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Comparative Clinicopathological Study of Salmonellosis in Integrated Fish-Duck Farming

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ABSTRACT

Poultry litter is used in fish farms as fertilizer thus integrated fish-duck farming is common in some areas of Egypt. *Salmonella* bacteria may be present in poultry litter and contaminate fish ponds and infect duck farms. To investigate incidence and prevalence of *Salmonella* infection in integrated duck-fish farms, 50 litter samples, 200 cloacal swabs from integrated duck farms, 60 liver samples from integrated duck farms and 69 water samples from the fish pond were collected. Results revealed the isolation and identification of 19 *Salmonella* spp. belonging to 14 different serotypes (4 isolates from litter, 2 isolates from fish pond water, 8 isolates from cloacal swabs of ducks and 5 isolates from ducks liver). Fifty, one-day-old Pekin ducks were experimentally infected with five chosen *Salmonella* serotypes (*S. Bargny, S. Tshingwe, S. Uganda, S. Kentucky*, and *S. Enteritidis*). The results from experimental infection revealed clinicopathological findings including degeneration and necrosis in the liver, lymphoid depletion and macrophage infiltration in the spleen and enteritis. Mortality ranged from 28.6% in *S. Bargny, S. Enteritidis and S. Kentucky* and increased to 42.9% in *S*. Uganda and reached up to 100% in *S*.Tshingwe. Body weight gain decreased by 16% in *S*. Uganda and exceeded to 23.9% in *S*. Kentucky and decreased by 31% in *S. Bargny and S. Enteritidis* as compared to the control group. Feed conversion ratio was recorded and ranged from 5.1, 5.11, 4.98, 5.15 and 4.02 in *S. Bargny, S. Uganda, S. Kentucky, S. Enteritidis*, and control group, respectively. In conclusion, different species of *Salmonella* can affect integrated duck-fish farms and cause high mortality as well as a decrease in feed intake, feed conversion ratio, and body weight gain.

Key words: Histopathology, Integrated duck-fish farms, Pathogenicity, *Salmonella* spp.

INTRODUCTION

Some of the fish farms are integrated with waterfowl as integrated duck-fish farms are preferred as ducks fit easily into aquaculture facilities, inducing, vegetation, pest control, and fertilization roles, in the same time this system needs minimum requirement concerning facilities and expenditure in this warm water system (Little and Edwards, 2003; Majhi, 2018). Poultry litter is used by some farms as a fertilizer due to the non-digested feed, metabolic excretory products and residues in poultry litter resulting in a microbial synthesis that can be utilized to replace reasonable parts of feedstuff used in conventional fish production cost (Bekibele and Onunkwo, 2007; Hirpo, 2017). The microbiological examination of poultry litter exhibits various pathogenic microorganisms. Existence of pathogens in litter and in the aqua-system is considered one of the critical reasons for infection transmission (Guan and Holley, 2003; Soliman et al., 2018).

The objective of the present study was to identify *Salmonella* species which may be present in poultry litter and can access to fish ponds during fertilization of the ponds and consequently infect ducks integrated with aquaculture. Moreover, the clinicopathological aspects of salmonellosis were evaluated by experimental infection of ducklings with isolated *Salmonella*.

MATERIALS AND METHODS

Ethical approval

The animal use protocol in this study approved by the Institutional Animal Care and Use Committee (Vetcu02122019102).

Experimental design and sampling

Samples were obtained according to the research design from the different districts in Kafr El-Sheikh Governorate, Egypt. In total, 50 litter samples, 200 cloacal swabs from integrated duck farms and 69 water samples from fish ponds, 60 liver samples were taken from sacrificed ducks from different fish farms. All samples were labeled and transported to the laboratory. (Animal Health Research Institute, Kafr El-Sheikh provisional laboratory, Egypt). The samples were subjected to *Salmonella* isolation and identification.

