

A Field Study on Biochemical Changes Associated with *Salmonella* Infection in Ducklings

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ABSTRACT

The present study aimed to investigate the incidence of *Salmonella* infection in diarrheic ducklings in Kafr El Sheikh Governorate, Egypt. A total of 100 samples were collected from ducklings suffered from diarrhea and mortality. Also, 50 litter samples were collected from duck farms. All specimens were collected under aseptic conditions for the isolation of *Salmonella* spp. The incidence of *Salmonella* was 7% in pooled samples from cecum, liver, spleen and gall bladder and 6% in litter samples. Ten strains of *Salmonella* spp. were serotyped, of which, *S. Salamae* (1 strain), *S. Miami* (2 strains), *S. Kentucky* (4 strains), *S. Paratyphi A* (2 strain) and *S. Magherafelt* (1 strain) were detected. Susceptibility of *Salmonella* isolates to 10 antimicrobial agents showed that *Salmonella* isolates were highly sensitive to amikacin (100%), followed by trimethoprim/sulphamethoxazole and gentamicin (50%). While isolates showed the highest percentage of resistance to norfloxacin (90%), followed by ciprocin (70%), flumox (70%) and amoxicillin-clavulanic acid (70%). Virulence genes (*invA*, *hila*, and *fimA*) were detected by PCR assay, all 10 *Salmonella* isolates showed positive results for three virulence genes, which gave specific amplicon at 284, 150, and 85 base pairs, respectively. Lethality test in five groups of three-day-old ducklings with different five isolated strains indicated a mortality rate ranged from 20-30 % in three isolates only. The most lethal strain *S. Paratyphi A* was chosen for further investigation as a pathogenicity test. IL-6 slightly decreased in the infected group in comparison to control. The results indicated that ducks infected with *Salmonella* spp. significantly showed lower RBCs, Hb, PCV, Phagocytic activity, phagocytic index, and serum albumin while, significantly had higher WBCs, neutrophil, lymphocyte, serum globulin, uric acid, creatinine, AST and ALT concentrations compared to non-infected. It could be concluded that *Salmonella* has hepatic and renal destructive effects and immunosuppressive effects.

Keywords: Biochemical changes, Ducklings, *Salmonella*.

INTRODUCTION

Salmonella infections are a major problem in the poultry industry. These bacteria enter the human food chain through poultry products. Human *Salmonella* infections and food-poisoning take the form of gastroenteritis, which can result in death in highly susceptible individuals (Here et al., 2003). *Salmonella* is a significant source of foodborne maladies that cause morbidity and mortality around the world. Among 94 million cases of non-typhoid *Salmonella* contaminations, it was assumed that roughly 85% of the cases were initiated by nourishment root *Salmonella* (Chiu et al., 2010).

Salmonella contaminations are too vital as both a cause of clinical infection in duck and as a source of nourishment borne transmission of sickness to people. Overwhelming financial problems happen due to

morbidity, mortality, decreased egg and meat creation in duck. Mortality may shift from 10% to 80% or higher in extreme episodes (Kleven and Yoder, 1998). Numerous *Salmonella* serovars exist. More than 2,600 serovars are grouped depending on the reactivity of antisera to O and H antigens (Stevens et al., 2009), and the serovars from ranches have a critical cover with those causing sicknesses in people (Alcaine et al., 2006). For the control and treatment of *Salmonella*, antimicrobials use is important. However, multidrug-resistant *Salmonella* has emerged and lead to treatment failure (Gong et al., 2013).

The *Salmonella* virulence is linked to a combination of chromosomal and plasmid factors. There are different genes such as *inv*, *spv*, *fim A* and *stn* have been identified as major virulence genes responsible for salmonellosis. *Salmonella* pathogenicity islands are huge gene tapes inside the *Salmonella* chromosome that encode

determinants liable for building up particular associations with the host. Also, it required for bacterial virulence (Sabbagh et al., 2010).

Salmonella spp. enter the intestinal epithelium and penetrates the Peyer's patches and from the Peyer's patches, *Salmonella* spp. go toward the mesenteric lymph nodes where it spreads to the circulatory system, leading to transient bacteremia (Smith and Beal, 2008). In this phase, there is massive chemotaxis of chemokines (IL-8, CXC, MIP-1 β) together with IL-1 and IL-6 into intestinal mucosa. Bacteria are rapidly cleared from the blood by phagocytes in the spleen and liver, and a large fraction of bacteria are killed by these cells (Coble et al., 2011).

The current study was performed to isolate and identify *Salmonella* serovars isolated from ducks using serological techniques and also to study the antibiotic sensitivity of the isolates. PCR assay was used for detecting *Salmonella* virulence genes. Also, the pathogenicity of isolated strains and changes of biochemical parameters and immune response during *Salmonella* infection in ducks were investigated.

MATERIAL AND METHODS

Ethical approval

The study was conducted according to the institutional Animal Care and Use Committee (Vet. CU20022020149)

Collection of samples

A total of 100 samples from 100 ducklings which suffer from diarrhea and mortality were collected from different farms and transported to Animal Health Research Institute Kafr El sheikh branch for examination. Samples from live and fresh dead birds were taken for isolation and identification of *Salmonella* spp. as pooled samples from the cecum, liver, spleen and gall bladder were collected in sterile containers to be cultured bacteriologically. Also, 50 Litter samples were collected from duck farms from dry areas of floor litter from the upper 2.5-5 cm of litter in sterile plastic bags and transported to the lab for bacterial examination.

Isolation of *Salmonella* spp.

Samples were cultured in Rappaport Vassiliadis Broth at 37°C for 18 hrs. and then subcultured on *Salmonella* and *Shigella* agar and incubated at 35°C for 24 hrs and XLD agar at 37°C for 24-48 hrs. *Salmonella* was isolated from poultry litter according to the American Association of Avian Pathologists (AAAP) (1989).

