. A: PCR results for the *bcfC* gene showing positive amplification of 467 bp. B: PCR results for the *stn* gene showing positive amplification of 617 bp in all samples. C: PCR results for the *sopB* gene showing positive amplification of 517 bp. D: PCR results for the *spvC* gene showing positive amplification of 467 bp.

Salmonella isolates were serotyped using poly and monovalent "O" and "H" antisera and the result of this study revealed that 16 strains were isolated from chickens from different Governorates. The results in this study revealed that *S. Typhimurium* predominated other serotypes. These results agreed with Hoszowski *et al.* [9] who reported that *S. Enteritidis* and *S. Typhimurium* were the most common serotypes isolated from poultry. Whereas, Dahal [40] recorded that *S. Enteritidis* is the most frequently isolated serotype (84.62%) followed by *S. Typhimurium* (15.38%).

The results of serotyping of 7 different Salmonellae from ducks in the current study showed that *S. Enteritidis* (28.6%) and *S. Typhimurium* (14.3%) predominated other serotypes. These results coincide with Hoszowski and Wasyl [33] who detected Salmonella in duck broilers with the percentage of 14.3% and the most frequent serovars were *S. Enteritidis*, *S. Infantis*, *S. Hadar* and *S. Typhimurium*. However, El-Sawy [41] isolated *Salmonella* spp. from ducklings in Kalioubia Governorate and they were identified as: *S. Typhimurium*, *S. Tshiongwe*, *S. Newport*, *S. Nchanga*, *S. Tuebingen* and *S. Bovis-mobificans*.

Regarding serotyping of 3 different Salmonella isolates from quails, 3 different serogroups were identified as *S. Virchow* (33.3%), *S. Tamale* (33.3%) and *S. Typhimurium* (33.3%). Different *Salmonella* spp. were previously serotyped by Neto *et al.* [37] who reported *S. Corvalis*; *S. Give*; *S. Lexington*; *S. Minnesota*; *S. Schwarzengrund*; *S. Rissen* and *S. Typhimurium* from meat-type quails in Brazil.

In the present study, 2 different serogroups were identified as *S. wingrove* (50%) and *S. Kentucky* (50%) from turkeys. Hird *et al.* [42] reported that *S. Kentucky*, *S. Anatum*, *S. Heidelberg*, *S. Reading*, and *S. Senftenberg* were identified from turkeys at the California Veterinary Diagnostic Laboratory System. The variation of prevalence might be due to geographical variation, differences in management, type of samples, age of examined birds, season, poor hygienic conditions and inadequate nutrition.

Results of PCR for the detection of *bcfC* from 28 isolated strains showed that it was present in all the isolates (100%). Nearly similar results were obtained by Osman *et al.* [26] who reported *bcfC* gene in 100% of the Salmonella serovars isolated from humans and day-old ducklings. Also, Alatifehy [39] recorded that *bcfC* gene with the percentage of 95.7% was identified in Salmonella isolates from poultry. However, El-Sayed [43] reported the absence of *bcfC* gene in Salmonella strains isolated from ducklings.

The results of our study revealed that *sopB* gene was detected in 18 isolates with the percentage of 64.3%. Nearly similar results were obtained by Osman *et al.* [26] who detected *sopB* gene with the percentage of 54.3%. However, lower percentage (15.4%) was reported by El-Sayed [43].

In the present study, *spvC* gene was detected in 3 isolates only with the percentage of 10.7%. Similar results were obtained by El-Azzouny [28] who identified *spv* gene in 6% of Salmonella isolates. The results disagreed with Amini *et al.* [44] who detected *spv* gene in 30% of Salmonella strains isolated from poultry and Moussa *et al.* [45] who reported that *spv* gene was present in 31.5% in *S. Enteritidis* and 30% in *S. Typhimurium* isolated from poultry. In addition, the result of our study revealed that the *stn* gene was present in all of the isolates (100%). In accordance, Murugkar *et al.* [21] and Shalaby [46] reported that *stn* gene was detected in all the isolated Salmonella strains. Moreover, Zou *et al.* [16] identified *stn* gene in all 425 isolates (100%) of poultry origin.

