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Isolation, Characterization and Pathogenicity of the Most Common Bacteria Associated with Gut Health in Egyptian Broiler Chicken Flocks

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ABSTRACT

This study was designed to isolate and characterize the pathogenicity of the most common bacteria causing enteric diseases in broilers in some Egyptian governorates. Enteric bacterial organisms like *Escherichia coli* (*E. coli*), *Salmonellae*, and *Clostridia perfringens* (*C. perfringens*) were isolated and identified from 100 dead and diseased broilers in Giza, El-Kalubia and El-Sharqia governorates. The samples were subjected to conventional isolation as well as biochemical and serological identification techniques. Molecular and toxigenic detection of *C. perfringens* were performed on one selected isolate by multiplex PCR. The pathogenicity test of some isolated bacterial strains was done on 80 a-day-old broiler chicks. The clinical observation, performance parameters, bacterial re-isolation and histopathological examination were carried out after bacterial challenge. The results revealed isolation of *E. coli*, *Salmonella* and *C. perfringens* in rates of 17, 11 and 39%, respectively. Serological identification of *E. coli* revealed that O78 (35.2%) and O1 (23.5%) were the highest isolated serotypes, while O117 (17.6%), O91 (11.7%), O112, and O146 (5.8% each) were the lowest ones. *Salmonella enteritidis* (*S. enteritidis*) was the most prevalent (36.3%) followed by *S. kentucky*, *S. larochelle* (27.2%, each) and *S. inganda* (9%). Multiplex PCR of *C. perfringens* strains revealed presence of both *cpa* and *cpb* genes that encoding to alpha (α) and beta (β) lethal toxins, respectively. The pathogenicity of *E. coli* O78, *S. enteritidis*, and *C. perfringens* were tested in broiler chickens. The results showed that *E. coli* O78, *S. enteritidis* and *C. perfringens* were pathogenic strains. It was concluded that enteric bacterial pathogens especially *E. coli* O78, *S. enteritidis*, and *C. perfringens* type C are widely distributed in Egyptian broiler chicken flocks and still causing severe losses because of mortalities and decreasing in production.

Key words: *E. coli*; *Clostridium;* Broiler; Egypt; *Salmonella.*

INTRODUCTION

Animal production especially poultry sector represents as a major source of high-quality human's food worldwide. This sector is subjected for infectious and non-infectious constrains that adversely affect the productivity. Enteric infection is regarded as one of the most important problem that causes severe damage of the digestive tract of poultry (Reynolds 2003). Poor feed efficiency parameters as well as high mortalities of birds resulting from intestinal damage due to bacterial enteric infections (Awaad et al. 2019). Enteric diseases like salmonellosis, colibacillosis, and Clostridial infection have a significant and adverse impact on poultry industry as a result of severe worldwide economic losses. These losses include poor growth performance, increased mortality, zoonotic significance as well as increased medication costs (Hussein et al. 2013; Soliman et al. 2018).

Avian colibacillosis caused by *Escherichia coli* (*E. coli*) is one of the main widespread bacterial infection that causes losses and a decrease in the production (Ronco et al. 2017; Ellakany et al. 2019; Mehmood et al. 2020). Some strains of *E. coli* are presented as intestinal commensal of birds, but in weak immune hosts, the pathogenic or even non-pathogenic strains can cause diseases conditions (Sarba et al. 2019). Infection with *E. coli* in poultry may induce acute septicemia with high mortality or appear in subacute form with polyserositis (Calnek et al. 1997). The most prevalent serogroups of pathogenic *E. coli* in chickens are belonging to O78, O1, and O2, and to a lesser extent, O15 and O55 (Rahman et al. 2004; Ali et al. 2019).

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Salmonellosis caused by paratyphoid *Salmonella* species, is one of the major worldwide foodborne pathogens that transmit to human through consumption of raw or insufficient prepared poultry meat. In addition to the significant public health problem, paratyphoid *Salmonella* species have adverse economic impact on poultry industry that represented by high mortality and morbidity, and impaired production (Shoaib et al. 2017; Alam et al. 2020). Multiple *Salmonella* serotypes induce different diseases conditions in nearly all types of avian species (Su et al. 2011). However, *Salmonella enteritidis* (*S. enteritidis*) is regarded as the most dominating serovar that affecting poultry flocks (Schlundt 2002; Afshari et al. 2018; Yasmin et al. 2020; Ahmad et al. 2020).