Isolation and identification of different *Salmonella* serotypes

Isolation of different *Salmonella* was applied on litter. Briefly, 25 g of litter samples were prepared by mixing in a sterile flask with 225 ml phosphate buffer saline (PBS, Bio Basic, Canada). Water samples obtained through inverting a 500 ml sterilized flask in 30 cm (Abd-Elghany et al., 2015) depth water surface. Then, 30 ml of water samples were clarified by centrifugation (centrifuge-Universal- Germany) at 5000 rpm for 5 minutes. Cloacal swabs and liver samples (A sterile cotton swabs stabbed into liver parenchyma) by using nutrient broth (Oxoid, UK), where 1 ml of all of these samples inoculated in nutrient broth and incubated at 37ºC for 24 hr. Then, 1 ml of incubated broth was inoculated into selenite F broth (Oxoid, UK) and incubated at 37 ºC for 24 hr. a loopful from this broth were streaked onto *Salmonella*-shigella (SS) agar (Oxoid, UK) and incubated at 37 ºC for 24 hr. All the suspected pure colonies of salmonellae were furtherly subjected to biochemical reactions (methyl-red, Voges-Proskauer, indole and urea tests) according to Cheesbrough (1985). Biochemically positive reaction for *Salmonella* isolates was finally identified according to (Grimont and Weill, 2007) using *Salmonella* poly ”O” antiserum and *Salmonella* monovalent ”O and H” antiserum (SINIF Co., Germany). Then a five *Salmonella* isolates were employed to study the clinicopathological picture in duckling.

Comparative clinicopathological effects of *Salmonella* isolates

Fifty, one-day-old Pekin duckling were employed to study the clinicopathological effects of the different *Salmonella* isolates including *S. Bargny, S. Tshingwe, S. Uganda, S. Kentucky* and *S.* Enteritidis in susceptible one-week-old ducklings through oral inoculation. Eight experimental ducklings were bacteriologically examined and proved to be free from *Salmonella*. The remaining 42 ducklings subdivided into six equal groups (1-6) by ranking methods. At the 7th day, the first five groups were inoculated orally (using 1-ml sterile feeding tube via crop) containing (1x10 ^{9} cfu**)** / duckling (Barrow et al., 1999) of each of *S. Bargny, S. Tshingwe, S. Uganda, S. Kentucky, and S. Enteritidis* respectively, while the 6th group kept as uninfected control and was similarly inoculated orally with physiological saline. Each group was reared separately in wire-floored batteries and fed on commercial ration which contain the nutritional requirement for Pekin duckling. Feed and water were given *ad.lib*. All ducklings were kept under observation for signs and deaths up to 3 weeks of age. Cloacal swabs were collected for detection of fecal shedding from all groups during the first 3 days post-inoculation (PI), then at the weekly interval at the 2nd and the 3rd week PI. Moreover, at the end of the 2nd and 3rd week, two randomly selected ducks were sacrificed from each group for postmortem, bacteriological and histopathological examination. Initial and final body weight, feed consumption, body weight gain and feed conversion rate were calculated at a weekly interval as averages. Percentages of the average values of the infected groups were also calculated relative to the average values of the uninfected control group to allow better comparison. Also Re-isolation of salmonellae from dropping, liver, spleen and gall bladder of experimentally infected seven-day-old ducklings.

Statistical analysis

The obtained numerical data were statistically analyzed using SPSS software. Duncan's multiple range test was used for testing significance of differences among group means at p-value<0.05.

RESULTS

Results of salmonellae isolation are shown in table 1, which revealed that 19 *Salmonella* isolates were recorded from poultry litter, fish pond water, cloacal swabs of integrated ducks and liver of integrated ducks at rates of 8, 2.9, 4 and 8.3%, respectively.

All 19 *Salmonella* isolates were subjected to biochemical identification and the results are summarized and presented in table 2. The biochemically identified *Salmonella* isolates were serologically identified by using monovalent and polyvalent "O" and "H" *Salmonella* antisera. Results are summarized and presented in tables 3 and 4. Nineteen *Salmonella* isolates included *S. Kentucky*

(n=4), *S. Enteritidis* (n=2), *S.* Bargny (n=2) and one isolate for each of *S.* Belgdam, *S.* Cuckmere, *S.* Tshiongwe, *S.* Gueuletapee, *S.* Oxford, *S.* Atakpame, *S.* Ferruch, *S.* Uganda, *S.* Amsterdam, *S.* Brikama and *S.* Kulsrivier. *Salmonella* Kentucky was the most frequent isolate with a rate of 21%, followed by *S. Enteritidis* and *S.* Bargny with a rate of 10.5%. Table 4 shows that all *Salmonella* isolated were motile containing flagellar antigen "H" with its two phases "H1" and "H2" except *S.* Belgdam, *S.* Gueuletapee, *S. Amsterdam* and *S. Enteritidis* which contained "H1" only.