Isolates were identified as *Salmonella* spp. based on their colony morphology on selective media, biochemical testing (Edwards and Ewing, 1986). Isolates that were biochemically identified as *Salmonella* spp. were confirmed serologically by using the Polyvalent *Salmonella* (A-E and Vi) antisera (Benex Ltd., Shannon, Ireland). Serological identification of *Salmonella* was performed according to Grimont and Weill (2007).

Antimicrobial susceptibility test of isolated *Salmonella* spp.

Antimicrobial susceptibility was assessed using a disk diffusion method according to CLSI protocols (CLSI, 2018). Sensitivity discs with variable concentrations were used to determine the susceptibility of the isolated strains. The following antibiotics (Bioanalyse, Epico, and HiMedia) were used: norfloxacin, ciprocin, flumox, amoxicillin-clavulanic acid, ampicillin, trimethoprim/sulphamethoxazole, doxycycline, gentamicin, cefotaxime, and amikacin. The multidrug-resistant isolates which resistant to three or more kinds of antimicrobials (Schwarz et al., 2010).

Virulence genes of *Salmonella* detection by PCR

DNA extraction

DNA extraction from examined samples was done by using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with alterations from the manufacturer's suggestions. Briefly, 10 μ l of proteinase K and 200 μ l of lysis buffer was incubated with 200 μ l of the tested suspension at 56°C for 10 min. After incubation, 100% ethanol was put to the lysate by 200 μ l. The tested sample washed and then centrifuged following the manufacturer's order. 100 μ l of elution buffer was used to elute the nucleic acid which provided in the kit.

PCR amplification

The Primers which used were provided by Metabion (Germany) are listed in table 1. Primers were used in a 25 μ l of reaction mixture containing 12.5 μ l of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 μ l of each primer of 20 pmol concentrations, 4.5 μ l of water, and 6 μ l of DNA template. The reaction was performed in a T3 Biometra thermal cycler.

Analysis of the PCR Products

The PCR product was isolated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. To form gel analysis, about 20 μ l from products were loaded in each gel slot. A gel pilot 100 bp DNA Ladder

(Qiagen, Germany, GmbH) and gene ruler 50 bp ladders (Fermentas, Thermo) were used to detect the segment sizes. Gel documentation system (Alpha Innotech, Biometra) was used for photographing the gel and the computer software was used to analyze the data.

Lethality test

In this test, six groups of three-day-old duckling were used (ten ducks per group for the five isolates (*S. Salamae*, *S. Miami*, *S. Kentucky*, *S. Paratyphi A*, and *S. Magherafelt*) and last group as a negative control). A day before infection (challenge), randomly bacteriological samples were collected from ducks and tested for *Salmonella* free. Each duck was inoculated oral inoculation (using 1 ml sterile feeding tube introduced into the crop) with 1ml of overnight *Salmonella* isolates suspension (1×10^8 CFU/ml). The organism was prepared according to Osman *et al.* (2010). Morbidity and mortality rates following oral inoculation were observed until 15 days (Bjerrum *et al.*, 2003). The most lethal strain was chosen for further investigation as pathogenicity test (its effect on performance of duck, shedding and organ colonization)

Pathogenicity study

One group of 3-day old duckling and another group as control (20 birds for each) were separately housed in controlled biosafety isolator. Birds were fed rations of antibiotic-free. A day before infection (challenge) samples were collected and tested for *Salmonella* free. Birds were fasted for 12 hours to decrease crop bulk, thus expediting the flushing of the crop. The organism was prepared according to Osman *et al.* (2010). Infection dose was 1 milliliter of dilution introduced orally for all infected ducks with 1×10^8 CFU/ml *Salmonella* concentration for studying morbidity and mortality rates following oral inoculation were observed to 45 days, The control group was inoculated oral inoculation with 1 milliliter of sterile saline. Fecal swabs were collected for detection of fecal shedding from all groups during the first 3 days PI, then at weekly interval till 45 days. Moreover, at the end of each week till 45 days, two randomly selected ducks were sacrificed from each group for postmortem and bacteriological examination (organ colonization).

Detection of interleukin 6 by real-time PCR

A. RNA extraction: RNA performed from spleen tissue samples by using a QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH) when 30 mg of the tissue sample was added to 600 μ l RLT buffer containing 10 μ l β -mercaptoethanol per 1 ml. To form the homogenization

of the samples, the tubes were put in the adaptor sets, where it fixed into the clamps of the Qiagen tissue Lyser. Disruption was done in 2 minutes by high-speed (30 Hz) shaking step. One size of 70% ethanol was put on the cleared lysate, and the steps were done concurring to the Decontamination of Add up to RNA from Animal Tissues system of the QIAamp RNeasy Mini kit (Qiagen, Germany, GmbH). N.B. On column DNase, assimilation was done to evacuate leftover DNA.

B. Oligonucleotide Primers: Primers which used were provided from Metabion (Germany) are listed in table 2.

C. Taqman RT-PCR: PCR extension was done in a volume of 25 μ l containing 3 μ l of RNA format, 12.5 μ l of 2x QuantiTect Probe RT-PCR Master Mix, 8.125 μ l PCR grade water, 0.5 μ l from each primer of 20 pmol condensation and 0.125 μ l of each probe (30 pmol cons.) and 0.25 μ l of QuantiTect RT Mix. On the Stratagene MX3005P real-time PCR machine The reaction was performed.

D. Analysis of RT-PCR results: Amplification curves and cycle threshold (CT) values were detected by the Stratagene MX3005P software. The gene expression difference on the RNA of the different samples was assessed, the CT of the tested sample was compared with the positive control group, according to the " $\Delta\Delta CT$ " method mentioned by Yuan *et al.* (2006).