Conclusion

In conclusion, different *Salmonella* species of different serotypes carrying common virulence genes were recovered from domestic birds in the examined areas of Delta, Egypt. Therefore, strictly hygienic and biosecurity measures must be applied in poultry management to avoid spread of salmonellae.

Conflict of interest

All the authors have no conflict of interest to declare.

References

- [1] Rao, V. and Chauhan, H.V. (1987): The pathology and pathogenesis of *Salmonella* Stanley infection in experimental chicks. *Res Vet Sci*, 42(3):287-293.
- [2] Nagaraja, K.V.; Pomeroy, B.S. and Williams, J.E. (1991): Paratyphoid infection In *Diseases of poultry*, 9th ed. B. W. Calnek, H.J. Barnes, C. W. Beard, W. M. Reid, and H. W. Yoder, Jr., eds. Iowa state university press, Ames, Iowa. PP 99-130.
- [3] Gast, R. K. and Beard, C. W. (1990): Production *S. Enteritidis* contaminated eggs by experimentally Infected hens. *Avian Dis*, 34:438-446.
- [4] Shivaprasad, H. L.; Timoney, J. F.; Morales, S.; Lucio, B. and Baker, R. (1990): Pathogenesis of *S. Enteritidis* infection in laying chickens. I. Studies on egg transmission, clinical signs, fecal shedding, and serologic responses. *Avian Dis*: 548- 557.
- [5] Gast, R. K. and Beard, C. W. (1992): Evaluation of a chick mortality model for predicting the consequences of *Salmonella* Enteritidis infections in laying hens. *Poult Sci*, 71(2): 281-287.
- [6] Ibrahim, I. A. (2003): Diarrhea in duckling. M V Sc thesis, Fac. Vet. Med., Zagazig univ, Benha branch.
- [7] Hoop, R. K. and Pospischill, A. (1993): Bacteriological, serological, histological and immunohistochemical findings in laying hens with naturally acquired *Salmonella* Enteritidis phage type 4 infection. *Vet Rec*, 133(16): 391-393.
- [8] Hendriksen, R. S. (2003): Global Salm-surv A global *Salmonella* surveillance and laboratory support project of the world health organization. Laboratory protocols level 1 Training Course Identification of *Salmonella* 4th Ed. April 2003.
- [9] Hoszowski, A.; Wasly, D. and Trusczyrski, M. (2000): *Salmonella* serovars determined in the National Veterinary Research Institute in 1994-1998. *Bull Vet Instit Putawy*, 44 (1): 33-38.
- [10] Falkow, F. (1996): The Evolution of pathogenicity in *Escherichia, shigella* and *Salmonella* In (ASM Press) *Escherichia Coli* and *Salmonella* chapter 149, P: 2723 – 2729.
- [11] Groisman, E. A. and Ochman, H. (1997): How *Salmonella* became a pathogen. *Trends in Microbiology*, 5(9):343-348.
- [12] Huehn, S.; La Ragione, R.M.; Anjum, M.; Saunders, M.; Woodward, M.J.; Bunge, C.; Helmuth, R.; Hauser, E.; Guerra, B.; Beutlich, J.; Brisabois, A.; Peters, T.; Svensson, L.; Madajczak, G.; Litrup, E.; Imre, A.; Herrera-Leon, S.; Mevius, D.; Newell, D.G. and Malorny, B. (2010): Virulotyping and Antimicrobial Resistance Typing of *Salmonella* enterica Serovars Relevant to Human Health in Europe. *Food Borne Pathog Dis*,7(5): 523-535.
- [13] Galán, J. E. and Zhou, D. (2000): Striking a balance: modulation of the actin cytoskeleton by *Salmonella*. *Proc Natl Acad Sci*, 97(16): 8754-8761.
- [14] Borges, K. A.; Furian, T. Q.; Borsoi, A.; Moraes, H. L. S.; Salle, C. T. P. and Nascimento, V. P. (2013): Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in South of Brazil. *Pesquisa Veterinária Brasileira*, 33(12): 1416-1422.
- [15] Swamy, S. C.; Barnhart, H. M.; Lee, M. D.; Dreesen, D. W. (1996): Virulence Determinants *invA* and *spvC* in salmonellae isolated from Poultry Products, Waste water and Human Sources. *Appl Environ Microbiol*, 62(10):3768-3771.
- [16] Zou, M.; Keelara, S. and Thakur, S. (2012): Molecular Characterization of *Salmonella* enterica Serotype Enteritidis Isolates from Humans by Antimicrobial Resistance, Virulence Genes, and Pulsed-Field Gel Electrophoresis. *Foodborne Pathogens and Disease*, 9 (3):232-238
- [17] ISO 6579 (2002): Microbiology of food and animal feeding stuffs- horizontal method for the detection of *Salmonella*