Table 1. Numbers and percentage of isolated salmonellae from different samples in integrated duck-fish farming

Types of samples	Total number of samples	*Salmonella*	
		Number of isolates	%
Litter samples	69	2	2.9
Water samples	50	4	8
Liver [a]	60	5	8.3
Fecal swabs [b]	200 (pooled sample)	8	4
Total	379	19	5

[a] from scarifying ducks and duckling [b] 3 pooled sample (2-3 individual samples) were taken from each farm

Clinical signs, postmortem findings and mortality rate during experimental infection with chosen *Salmonella* isolates

Clinical signs were recorded as mentioned in table 5, from this table it is clear that the clinical signs were detected in all infected groups 24-48 hours PI in the form of extreme thirst, profuse diarrhea, huddling together as chilled, ruffled feather in some of them, lameness appeared in *S.Bargny*. Staggering gait appeared in *S.Tshingwe* 24hrs PI and in *S.Enteritidis* 72 hrs. PI. This was followed by retraction of the head towards the chest, later by tremors, retraction of the neck backward, paddling movement, coma, and death. Gross lesions of dead and/or sacrificed birds from the five infected groups were recorded and mentioned in table 5, from this table it is clear that the gross lesions revealed severe congestion of all internal organs, enlargement of the spleen, enlargement, and lobulation of the kidney, distention of the ureters with urates and typhlitis with frothy content. *S.* Bargny and, *S.Enteritidis* groups appeared to have necrotic foci on liver. Also, liver appeared very pale in third week PI in each of *S. Uganda, S. Kentucky*, and *S.Enteritidis* groups. Mortality ranged from 28.6% in *S.* Bargny, *S. Enteritidis* and *S.* Kentucky and increased to 42.9% in *S.* Uganda and reached up to 100% in *S.Tshingwe* as mentioned and recorded in table 6.

Also shedding pattern (Table 7), organ colonization (Table 8), initial body weight, final body weight, feed consumption, body weight gain, and feed conversion rate (Table 9) were measured and calculated.

Histopathological findings in ducklings infected with salmonellae

Generally, *Salmonella* infection in ducklings produced marked tissue alterations as compared to the negative control group. The main lesions were recorded in liver, spleen, and intestine. Regarding to the experimental infection by using different *Salmonella* species including *S. Bargny, S. Tshingwe, S. Uganda, S. Kentucky, and S. Enteritidis*, the histopathological finding are summarized and presented in table 10 and from this table, it is clear that the degenerative effect and necrotic effect in liver, also depletion and macrophage infiltration were more remarkable in *S. Tshingwe* (Figure 1) than *S. Bargny* (Figure 2) and *S. Uganda* (Figure 3) in the first week post-infection, also the degenerative effect in the liver in second week post-infection was clearer in *S. Bargny* (Figure 4) than *S. Uganda, S. Kentucky* (Figure 5) and *S. Enteritidis*, macrophage infiltration in spleen is clear in *S.* Bargny and *S.* Uganda than *S. Kentucky* and *S. Enteritidis* (Figure 6).

Also, hyperplasia in lining epithelium of examined intestine was higher in *S.* Bargny followed by *S. Enteritidis* as compared with each of *S.* Uganda and *S.* Kentucky while the histopathological changes in the third week post-infection were less remarkable than the previous weeks. Hyperplasia of the lining epithelium of examined intestine was more remarkable in cases infected with *S.* Bargny and *S.* Uganda compared to each of *S.* Kentucky and *S. Enteritidis* while enteritis was not detected in cases infected with *S.* Uganda and *S.* Kentucky.

Also, ducks infected with S. Kentucky from two weeks post-infection showed hepatic vacuolation and a mild degree of histiocytic proliferation in spleen (Figure 7).

Table 2. Biochemical characters of isolated *Salmonella* from different samples in integrated duck-fish farming

Items	Motility	Indole	M.R	V.P	TSI				urea
					H_2S	gas	Butt	slant	
Salmonella isolates	+	-	+	-	+	+	Y	R	-

M.R: methyl red. V.P: Voges-Proskauer. TSI: triple sugar iron. H_2S: hydrogen sulfide. The samples used were poultry litter samples and water samples from fish farms as well as cloacal swabs and liver samples from ducks.