Serum biochemical parameters

Biochemical examinations of the one ml of blood samples were withdrawn from 3selected ducks of each treatment via brachial vein puncture into EDTA tubes for hematological analysis and were placed inside an icebox and transferred to the laboratory. Hemoglobin (Hb) according to the cyanomethemoglobin technique (Jain, 1986), red blood cell and white blood cell counts using a Neubauer hemocytometer (Natt and Herrick, 1952) and packed cell volume (PCV) (Britton 1963) were measured. Differential leukocyte count was performed using blood smears stained according to the Rosenfeld method (Lucas and Jamroz, 1961). Determination of phagocytic activity and phagocytic index (Richardson and Smith, 1982). Also, the blood samples were kept for 30 min at room temperature and the serum was collected through centrifugation at 3000 RPM for 15 min and was used for Activities of Alanine Amino Transferase (ALT) and Aspartate Amino Transferase (AST) were determined according to Reitman and Frankel (1957). Uric acid and creatinine were determined according to Arliss and Entwistle, (1981), and Michael and Malcolm (2006) respectively. Also, the serum

used to determine the total protein (TP) according to (Doumas et al., 1981), albumin (Alb) according to Henry et al., (1974), Globulins concentration (Glob) in serum was computed by subtracting albumin concentration from total Proteins, albumin to globulin ratio (A/G) was calculated according to Kaneko (1989).

Statistical analysis

Statistical analysis was performed using one-way analysis of variance using SAS software.

RESULTS

The result of serotyping of *Salmonella* isolates revealed five different *Salmonella* serotypes (*S. Salamae* , *S. Miami*, *S. Kentucky*, *S. Paratyphi A*, and *S. Magherafelt*) with 1, 2, 4, 2 and 1 strains, respectively.

Pathogenicity study

Clinical signs, Postmortem findings and mortality rate

Clinical signs were observed in the group infected with *S. Paratyphi A* 48 hours post-inoculation (PI) in the form of extreme thirst, profuse diarrhea, huddling together as chilled, ruffled feathers in some of them, lameness. Staggering gait, tremors, retraction of the neck backward, paddling movement, coma, and death. PM lesions revealed severe congestion of all internal organs, enlargement of the spleen, enlargement, and lobulation of the kidney, distention of the ureters with urates. Also, the liver appeared very pale. The mortality rate was 30%.

Quantification of interleukin-6 mRNA expression

A linear relationship between the amount of input RNA and the CT values for the various reactions was seen as expected in a log10 dilution series of standard samples for Interleukin 6 that also acted as positive controls for RT and PCR. Regression analysis of the CT values generated with the log10 dilution series of standards gave R2 values for all reactions that were greater than 0.97. To account for the variation in sampling and RNA preparations, the CT values for cytokines and chemokines specific for each sample were standardized using the CT value for 28S rRNA for the same sample from a reaction completed at the same time. Using the slopes of the Interleukin 6 and 28S rRNA log10 dilution series regression lines, the difference in input total RNA, as represented by the 28S rRNA, was then used to adjust cytokine- and chemokine-specific CT values (Table 8).

Blood Parameters

Infection with *Salmonella* spp. in ducks significantly (P<0.05) decreased RBCs, Hb, PCV, Phagocytic activity % and Phagocytic index while, significantly (P<0.05) increased WBCs, neutrophil, and lymphocyte compared with non-infected (Table 10).

Kidney and liver functions related to serum parameters

Infection with *Salmonella* spp. in ducks significantly (p<0.05) decreased serum albumin while, significantly (p<0.05) increased blood serum globulin, uric acid, creatinine, AST and ALT concentrations compared with non-infected (Table 12).

Table 1. Primer sequences, target genes, amplicon sizes, and cycling conditions.

Target gene	Primer sequences (5'-3')	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
<i>invA</i>	F:GTGAAATTATCGCCACGTTCCGGCAA R:TCATCGCACCGTCAAAGGAACC	284	94°C 5 min.	94°C 30 sec.	55°C 30 sec.	72°C 30 sec.	72°C 10 min.	Oliveira et al. (2003)
<i>hilA</i>	F:CATGGCTGGTCAGTTGGAG R:CGTAATTCATCGCCTAAACG	150	94°C 5 min.	94°C 30 sec.	60°C 30 sec.	72°C 30 sec.	72°C 7 min.	Yang et al. (2014)
<i>fimA</i>	F:CCT TTC TCC ATC GTC CTG AA R:TGG TGT TAT CTG CCT GAC CA	85	94°C 5 min.	94°C 30 sec.	50°C 30 sec.	72°C 30 sec.	72°C 7 min.	Cohen et al. (1996)

R: reverse, F: forward

Table 2. Primer sequences, target genes and cycling conditions for TaqMan RT-PCR.

Target gene	Primers and probes sequences (5'-3')	Reverse transcription	Primary denaturation	Amplification (40 cycles)		Reference
				Secondary denaturation	Annealing and extension	
28S rRNA	F:GGCGAAGCCAGAGGAAACT	50°C 30 min.	94°C 15 min.	94°C 15 sec.	60°C 1 min.	Suzuki et al. (2009)
	R:GACGACCGATTTGCACGTC					
IL-6	TaqMan probe: (FAM)AGGACCGCTACGGACCTCCACCA (TAMRA)	50°C 30 min.	94°C 15 min.	94°C 15 sec.	60°C 1 min.	Suzuki et al. (2009)
	F:GCTCGCCGGCTTCGA					
	R:GGTAGGTCTGAAAGGCGAACAG					
	TaqMan probe: (FAM)AGGAGAAATGCCTGACGAAGCTCTCCA (TAMRA)					

R: reverse, F: forward

Table 3. Incidence of *Salmonella* spp. isolated from ducklings and duck farms, Egypt

Types of samples	No. of samples	No. of positive samples	Percentage %
Pooled samples from cecum, liver, spleen and gall bladder	100	7	7
Litter samples	50	3	6

Table 6. Mortality rates in ducks infected with different *Salmonella* isolates through oral inoculation.