- SPP, International standard, 4th Ed., pp: 7- 15.
- [18] Kauffman, F. (1974): Serological diagnosis of *Salmonella* species. Kauffman white scheme Minkagaord, Copenhagen, Denmark.
- [19] World health organization WHO (2007): *Salmonella* epidemiology. <http://www.safe-poultry.com/Salmonella-epidemiology.asp>.
- [20] Oliveira, S. D.; Rodenbusch, C. R.; Cé, M. C.; Rocha, S. L. and Canal, C. W. (2003): Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. *Lett Appl Microbiol*, 36(4):217-221.
- [21] Murugkar, H.; Rahman, H. and Dulta, P. (2003): Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. *Indian Journal of Medical Research*, 117:66-70.
- [22] Shalaby, N. A. and Abdel-Hamid, H. S. (1987). Microbial agents responsible for embryonic mortalities in hatcheries in Gharbia Province. *Zagazig VetJ*, 5(2): 165-175.
- [23] Abd El-Nasser, A.; Mahmoud, F. M.; El-Shabiny, L. M.; Hassanein, Z. A. and Abbas, A. A. (1994): Studies on major bacterial agents causing arthritis in chickens in Qalyoubia province. *Veterinary Medical Journal Giza*, 42(1 B):277-285.
- [24] Taha, N. A. A. (2002): Zoonotic importance of enteropathogenic *E. coli* (EPEC). Ph.D. Thesis, Fac. Vet. Med. Zagazig Univ.
- [25] Roy, P.; Dhillon, A. S.; Lauerman, L. H.; Schaberg, D. M.; Bandli, D. and Johnson, S. (2002): Results of *Salmonella* isolation from poultry products, environment, and other characteristics. *Avian Dis*, 46(1):17-24.
- [26] Osman, M.M. (1992): Studies on bacterial causes of early poultry mortality in Sharkia Governorate. M.V.Sc., Thesis, Fac. Vet. Med., Zagazig Univ.
- [27] El-Zeedy, S. A.; Eldeen, H. K. and Badr, G. M. (2007): Protein analysis for comparison between salmonellae isolated from different poultry species. *BSVet Med J*, 17(2):1-9.
- [28] El-Azzouny, M. M. E. (2014): Occurrence of virulence genes among multidrug resistant *Salmonellae* isolated from broilers. Ph.D. Thesis, Fac. Vet. Med. Zagazig Univ.
- [29] Rehan, H.M.A. (2004): Application of PCR identification of *Salmonella* in chickens. M.V.Sc., Thesis, Fac. Vet. Med., Mansoura Univ.
- [30] Sadoma, A. M. (1997): *Salmonella* in chicken in connection with human infection. M.V.Sc. Thesis, Fac. Vet. Med., Tanta Univ.
- [31] Mohamed, L.N.; Samaha, H.A.; Draz, A.A. and Haggage, Y.N. (1999): *Salmonellae* among birds and human beings. *Alex J Vet Sci*, 15 (1): 147-154.
- [32] Abd El-Tawab, A. A.; Ammar, A. M.; Soad, A. N., El-Hofya, F. I. and Nabil, N. M. (2015): Studies on different *Salmonella* serotypes isolated from poultry in different governorates in Egypt. *Benha VetMed J*, 28 (2):169-175.
- [33] Hoszowski, A. and Wasyl, D. (2005): *Salmonella* prevalence and resistance to antibiotics in Poland. *Medycyna Weterynaryjna*, 61(6): 660-663.
- [34] Ismail, R. H. I. (2013): Some studies on bacterial causes of respiratory troubles in ducklings. M. V. Sc. Thesis, Fac. Vet. Med., Zagazig Univ.
- [35] Palanisamy S. and Bamaiyi P. H. (2015): Isolation and Antibigram of *Salmonella* spp. from Quails in a Farm from Kelantan, Malaysia. *J Vet Adv*, 5(12): 1191-1198.
- [36] Jalali, M.; Abedi, D.; Pourbakhsh, S. A. and Ghokasin, K. (2008): Prevalence of *Salmonella* species in raw and cooked foods in Isfahan, Iran. *J of Foods Safety*, 28 (3): 442-452.
- [37] Neto, F.; Angela, H.L.; Guastalli, S.; Almeida, A.M. and Junior, B. (2013): *Salmonella* spp. in Meat-type Quails (*Coturnix coturnix coturnix*) in the State