Table 3. Serotypes of isolated *Salmonella* from different samples in integrated duck-fish farming

Types of samples	Number of samples	Number of isolates	*Salmonella* incidence	Identified serotypes
Water samples	69	2	2.89%	*S.* Gueuletapee *S.* Tshiongwe
Litter samples	50	4	8%	*S.* Bargny *S.* Cuckmere *S.* Belgdam *S.* Kentucky
Liver samples [a]	60	5	8.3%	*S.* Enteritidis *S.* Brikama *S.* Amesterdam *S.* Kentucky *S.* Kulsrivier
Cloacal swab samples [b]	200 Pooled samples	8	4%	*S.* Atakpame *S.* Kentucky (2 isolates) *S.* Oxford *S.* Enteritidis *S.* Ferruch *S.* Uganda *S.* Bargny

[a] from scarifying ducks and duckling [b] 3 pooled sample (2-3 individual samples) were taken from each farm

Table 4. Antigenic profile of isolated *Salmonella* from different samples in integrated duck-fish farming

Serotype	Antigenic structure profile		
	O antigen	H antigen	
		Phase I	Phase II
S. Ferruch	8	e,h	1,5
S. Bargny	8,20	1	1,5
S. Brikama	8,20	r,i	l,w
S. Tshiongwe	6,8	e,h	e,n,z15
S. Amesterdam	3,10,(15),(15,34):	g,m,s	-
S. Uganda	3,10,(15)	1,Z13	15
S. Belgdam	9,46	g,m,q	-
S. Atakpame	8,20	e,h	1,7
S. Gueuletapee	1,9,12	g,m,s	-
S. Oxford	3,10,(15),(15,34)	A	1,7
S. Cuckmere	3,10	I	1,2
S. Kulsrivier	1,9,12	g,m,s,t	e,n,x
S. Enteridídis	1,9,12	g,m	-
S. Kentucky	8,20	I	Z6

O antigen: somatic antigen. H antigen: flagellar antigen. The samples used were poultry litter samples and water samples from fish farms as well as cloacal swabs and liver samples from ducks.

Table 5. Clinical signs and postmortem lesions in experimentally infected 7-day-old ducks with different *Salmonella* serovars.

Symptoms	**Experimentally infected groups with different *Salmonella* serovars**					**Control**
	Group 1 (*S.* Bargny)	**Group 2 (*S.* Tshingwe)**	**Group 3 (*S.* Uganda)**	**Group 4 (*S.* Kentucky)**	**Group 5 (*S.* Enteritidis)**	
Diarrhea	+ve	+ve	-ve	+ve	+ve	-ve
Huddling	+ve	+ve	+ve	+ve	+ve	-ve
Weakness	+ve	+ve	+ve	+ve	+ve	-ve
Ruffled feathers	-ve	+ve	-ve	+ve	+ve	-ve
Nervous manifestation	-ve	+ve	-ve	-ve	+ve	-ve
Increase thirst	+ve	+ve	-ve	-ve	+ve	-ve
Reduced feed intake	-ve	-ve	+ve	+ve	-ve	-ve
Lameness	-ve	+ve	+ve	+ve	+ve	-ve
Post mortem lesions	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Control**
Congested liver	+ve	+ve	+ve	+ve	+ve	-ve
Congested spleen	+ve	+ve	+ve	+ve	-ve	-ve
Enlarged kidney	-ve	+ve	-ve	-ve	+ve	-ve
Ureter filled with urates	-ve	+ve	+ve	-ve	+ve	-ve
Necrosis of liver	+ve	-ve	-ve	-ve	+ve	-ve
Pale liver (3rd week)	-ve	-ve	+ve	+ve	+ve	-ve

+ve: positive. -ve: negative.

Table 6. Mortality pattern and mortality rate in experimentally infected 7-day-old ducks with different *Salmonella* serovars.

Time / **Groups**	**1st week**				**2nd week**	**3rd week**	**Mortality rate**
	1st day	**2nd day**	**3rd day**	**4th day**			
Group 1 (*S.*Bargny)	1	-	1	-	2(sacrified)	2(sacrified)	28.6%
Group 2 (*S.*Tshingwe)	1	2	3	1	-	-	100%
Group 3 (*S.*Uganda)	-	1	1	1	2(sacrified)	2(sacrified)	42.9%
Group 4 (*S.*Kentucky)	-	-	1	1	2(sacrified)	2(sacrified)	28.6%
Group 5 (*S.*Enteritidis)	-	1	-	1	2(sacrified)	2(sacrified)	28.6%
Control group	-	-	-	-	2(sacrified)	2(sacrified)	0%

Table 7. Duration of fecal *Salmonella* shedding in experimentally infected ducks with different *Salmonella* serovars