Strain	No. of infected duck	No. of deaths	Percentage %
<i>S. Salamae</i>	10	0	0
<i>S. Miami</i>	10	2	20
<i>S. Kentucky</i>	10	0	0
<i>S. Paratyphi A</i>	10	3	30
<i>S. Magherafelt</i>	10	2	20

Table 4. The results of antimicrobial susceptibility test of *Salmonella* spp. (n=10) isolated from ducklings, Egypt

Antimicrobial agent	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Norfloxacin	1	10	-	-	9	90
Ciprocin	-	-	3	30	7	70
Flumox	-	-	3	30	7	70
Amoxicillin-Clavulanic acid	3	30	-	-	7	70
Ampicillin	3	30	-	-	7	70
Trimethoprim / Sulphamethoxazole	5	50	-	-	5	50
Doxycycline	3	30	2	20	5	50
Gentamicin	5	50	4	40	1	10
Cefotaxime	2	20	8	80	-	-
Amikacin	10	100	-	-	-	-

Table 7. Fecal shedding and mortality of experimentally-infected ducks with *Salmonella* Paratyphi A

Time	Mortality (number)	Number of positive ducks for <i>Salmonella</i> shedding (%)
1st day	-	12 (60)
2nd day	2	13 (72.2)
3rd day	1	15 (88.2)
End of 1 st week	1	14 (100)
End of 2 nd week	2	7 (70)
End of 3 rd week	-	3 (37.5)
End of 4th week	-	3 (50)
End of 5th week	-	1 (25)
After 45 days	-	0

Table 5. Detection of *invA*, *hilA*, *fimA* virulence genes by PCR in *Salmonella* serotypes isolated from ducklings and litter duck farms, Egypt.

Serotype	Gene		
	<i>invA</i>	<i>hilA</i>	<i>fimA</i>
<i>S. Salamae</i>	1+ve strain	1+ve strain	1+ve strain
<i>S. Miami</i>	2+ve strain	2+ve strain	2+ve strain
<i>S. Kentucky</i>	4+ve strains	4+ve strains	4+ve strains
<i>S. Paratyphi A</i>	2+ve strain	2+ve strain	2+ve strain
<i>S. Magherafelt</i>	1+ve strain	1+ve strain	1+ve strain

+ve: positive

Table 8. Reisolation of *Salmonella* from internal organs of experimentally infected ducks with *Salmonella* Paratyphi A (n=18)

Organ	<i>S. Paratyphi A</i>	
	No	%
Liver	10	55.5
Spleen	12	66.6
Gall bladder	7	38.8
Cecum	11	61.1

Table 9. Detection of interleukin-6 mRNA and 28S rRNA in spleen of control and *Salmonella* infected ducklings by real-time PCR

Groups	Sample No.	28S rRNA		Interleukin-6			
		Individual CT	Mean CT	Individual CT	Mean CT	Fold change	
						Individual	Collective
Control	1	20.53	20.42	23.68	23.59	-	-
	2	20.30		23.49		-	-
Infected	1	20.38	20.60	20.50	20.81	8.2821	7.7633
	2	20.64		20.89		7.5685	
	3	20.77		21.04		7.4643	

CT: cycle threshold

Table 10. Effect of *Salmonella* challenge on some blood parameters of ducklings

Items	Non-infected ducks	Infected ducks
RBCs x106 /mm3	2.12± 0.06 ^a	1.12 ± 0.12 ^b
WBCs x103 /mm3	26.5 ± 0.87 ^b	51 ± 0.32 ^a
Hb (g/dl)	8.08 ± 0.12 ^a	5.6± 0.32 ^b
PCV%	34.98 ± 0.33 ^a	18.48 ± 1.76 ^b
Lymphocyte%	29.93±1.81 ^b	36.38±1.11 ^a
Neutrophil%	54.3±1.61 ^b	63.4±1.75 ^a
Monocyte%	6.12±0.20 ^a	5.40±0.22 ^a
Esinophil%	2.8±0.09 ^a	2.01±0.05 ^a
Basophil%	5.6±0.53 ^a	4.08±0.23 ^a
Phagocytic activity%	35.23±1.61 ^a	25.93±1.22 ^b
Phagocytic index	1.96± 0.12 ^a	1.180.32 ^b

Values are expressed as mean ± standard error. Different superscript letters within the same row indicate a significant difference (p ≤0.05).

Table 11. Effect of *Salmonella* challenge on some serum biochemical parameters of ducklings

Items	Non-infected ducks	Infected ducks
Total protein (g/dl)	5.94 ±0. 08 ^a	5.86 ±0. 05 ^a
Albumin (g/dl)	4.8 ±0. 03 ^a	2.69±0.09 ^b
Globulin (g/dl)	0.80 ±0. 08 ^b	1.1366 ±0. 11 ^a
Uric acid (mg/dl)	7.74 ±0. 05 ^b	11.04 ±0. 02 ^a
Creatinine (mg/dl)	0.67 ±0. 08 ^b	1.46 ±0. 03 ^a
AST (u/ml)	42 ±0. 46 ^b	67.3 ±0. 38 ^a
ALT (u/ml)	49±0.27 ^b	82.3 ±1. 2 ^a

Values are expressed as mean ± standard error. Different superscript letters within the same row indicate a significant difference (p ≤0. 05).