- of São Paulo, Brazil. *Brazilian J of Poultry Science*, 15(3): 277-281.
- [38] Tel, O. Y.; Bozkaya, F. and Keskin, O. (2013): *Salmonella*, *Campylobacter*, and *Chlamydophila* in bald ibis (*Geronticus eremita*) feces in Turkey. *J Zoo Wildl Med*, 44(1):21-26.
- [39] Alatfeh, N. M. M. M. (2012): Genomic comparison and characterization of *Salmonella* enterica serovars by the use of different molecular techniques. Ph.D. Thesis, Fac. Vet. Med., Cairo Univ.
- [40] Dahal, N.; Ellerbroek, L. and Poosaran, N. (2007): Prevalence and Antimicrobial Resistance of *Salmonella* in Imported Chicken Carcasses in Bhutan. *National Cent Anim Health*, 1:1-92
- [41] El-Sawy, E. (1976): Studies on Salmonellosis in ducks. M. V. Sc. Thesis, Fac. Vet. Med., Cairo Univ.
- [42] Hird, D. W.; Kinde, H.; Case, J. T.; Charlton, B. R.; Chin, R. P. and Walker, R.L. (1993): Serotypes of *Salmonella* isolated from California turkey flocks and their environment in 1984-89 and comparison with human isolates. *Avian Dis*, 37(3):715-719.
- [43] El-Sayed, Z. A. E. (2014): Bacteriological Studies on *Salmonella* isolated from imported One-day old Ducklings. M. V. Sc. Thesis, Fac. Vet. Med., Cairo Univ.
- [44] Amini, K.; Salehi, T.Z.; Nikbakht, G.; Ranjbar, R.; Amini, J. and Ashrafganjooe, S.B. (2010): Molecular detection of *invA* and *spv* virulence genes in *Salmonella* Enteritidis isolated from human and animals in Iran. *Afr J Microbiol Res*, 4 (21): 2202-2210.
- [45] Moussa, I. M.; Aleslamboly, Y. S.; Al-Arfaj, A. A.; Hessain, A. M.; Gouda, A. S. and Kamal, R. M. (2013): Molecular characterization of *Salmonella* virulence genes isolated from different sources relevant to human health. *J Food Agriculture and Environment*, 11(2):197-201.
- [46] Shalaby, A. G. A. (2012): Further studies on *Salmonella* species isolated from wild birds. Ph.D. Thesis, Fac. Vet. Med., Cairo University.

الملخص العربي

التوصيف المظهري والوراثي للسالمونيلا باراتيفويد المعزولة من الدواجن في منطقة الدلتا- مصر

محمد عبد العزيز لبد^١، أمال انيس مهدي عيد^١، سعاد عبد العزيز عبد الونيس^٢، ايناس محمد علي حماد^٣

^١ قسم طب الطيور و الارانب-كلية الطب البيطرى -جامعة الزقازيق - مصر

^٢ المعمل المرجعى للرقابه البيطريه على الانتاج الداجنى- معهد بحوث صحة الحيوان - الدقى- الجيزة- مصر