Shedding	**Group 1 (*S.* Bargny)**			**Group 2 (*S.* Tshingwe)**			**Group 3 (*S.* Uganda)**			**Group 4 (*S.* Kentucky)**			**Group 5 (*S.* Enteritidis)**			**Control**
	+ve	**total birds**	**%**	**+ve**	**total birds**	**%**	**+ve**	**total birds**	**%**	**+ve**	**total birds**	**%**	**+ve**	**total birds**	**%**	**+ve**
First day	4	7	57	2	7	28.6	3	7	42.9	1	7	14.3	1	7	14.3	0
Second day	2	6	33	4	6	66.6	2	7	28.6	3	7	42.9	1	7	14.3	0
Third day	1	6	16.6	1	4	25	2	6	33.3	2	7	28.6	3	6	50	0
Fourth day *	-	-		1	1	100	1	1	100	1	1	100	1	1	100	0
Second week	2	5	40	-	-	-	1	4	25	2	5	40	1	5	20	0
Third week	3	3	100	-	-	-	1	2	50	2	3	66.6	1	3	33.3	0
Total	12	27	44.4	8	18	44.4	10	27	37.4	11	30	36.6	8	29	27.5	0

*cloacal swabs collected in 4th day post-infection only from dead ducks. +ve: positive. -ve: negative

Table 8. Recovery of *Salmonella* from different organs of freshly dead and /or sacrificed ducks after oral experimental infection with different *Salmonella* serovars

Organ	Group 1 (*S.* Bargny)		Group 2 (*S.* Tshingwe)		Group 3 (*S.* Uganda)		Group 4 (*S.* Kentucky)		Group 5 (*S.* Enteritidis)		Control	Total	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	No.	%
Liver	3/6	50	3/7	42.9	2/7	28.6	2/6	33	2/6	33	-	12/32	37.5
Spleen	3/6	50	4/7	57.1	4/7	57.1	3/6	50	4/6	66.7	-	18/32	56.2
Gall bladder	2/6	33.3	2/7	28.6	3/7	28.6	1/6	16.6	2/6	33	-	10/32	31.2
Heart blood	2/6	33.3	2/7	28.6	2/7	28.6	1/6	16.6	1/6	16.6	-	8/32	25
Total	10	41.7	11	39.3	11	39.3	7	29.2	9	37.5	-		

No.: Number

Table 9. Performance analysis of seven-day-old duckling under *Salmonella* infection

Parameters \ Groups	Group 1 (*S.* Bargny)	Group 2 (*S.* Tshingwe)	Group 3 (*S.* Uganda)	Group 4 (*S.* Kentucky)	Group 5 (*S.* Enteritidis)	Control
Initial body weight (g; M±SE)	170±5.3	172±5.7	172±5.5	170±6.2	171.71±6.8	173±8.7
Final body weight (g; M±SE)	336±8.7***	0	365.7±8.3***	359±17***	332±6.6***	415±13.1
Relative average final body weight (%)	81	0	88	86.5	80	100
Body weight gain (g; M±SE)	158±5.7***	0	192±3.9***	175.6± 2.48***	158.2±3.52***	230±2.26***
Relative average body weight gain (%)	68.6	0	79.8	78.1	66.5	100
Feed intake (g)	846	0	986	941	829	972
Relative average feed intake (%)	87	0	101.4	96.8	85.5	100
Feed conversion ratio	5.1	0	5.11	4.98	5.15	4.02
Relative average feed conversion ratio (%)	126.9	0	127.1	123.9	128.1	100

*** Significant difference ($p<0.001$) compared to control; group. M ± SE: mean ± standard error

Table 10. Semi-quantitative assessment of the histopathological score in experimentally infected ducks with different *Salmonella* serovars

Isolates	Sampling time (week post-infection)	Liver		Spleen		Intestine	
		Degeneration	Necrosis	Lymphoid depletion	Macrophage infiltration	Enteritis	Hyperplasia of the lining epithelium
S. Bargny	1	++++	+++	+++	++		
*S.*Tshingwe	1	++++	++++	++++	+++		
*S.*Uganda	1	+++	++	++	+++		
*S.*Bargny	2	+++	++	++	+++	++	++++
*S.*Uganda	2	++	++	++	+++	+	++
*S.*Kentucky	2	++	+	+	++	+	++
*S.*Enteritidis	2	++	+	+	++	+	+++
*S.*Bargny	3	++	+	+	++	+	++
*S.*Uganda	3	++	+	+	++	+	++
*S.*Kentucky	3	++	Not detected	Not detected	++	Not detected	+
*S.*Enteritidis	3	++	Not detected	Not detected	++	Not detected	+