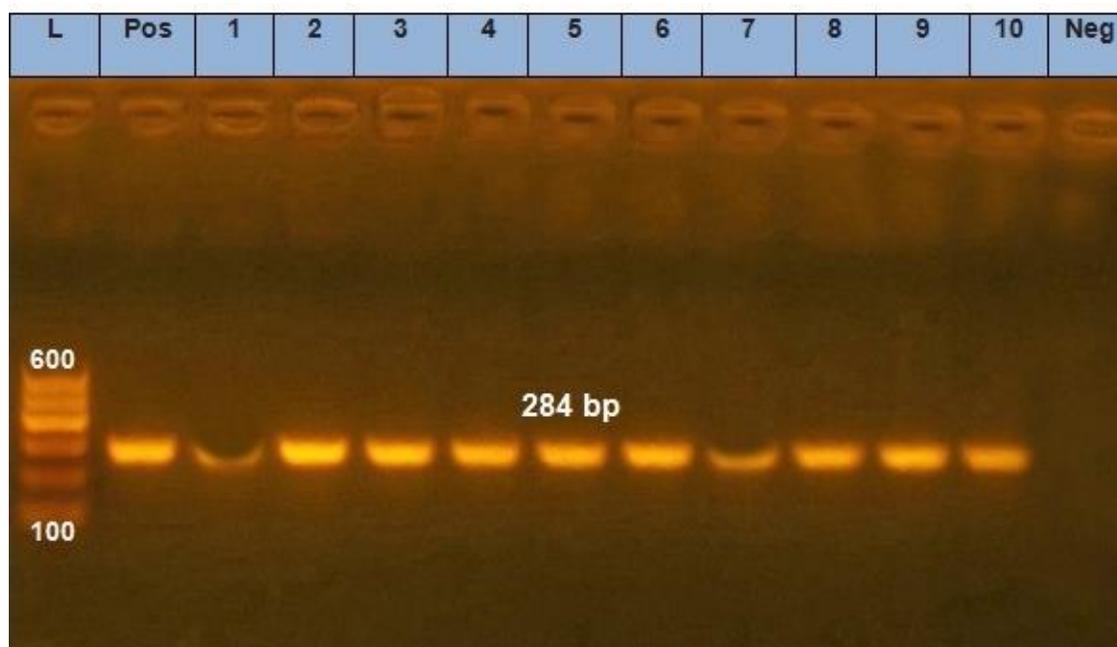


Figure 1. Detection of *invA* virulence gene in *Salmonella* isolates. Agarose gel showing polymerase chain reaction amplification of products of *invA* virulence gene of *Salmonella*. Lane L: 100-600 bp molecular size marker. Lane Pos: Control positive *Salmonella invA* virulence gene at 284 bp. Lane 1,2,3,4,5,6,7,8,9 and 10: samples positive for *invA* gene.

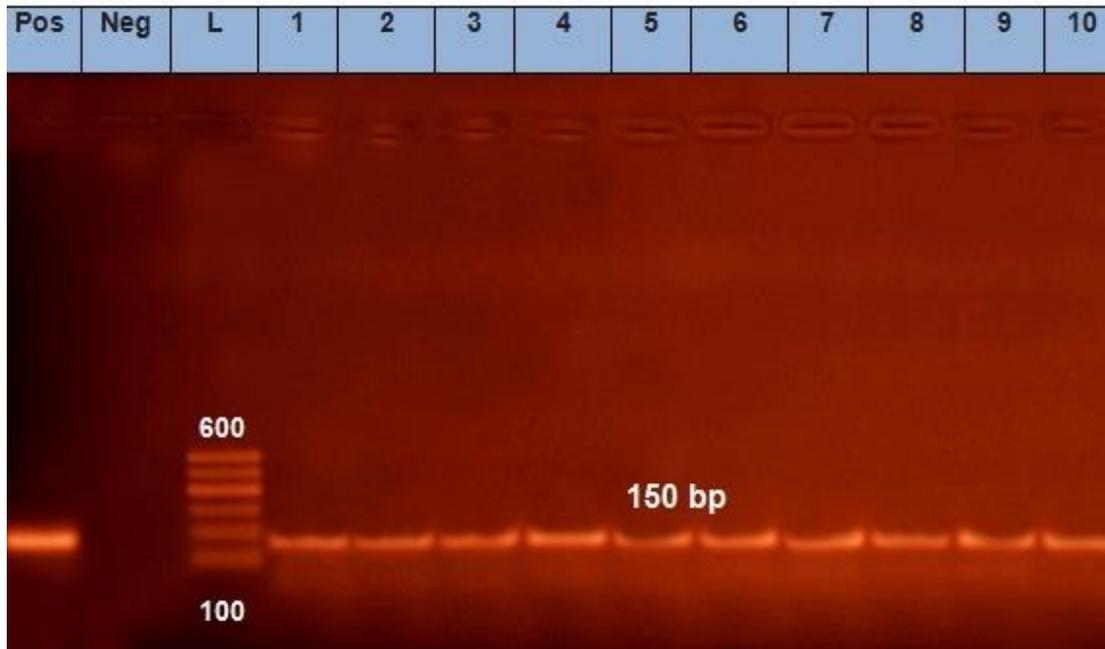


Figure 2. Detection of *hilA* virulence gene in *Salmonella* isolates. Agarose gel showing polymerase chain reaction amplification products of *hilA* virulence gene of *Salmonella*. Lane L: 100-600 bp molecular size marker. Lane Pos: Control positive *Salmonella hilA* virulence gene at 150 bp. Lane 1, 2, 3,4,5,6,7,8,9 and 10: samples positive for *hilA* gene.