Necrotic enteritis is an important disease condition that is characterized by high mortality, poor feed conversion, weight losses as well as decreasing in egg production of the affected birds (Sarkar et al. 2013; la Mora et al. 2020). Coccidial affection is the main predisposing factor for initiation of necrotic enteritis (Palliyeguru et al. 2010). The disease in poultry is regards as enterotoxaemia caused by *Clostridium perfringens* (*C. perfringens*) (Williams 2005). Based on the production of major lethal toxins α, β, Ɛ and iota (ι). *Clostridium perfringens* can be classified into five toxin production biotypes (A, B, C, D, and E) (Uzal et al. 2014). Types A and C of *C. perfringens* are the most important types that affecting poultry. However, *C. perfringens* type A is regarded as the most common type and to a lesser extend type C. Strains of *C. perfringens* type A have gene coding α-toxin (*cpa*), while type C have both α-toxin (*cpa*) and β-toxin (*cpb*) gene encoding (Enstrom et al. 2003; Abd El-Hamid et al. 2015). Accordingly, this work was designed to isolate, characterize, and detect the pathogenicity of the most common bacteria causing enteric diseases in broiler chickens in some Egyptian governorates.

MATERIALS AND METHODS

The pathogenicity test was carried out after the approval of the Institutional Animal Care and Use Committee, Cairo University (CU-IACUC) (CU II F 3 20).

Samples Collection

This research work was conducted during the period from April till September 2019. A total of 100, 4-6-weeks-old diseased and freshly dead broilers with a history of diarrhea, depression, and reduced growth performance were collected from different flocks in Giza, El-Kalubia, and El-Sharkia governorates of Egypt. The examined dead birds showed severe enteritis, general congestion of the internal abdominal viscera as well as perihepatitis, pericarditis and peritonitis. Samples including heart, liver, and intestine were collected, identified, and rapidly transported to the laboratory in an icebox for further processing.

Isolation and Characterization of the Enteric Bacterial Strains

Isolation of *E. coli* was done according to Lee and Nolan (2008). Collected samples were cultured on tryptic soy broth and incubated at 37°C for 18 hours. A loop full of incubated broth was streaked into MacConkey's agar and incubated at 37°C for 18-24 hours. The suspected *E. coli* colonies were subjected for morphological and biochemical identification. The isolates were serologically identified as Kok et al. (1996) by using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co, Japan).

Besides, isolation and identification of *Salmonella* species were done according to standard methods (ISO 6579 2002). Rappaport-Vassiliadis enrichment broth was used for isolation of *Salmonella* and incubated at 37°C for 18 hours. A loop-full of culture was streaked into Xylose Dextrose (XLD) and MacConkey agar and incubated at 37°C for 18-24 hours. The morphological and bio-chemical identification were carried out on the suspected colonies, while the serotyping was done as described by Kauffman-white scheme (Kauffman 1974; Popoff 2001) for somatic (O) and flagella (H) antigens using *Salmonella* antiserum (DENKA SEIKEN Co. Japan).

For *Clostridia* isolation, the samples were inoculated into a cooked meat broth medium and incubated in an anaerobic gas pack jar for 24 hours at 37°C. A loop full of broth culture was streaked onto 10% sheep blood agar, supplemented with neomycin sulfate (200µg/ml) (Carter and Cole 1990) and incubated anaerobically at 37°C for 24 hours. Gram staining from suspected colonies were prepared for microscopical examination. Biochemical identification was applied (Koneman et al. 1988). The strains were maintained in 25% glycerol stock at −80°C until further use.

Detection of *Clostridium perfringens* Toxins using Multiplex PCR

Genomic DNA was extracted from *C. perfringens* strain by culturing on blood agar media using QIAamp Mini Kit (Cat No. 51304). The specific three primer sets design corresponding to each α, β and Ɛ toxins of *C. perfringens* (Komoriya et al. 2007; van Asten et al. 2009) is shown in Table 1. The PCR was performed in a Thermal Cycler® (Bio-Rad, USA) in a total reaction volume of 50M containing 25M of 2x Master Mix (thermos scientific, 1M of 1pmol of each primer and 5M of template DNA). Cycling conditions of the primers during multiplex PCR is represented in Table 2. Then, 8M of the amplified product was electrophoresed in a 1.5% agarose gel and stained with ethidium bromide. Amplified bands were visualized and photographed under ultraviolet illumination.