+ mild, ++ moderate ,+++ severe focal and ++++ severe diffuse lesions

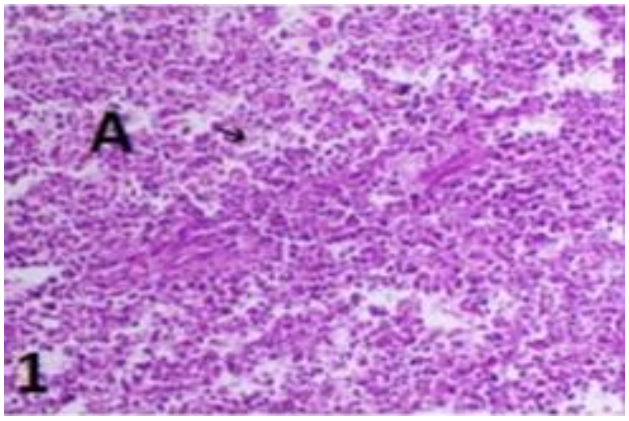

Figure 1. Diffuse necrosis of the lymphoid tissue mostly of liquefactive type (arrow A) in spleen of ducklings infected with *Salmonella* Tshingwe and sacrificed 7 days post-infection.

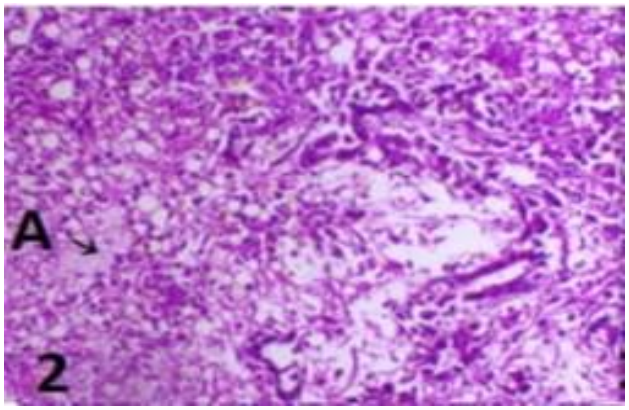

Figure 2. Large necrotic foci (arrow A) in liver of ducklings infected with *Salmonella* Bargny and sacrificed 7 days post-infection.

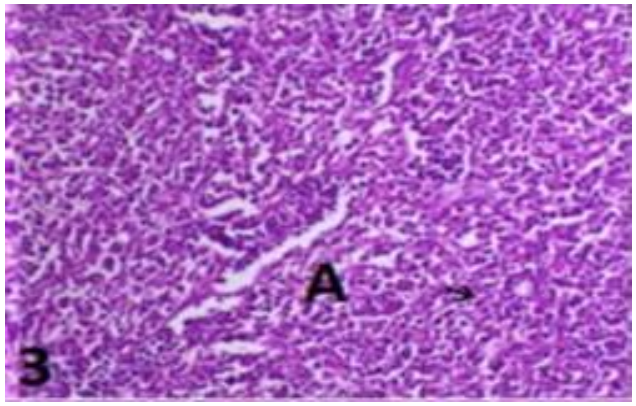

Figure 3. Lymphoid depletion associated with increase the inflammatory cell infiltration within the splenic parenchyma (arrow A) in spleen of ducklings infected with *Salmonella* Uganda and sacrificed 7 days post-infectionn

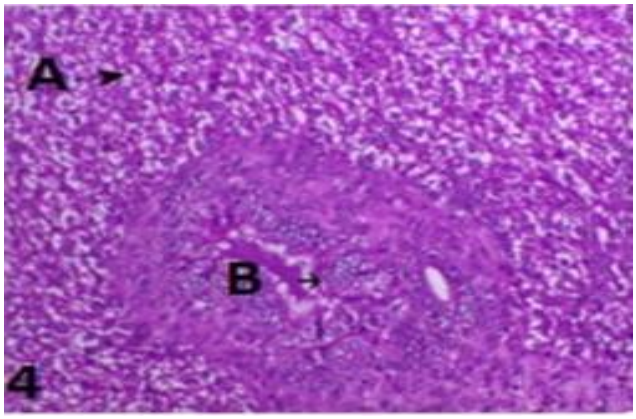

Figure 4. vacuolation of hepatocytes (arrow A) associated with bile duct lining epithelium hyperplasia (arrow B) in liver of ducklings infected with *Salmonella* Bargny and sacrificed 2 weeks post-infection.