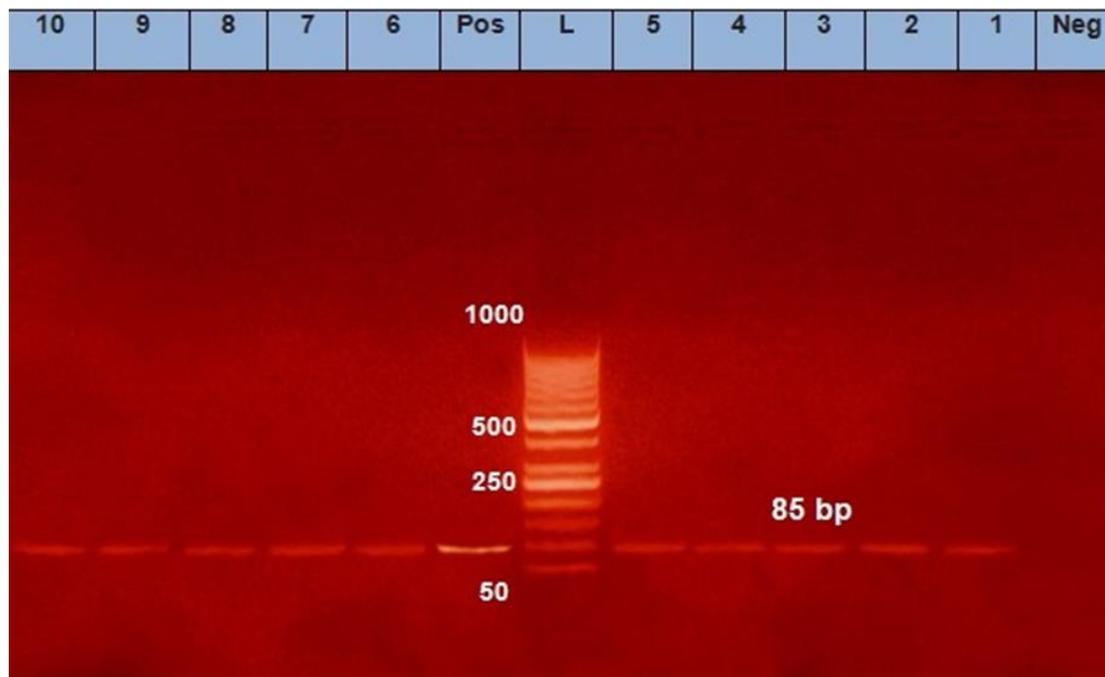


Figure 3. Detection of *fimA* virulence gene in *Salmonella* isolates. Agarose gel showing polymerase chain reaction amplification products of *fimA* virulence gene of *Salmonella*. Lane L: 50-1000 bp molecular size marker. Lane Pos: Control positive *Salmonella fimA* virulence gene at 85 bp. Lane 1, 2, 3,4,5,6,7,8,9 and 10: samples positive for *fimA* gene.

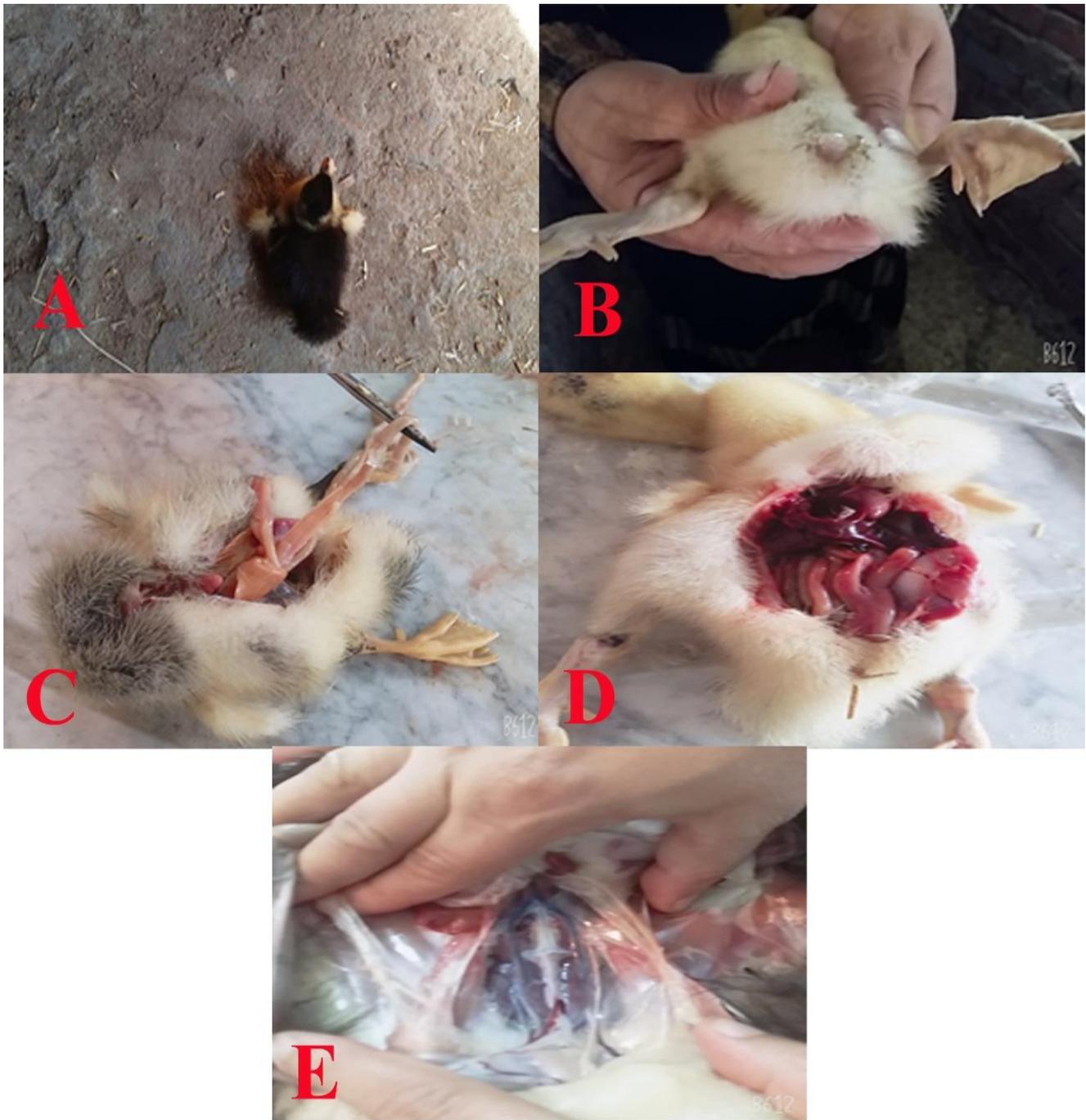


Figure 4. Clinical signs and postmortem lesions in experimentally *Salmonella* infected ducks. (A): staggering gait. (B): whitish diarrhea. (C): yellowish liver. (D): congested liver and intestine 1st week post-infection. (E): congested kidney after 45 days post-infection.