^٣ معهد بحوث صحة الحيوان - معمل فرعى المنصورة - مصر

تهدف هذه الدراسة الي وتصنيف ميكروب سالمونيلا باراتيفويد من الدجاج ,البط ,السمان و الرومى من خمسه محافظات مصريه. كما تم إستخدام تفاعل البلمرة المتسلسل لتحديد جينات الضراوة الشائعة في السالمونيلا. ولهذا الغرض تم تجميع عينات من ٢٦٥ مزرعة دواجن و كانت العينات مقسمه الى ١٥٠ من مزارع الدجاج , ٦٠ من مزارع البط , ٣٠ من مزارع السمان و ٢٥ من مزارع الرومى من محافظات الدقهليه, كفر الشيخ, دمياط, الشرقية و الغربية. تم فحص الطيور اكلينيكيًا وكذلك اجراء الصفة التشريحيو وعليه تم أخذ العينات من الكبد ,الرئه , الطحال , الاعورين و كيس المح لعزل السالمونيلا. كما تم اجراء الاختبارات البيوكيميائية للعزل كما تم تصنيف المعزولات سيرولوجيا بالاختبارات الخاصه لتصنيف السالمونيلا. تم عزل ٢٨ عينه ايجاييه من ٢٦٥ عينه من الدواجن بنسبه (١٠.٦%) حيث تم عزل ١٦ عترة بنسبه (١٠.٧%) من الفراخ. تم عزل ٧ عترات من البط بنسبه (١١.٧%), تم عزل ٣ عترات من السمان بنسبه (١٠%) و تم عزل ٢ عترة من الرومى بنسبه (٨%). و بإجراء الاختبارات السيرولوجيه للمعزولات تبين انها ٤ سالمونيلا تيفيموريم, ٣ سالمونيلا انترتيدس, ٣ سالمونيلا كنتاكي, ٢ سالمونيلا باراتايفاي ١, ١ سالمونيلا مولادى, ١ سالمونيلا هيدلبرج ١, سالمونيلا انفانتييس ١, سالمونيلا ابيبيمى من الدواجن بنسبه (٢٥%), (١٨.٨%), (١٨.٨%), (١٢.٥%), (٦.٢٥%), (٦.٢٥%), (٦.٢٥%), (٦.٢٥%) بالترتيب. و ايضا تم عزل ٢ سالمونيلا انترتيدس , ١ عترة سالمونيلا من كل من سالمونيلا تيفيموريم, سالمونيلا باراتايفاي ١, سالمونيلا كنتاكي, سالمونيلا انجاندا , سالمونيلا بارجنى من البط بنسبه (٢٨.٦%), (١٤.٣%), (١٤.٣%), (١٤.٣%), (١٤.٣%), (١٤.٣%), (١٤.٣%), (١٤.٣%) بالترتيب. تم عزل ١ عترة من كل من سالمونيلا فيرشاوا , سالمونيلا تامالى , سالمونيلا تيفيموريم بنسبه (٣٣.٣%) لكل منهما فى السمان. و ايضا تم عزل ١ عترة من كل من سالمونيلا انجروف و سالمونيلا كنتاكي بنسبه (٥٠%) لكل منهما فى الرومى. على الجانب الآخر تم إجراء اختبار تفاعل البلمره المتسلسل لعدد ٢٨ عترة معزوله من الدواجن لكل جين من جينات الضراوه الأكثر شيوعا و هم (*sopB*, *bcfC*, *spvC*, *stn*) و قد تبين تواجد جين *bcfC*, *stn* بجميع المعزولات بنسبه (١٠٠%) يليهما *sopB* و كانت نسبه تواجده (٦٤.٣%) من المعزولات ثم *spvC* بنسبه (١٠.٧%) من المعزولات. يستخلص من الدراسة أن عترات مختلفة من ميكروب السالمونيلا التي تحتوي علي جينات الضراوة الأكثر شيوعا منتشرة بين الطيور فى المزارع بمحافظات الدلتا بمصر مما قد يسبب أعباء اقتصادية وصحية والتي تتطلب الي التطبيق الصارم لإجراءات الأمن الحيوي في مزارع الدواجن.