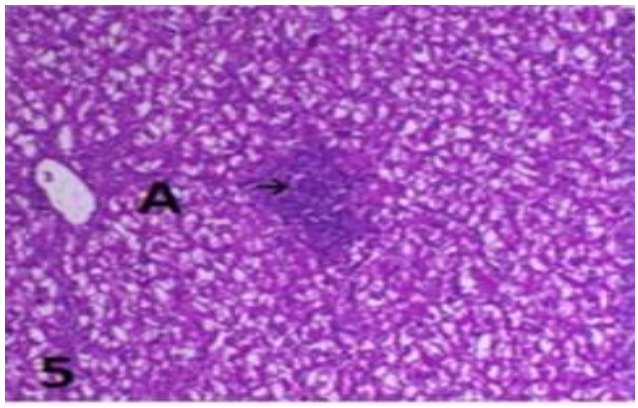

Figure 5. Mononuclear cells infiltration consisted mainly of lymphocytes and macrophages (arrows) and diffuse vacuolation of hepatocytes in liver of ducklings infected with *Salmonella* Kentucky and sacrificed 2 weeks post-infection.

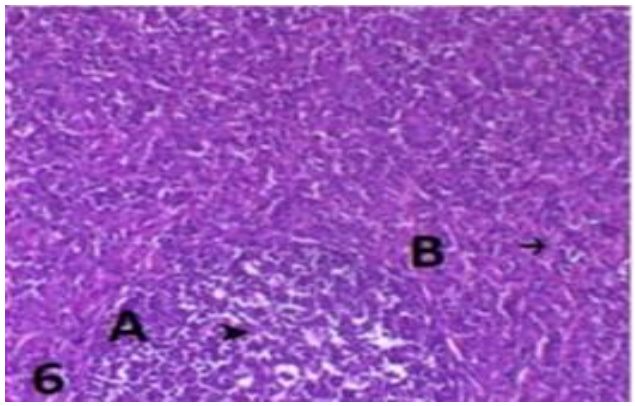

Figure 6. Mild degree of lymphoid depletion (arrow A) and minimal macrophages infiltration (arrow B) in spleen of ducklings infected with *Salmonella* Enteritidis and sacrificed 2 weeks post-infection.

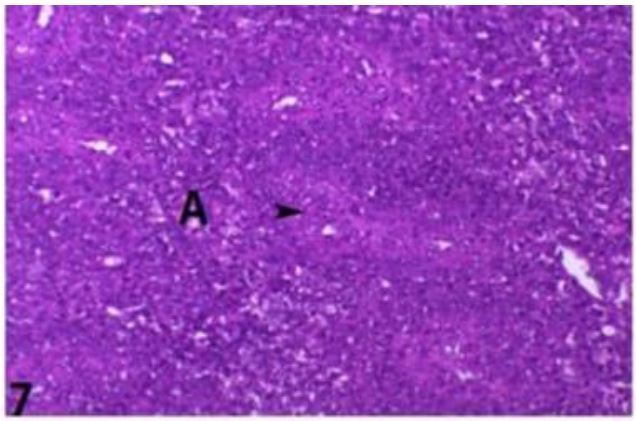

Figure 7. Perivascular histiocytic and macrophages infiltration (arrow A) in spleen of ducklings infected with *Salmonella* Kentucky and sacrificed 3 weeks post-infection.

DISCUSSION

Transmission of *Salmonella* species to waterfowls can be mediated by contaminated feeds, water, and litter (Gast 2003; Grigar et al., 2017). In the present work, *Salmonella* was isolated from litter with a rate of 8%, this rate appears to be higher than that previously reported in Egypt by Dahshan et al. (2015) who reported a rate of 4%. However, Rusul et al. (1996) reported that the isolation rate of *Salmonella* from broiler litter was 20% also Abunna et al. (2016) reported *Salmonella* from poultry litter with a rate of 40%. The recorded high rates of *Salmonella* in poultry litter in this study may be due to the different epidemiological picture of *Salmonella* infection and its shedding in poultry farms in governorates that integrated with fish farms breeding and waterfowls production 8% rather than other governorates with less integrated fish production and this indicates the role of integrated waterfowls and fish farms of the incidence and distribution of *Salmonella* infection and its ecological impact, also the unhygienic measurement in small-scale poultry farms in Kafr El-Sheikh Governorate and it poses a critical point for *Salmonella* transmission to fish farms in the integrated systems because the large scale farms are under veterinary supervision.

Salmonella spp. can be reached to aqua-system by fecal contamination and it has been reported in freshwater fish culture ponds in many countries and also may be present naturally in tropical aquatic environments (Musefiu et al., 2011; Lotfy et al., 2011).