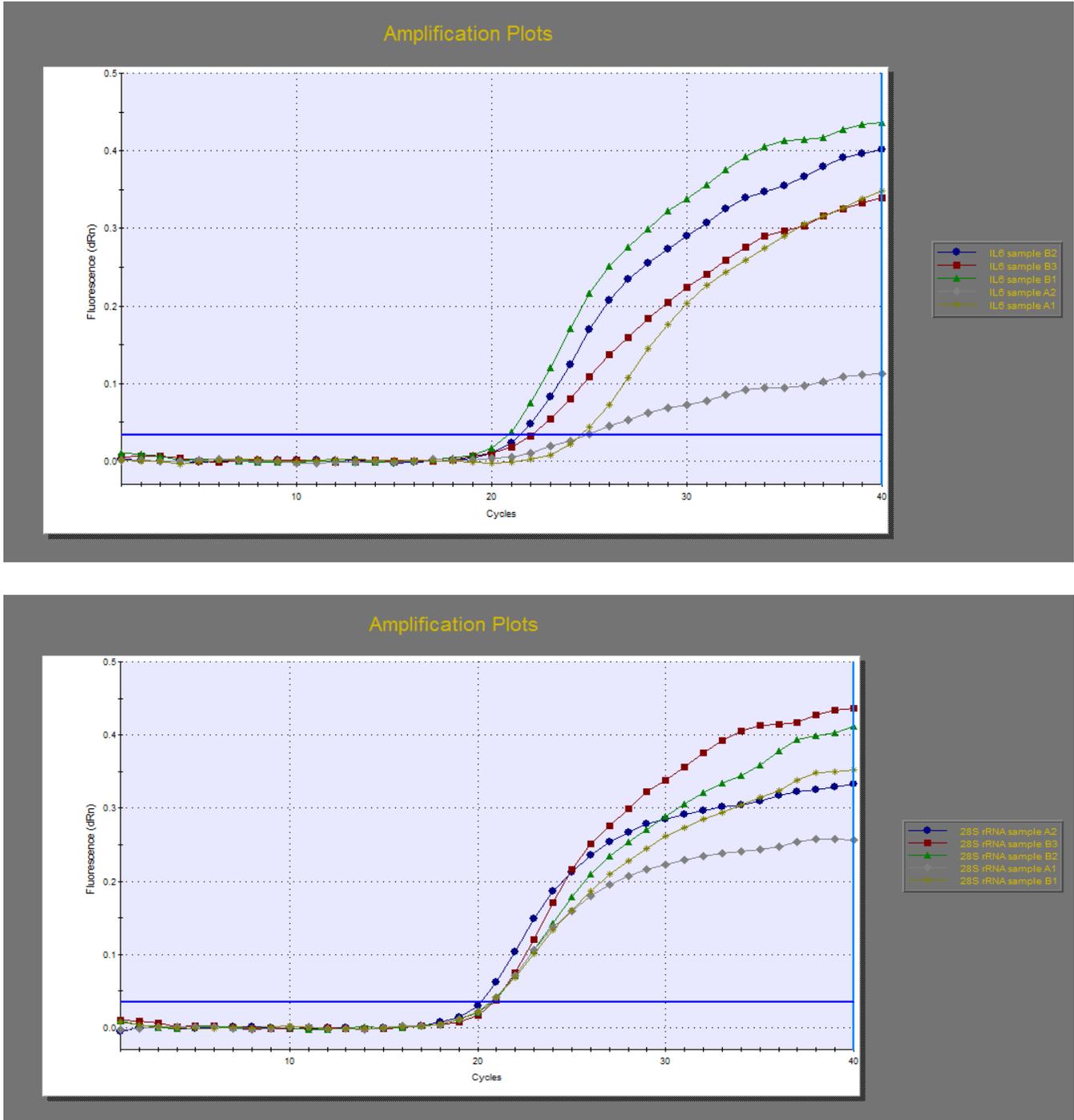


Figure 5. Expression of IL-6 and 28S rRNA in spleen of ducks following infection with *Salmonella*. The data are fold changes in mRNA determined by quantitative RT-PCR.

DISCUSSION

In the present work, *Salmonella* was isolated from ducklings and litter with a rate of 7% and 6%, respectively (Table 3), this rate appears to be similar to Abd El- Tawab et al. (2015) who isolated *Salmonella* from ducks in

Dakahlia and Damietta Governorates by 9.6% Batikh (2018), who isolated *Salmonella* from broiler chicken farm litter by 8%, but these results differ from Shamooun et al., (1998) who isolated *Salmonella* from ducks in open houses which was 16.6%, Abd-El-Rahman et al. (2000), who reported that the percentage of isolation was 20%

from 10 duck flocks in North Sinai. Hoszowski and Wasyl (2005), who detected *Salmonella* in duck broilers with a percentage of 14.3%, Adzitey and Huda (2012), who detected *Salmonella* in duck floor swab and transport crate swab with percentage of 13.3% for each.

The result of serotyping of 10 *Salmonella* isolates using "O", "H" and "Vi" antisera are illustrated, which clarified that the serotype of *Salmonella* spp obtained from positive *Salmonella* samples were *S. Salamae* (1 Strain), *S. Miami* (2 strain), *S. Kentucky* (4 Strain), *S. Paratyphi A* (2 Strain), and *S. Magherafelt* (1 strain). The most prevalent serovar was *S. Kentucky* (4 strains), these results agreed with Elgohary et al. (2017), who reported that *S. Kentucky* is the most prevalent serovar in diarrheic young duckling and slaughtered ducks (2 serovars) for each, but these results disagree with Guran et al. (2017), who found that *S. Kentucky* has been rarely reported in ducks, however, it has been reported in other animals, such as chicken