Table 1: Primers used in multiplex PCR for the detection of toxin's type of *C. perfringens*

Amplicon (bp)	Primer position	Sequence	Gene	Toxin
324	663-968	GCTAATGTTACTGCCGTTGA CCTCTGATACATCGTGTAAG	*Plc* (*cpa*)	α
196	871-1045	GCGAATATGCTGAATCATCTA GCAGGAACATTAGTATATCTTC	*cpb*	β
655	267-862	GCGGTGATATCCATCTATTC CCACTTACT TGTCCTACTAAC	*etx*	ε

The Pathogenicity Test

Eighty, day-old chicks obtained from local hatchery were divided into 4 equal groups consisting of 20 birds each. Each group was housed in a thoroughly cleaned and disinfectant pen, given feed and water *ad libitum* as well as vaccination programs according to the manufacturer's instructions. Group 1 was a negative control non-challenged birds, while groups 2, 3, and 4 were challenged orally at 2 weeks of age. Each bird of the challenged groups was inoculated with 1ml containing $1x10^8$ Colony Forming Unit (CFU) for *C. perfringens* type C and $1x10^9$ for *S. enteritidis* and *E. coli* O78 (Barrow et al. 1999; Botlhoko 2009; Johny et al. 2012; Abd El-Tawab et al. 2015b). Chickens in *C. perfringens* challenged group were orally inoculated with a mixture of *Eimeria* species oocysts (0.25ml/bird) at 12 days old to stimulate necrotic enteritis induction (Williams et al. 2003). The body weight and feed consumption of each group were taken weekly to measure the feed conversion ratio (FCR). Moreover, clinical signs, mortality rates, and post-mortem lesions of chickens in the challenged groups were recorded every day for 3 weeks later. Specimens including heart, liver, and intestine were collected from dead and euthanized birds (at the 3rd week post-challenge) for bacterial re-isolation and histopathological examination.

The Histopathological Examination

Specimens including liver, intestine and heart were collected and fixed in 10 % formalin solution, dehydrated in different grades of ethyl alcohol, and embedded in paraffin. Tissues of 5 microns thickness were stained with hematoxylin and Eosin (H&E) and microscopically examined using light microscope (Slaoui and Fiette 2011).

Statistical Analysis

Data were expressed as mean±SE. Statistical comparison between the mean of the different groups was made by One-Way Analysis of Variance (ANOVA) and multiple comparisons between groups (post hoc) LSD using SPSS version 26. A probability $P \leq 0.05$ was assumed for statistical significance.

RESULTS

The results of isolation of some enteric bacterial strains in different Egyptian governorates are illustrated in Table 3. From the table, it could be observed that the isolation rate of *E. coli* from 17 out of 100 samples was 17%. Culturing of suspected *E. coli* samples on MacConkey's agar showed medium-sized, rounded, and pink colonies. Gram's staining revealed Gram-negative, non-sporulated bacilli that arranged single, pairs, and in groups. In addition, the biochemical tests of the suspected isolates showed negative oxidase, urease, citrate utilization, and Voges Proskauer tests, but positive catalase, indole, methyl red, nitrate reduction and triple sugar iron tests. The isolates showed motility in semisolid agar media. The serological identification of the suspected *E. coli* isolates in different Egyptian governorates is shown in Table 4. The results demonstrated that the most predominant serotypes were O78 (6 isolates) followed by O1 (4 isolates), O117 (3 isolates), O91 (2 isolates), and O112, O146 (one isolate; each).