In the present study, salmonellae were isolated from ducks cloacal swabs with a rate of 4% (8 out of 200 pooled samples). This percent is lower than that reported by Mondal et al. (2008) who examined 65 fecal swabs from ducks and reported *Salmonella* with a rate 13.07 % but it is higher than that reported by Hegazy (1991) who isolate *Salmonella* from cloacal swabs of ducks and duckling with incidence 0.98% and 0.72% respectively.

Salmonellae have the ability to multiply and survive in internal organs particularly spleen and liver because these organs can provide places where bacterial multiplication can arise without interruption by host defense mechanism (Gast, 2003). Liver showed *Salmonella* isolation with an incidence of 8.3%. Many authors succeeded to isolate *Salmonella* from liver with a more or less identical incidence as Badr et al. (2015) reported four *Salmonella* isolates from the liver of ducks with a percentage of 6.45%. Also, this incidence is nearly similar to that isolated by Selvaraj et al. (2010) from poultry in India with incidence (6.25%). However, it was lower than that reported in Egypt by Abd-Elghany et al. (2015) who reported *Salmonella* from the chicken liver with an incidence of 32%.

In this study, a total of 13 *Salmonella* isolates were obtained from duck farms integrated with fish farms (200 cloacal swabs and 60 liver samples) with incidence (5%) but this percent is lower than that reported by Lebdah et al., (2017) from ducks in Dakahlia and Damietta Governorates in Egypt with a rate (11.7%), (Mahmoud and Moussa, 2000) reported 25 positive samples for *Salmonella* out of 125 samples from 10 duck flocks in North Sinai with 20% rate.

Salmonella Uganda isolated from ducks in this study with a rate of 7.7% and this may be the first report of *S.* Uganda isolation from ducks in Egypt according to the available literature. This serovar was predominantly isolated from pigs and it was responsible for 4 pork-associated outbreaks in humans between 1998 and 2008 in the USA (Jackson et al., 2013).

In this study, the mortality ranged from 28.6% in *S.* Bargny, *S.* Enteritidis, and *S.* Kentucky and increased to 42.9% in *S.*Uganda and reached up to 100% in *S.* Tshingwe. there is no report about the mortality rate of some of these serotypes according to the available literature, however, Osman et al., (2010) reported the mortality of *S.*Enteritidis in chicken with a rate 88% also they reported the mortality in case of *S.* Kentucky with a rate 40%. Hegazy (1991) reported mortalities from *S.* Enteritidis from ducklings with a rate of 10% while no mortalities were reported in *S.* Tshingwe infected group. In agreement with another investigator, Copper et al., (1992) and Barrow (2000) the postmortem lesions in dead and/or sacrificed ducks generally included congestion of

internal organs, enlargement of spleen, typhlitis and distention of ureter with urates.

Experimental *Salmonella* infection resulted in changes in feed consumption, body weight, and feed conversion. The relative average feed consumption for the infected groups was 13%, -1.4%, 3.2% and 14.5% for *S. Bargny, S. Uganda, S. Kentucky, and S. Enteritidis*, respectively with missing the data belonging to *S.Tshingwe* because the mortality in this group reached to 100% in the first 48 hr. relative average body weight was also affected and showed a reduction ranged from 20.2% to 33.5% among the infected groups at the end of the experiment. These data are in a general agreement with those reported by Levine and Graham (1942) and Williams (1978).

The histopathological picture was in general agreement with that described by several investigators for paratyphoid infection in ducks as El-Sawy, (1976) and chicken as Habib-ur-Rehman et al., (2003) and Haider et al., (2004). However, it is interesting to note that liver degeneration and necrosis with lymphoid depletion and macrophage infiltration were more severe in case of *S.* Tshingwe and this may explain the high mortalities that reached to 100% among this group.

CONCLUSION

Integrated duck farms can be infected with different species of *Salmonella*, which cause high mortality, reduced feed consumption and feed conversion ratio as well as decreased body weight gain. These consequences lead to negative economic impact, particularly when associated with high mortality. Therefore, salmonellosis in integrated duck farms should be investigated periodically.

DECLARATIONS

Competing interests

The authors have no competing interests

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Authors' contribution

Anwaar Mettwally El-Nabarawy contributed in planning, interpretation, and revision of the research, Mohamed Abdel Salaam Shakal design the idea for the research, Abdel-Haleem Mohamed Hegazy contributed in following up adoption of methodology and Mohamed Mohamed Ismail Batikh contributed through performing technical works including sampling, isolation and identification, experimental infection, collecting data and data analysis. All authors approved the final manuscript.

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