According to the results concerning antimicrobial susceptibility tests presented in table 4, 10 isolates showed the highest percentage of resistance (90%) to norfloxacin, followed by ciprocin, flumox, and amoxicillin-clavulanic acid by 70% for each. these results were higher than those reported by Abd El-Tawab et al. (2015), who detected that the resistance to amoxicillin and ampicillin /sulbactam was 50 % and 60% respectively in ducks isolates, but results of the present study were lower than Mohamed et al., (2015), who detected that the amoxicillin was 80% sensitive to *Salmonella* isolated from broilers, while in this study *Salmonella* isolates were amikacin sensitive by 100%, followed by trimethoprim/sulphamethoxazole, gentamicin by 50% for each. Similar result was reported by Abd El-Tawab et al. (2015), who reported that *Salmonella* was sensitivity to amikacin by 100% and sulfa+trimethoprim by 60% in ducks isolates. All isolates were screened by PCR analysis for the presence or absence of three selected virulence genes (*invA*, *hilA* and *fimA*) (Table 5; Figures 1, 2 and 3). The most common virulence gene which presents in *Salmonella*, *invA* gene, was used as a PCR target gene for the detection of *Salmonella* (Dong et al., 2014). Also, PCR screening analysis detected the presence of *invA*, *hilA*, and *fimA* in all *Salmonella* isolates. These result agreement with Abd El-Tawab et al. (2017), who detected that the percentage of *Salmonella* Typhimurium virulence genes *invA* and *hilA* were 100 % for each which isolated from clinically mastitic milk samples of cattle cows, Malorny et al. (2003), who revealed that in the studied strain that *invA* gene was detected in the rate of 100% and Thung et al. (2018), who detected *Salmonella invA* and *hilA* virulence

genes were detected by 100% and 82.61% respectively in retail beef meat samples. Transmission of infection is generally considered to occur orally. Enormous bacterial increase happens inside the intestine and tissue attack happens quickly. Under test conditions, mortality created by a harmful strain may change from 25% to 100% between distinctive inbred lines (Barrow et al., 1987).

This study examined the lethality of *Salmonella* strains using 3-day-old ducklings (Table 6). The observations were done during 15 days and showed a low rate of morbidity rate (weakness, lethargy, and low growth rate) while, the mortality rate reached 30%. Similar result was reported by Batikh (2018), who detected that the mortality was 28.6% in ducklings after inoculated orally with *S. Bargny*, *S. Enteritidis* and *S. Kentucky* strains.

The results indicated the pathogenesis of experimentally infected duckling with *Salmonella* Paratyphi A (Tables 7 and 8; Figure 4). Pathogenesis studies associated with virulent strains suggested that organisms multiply in the liver and spleen after the invasion and then disseminate to other organs, producing a systemic infection (Barrow et al., 1987). Ducks are very resistant to infection produced by *Salmonella*, they are possibly reservoirs of it and may shed it in the feces and pollute the environment (Barrow et al., 1999). The present study revealed that colonization of the cecum and shedding of *S. Paratyphi A* in the feces was detected in the feces since 24 h post-infection, a similar result was reported by Ribeiro et al. (2005), who detected *S. Kottbus* in the feces of broiler chicks since 24 h until 42 days post-infection.

Effect of *Salmonella* infection on interleukin 6

In the bacteremia phase, there is massive chemotaxis of chemokines (IL-8, CXCL1, MIP-1 β) together with IL-1 and IL-6 into the intestinal mucosa. Bacteria are rapidly cleared from the blood by phagocytes in spleen and liver, and a large fraction of bacteria are killed by these cells (Coble et al., 2011). IL-6 decreased in the infected group in compared to control one (Table 9, Figure 5), these differed from (Kaiser et al., 2000), who reported that IL-6 is usually indicative of the initiation of an acute-phase response and is produced following infection with *S. Typhimurium* in vitro model of avian cell culture.

Effect of *Salmonella* infection on hematological parameters

Infection with *Salmonella* spp. in ducks significantly ($P < 0.05$) decreased RBCs, Hb, PCV, Phagocytic activity % and Phagocytic index while, significantly ($P < 0.05$)

increased WBCs, neutrophil, and lymphocyte compared with noninfected (Table 10). Infection with *Salmonella* spp. in ducks significantly ($P < 0.05$) decreased serum albumin while, significantly ($P < 0.05$) increased blood serum globulin, uric acid, creatinine, AST and ALT concentrations compared with non-infected (Table 10).

Changes that happened in the blood picture and biochemical values are a mirror of the changes that occurred in the tissues and organs as a result of bacterial infection. These findings agreed with those reported by, (Assoku et al., 1970) and (Kokosharov, 2006), who discussed that there were decreased in RBCs, Hb and PCV in poultry infected with *S. Gallinarium*. Increased values of WBCs, neutrophil, and lymphocyte agreed with (Morgulis, 2002), who recorded that Leukocytosis is usually due to heterophilia, and common causes are general infections due to septicemias caused by infectious agents, such as *Salmonella* and disagreed with Allan and Duffus (1971), found no changes in lymphocyte counts during fowl typhoid and Assoku et al. (1970), worked at *S. Gallinarium* in birds, and noticed that the count of lymphocyte was lower than the normal values. There were no important changes in the percentage of eosinophil, monocyte, and basophil, these results were coordinated with previous results (Cardoso et al., 2003 and Freitas Neto et al., 2007). Decreased level in Phagocytic activity % and Phagocytic index agreed with (Belih et al., 2016).

There was a decrease in albumin, AST, ALT and increased globulin (Table 11), which agreed with (Freitas Neto et al., 2007), who reported that serum albumin was lower while ALT and AST were higher in *S. Gallinarium* infection. This may be due to the inability of protein synthesized by the liver which reflects lesion intensity, visibly proven by hepatomegaly and loss of protein by the affected kidney. Therefore, the damage in the glomerular filtration barrier, inflammation of the renal parenchyma or epithelial damage of the tubules leads to the presence of plasma proteins in the urine (Relford and Lees, 1996).

In the present work, *Salmonella* infection significantly increased serum creatinine and uric acid levels in *Salmonella* infected group, that agreed with Hegazy et al. (2014).

CONCLUSION

Results of antibiotic sensitivity demonstrated that amikacin could be used for ducks against *Salmonella* infection. Molecular analysis showed that virulence genes of *invA*, *hilA* and *fimA* were found in all *Salmonella* strains isolated from ducklings. The *invA* gene is present only in

Salmonella species and therefore is used as a golden marker in the genetic diagnosis of *Salmonella* species. It is concluded that *Salmonella* had immunosuppressive effects and destructive effects on the liver and kidney.

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