Regarding the isolation of *Salmonellae*, the isolation rate of *Salmonellae* was 11% as 11 out 100 samples were positive (Table 3). Colonies of suspected *Salmonellae* cultures appeared as pink with black center on XLD agar and colorless ones on MacConkey agar, while Gram-negative medium size bacilli were seen in stained culture smears. Biochemical identification of cultures showed positive catalase, methyl red, citrate utilization, H_2S production tests, while negative oxidase, indole, urease, Voges Proskauer, and gelatin liquefaction tests. Sugar fermentation was positive for glucose, maltose, and xylose, whereas negative for lactose and sucrose. Serological identification of the suspected *Salmonella* isolates in different Egyptian governorates was *S. enteritidis* (4), *S. kentucky* (3), *S. larochelle* (3), and *S. inganda* (1) as represented in Table 5.

Table 2: Cycling conditions of the primers during multiplex PCR for the detection of toxin's type of *C. perfringens*

Primary Denaturation	Secondary Denaturation	Annealing	Extension	No. of cycles	Final Extension
95°C	94°C	55°C	72°C	35	72°C
10 min.	45 sec.	30 sec.	90 sec.		10 min.

Table 3: The isolation rate of enteric bacterial strains in different Egyptian governorates

The governorate	Status of examined birds	No. of samples	The isolated enteric bacterial isolates					
			E. coli		*Salmonella*		*Clostridia*	
			No. of positive samples	% of positive samples	No. of positive samples	% of positive samples	No. of positive samples	% of positive samples
Giza	Diseased	20	2	10*	1	5*	5	25*
	Dead	6	1	16.7*	1	16.7*	2	33.3*
	Total	26	3	11.5**	2	33.3**	7	26.9**
El-Kalubia	Diseased	35	5	14.3*	4	11.4*	18	51.4*
	Dead	8	3	37.5*	2	25*	3	37.5*
	Total	43	8	18.6**	6	14**	21	48.8**
El-Sharqia	Diseased	25	4	16*	0	0	9	36*
	Dead	6	2	33.3*	3	50*	2	33.3*
	Total	31	6	19.9**	3	9.7**	11	35.5**
Total		100	17	17***	11	11***	39	39***

*The percentage was calculated according to each bird's status; **The percentage according to the total number in each governorate; ***The percent according to the total number of tested samples.

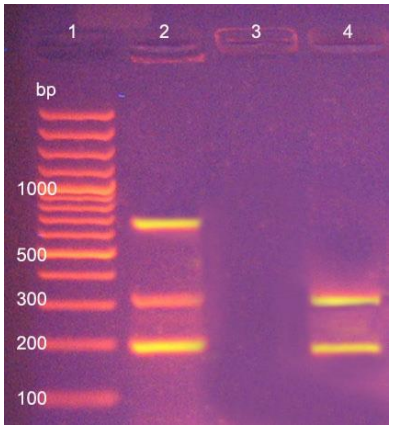

Fig. 1: Agarose gel (1.5%) electrophoresis of multiplex PCR products of *C. perfringens* toxins; Lane 1: DNA marker (Gene Ruler 100 bp DNA Ladder, Thermo-scientific); Lane 2: Control Positive (mix of various toxin types); Lane 3: Control negative and Lane 4: PCR product of the isolate toxin.

Table 4: The serological identification of *E. coli* isolates in different Egyptian governorates

The governorate	Total No. of isolated *E. coli*	Serogroup	No. of each serotype
Giza	3	O78	1
		O146	1
		O91	1
El-Kalubia	8	O78	3
		O1	3
		O91	1
		O112	1
El-Sharkia	6	O117	3
		O78	2
		O1	1
Total		17	

Table 5: The serological identification of *Salmonella* isolates in different Egyptian governorates

The governorate	Total No. of isolates	Serotypes	No. of each serotype
Giza	2	*S. kentucky*	1
		S. inganda	1
El-Kalubia	6	*S. kentucky*	2
		S. enteritidis	2
		S. larochelle	2
El-Sharkia	3	*S. enteritidis*	2
		S. larochelle	1
Total		11	

The results of *C. perfringens* isolation rate that presented in Table 3 demonstrated that out of 100 examined samples, 39 (39%) were positive. Anaerobic incubation of suspected samples on sheep blood agar showed presence of small, circular, smooth, flat, glistening and greyish colonies surrounded by double zones of hemolysis. Microscopically, Gram-positive spore-forming bacilli were noticed. Biochemically, positive results for lecithinase activity on yolk agar as well as sugar fermentation of glucose, lactose, maltose, and sucrose were demonstrated. Nevertheless, negative results for indole production, catalase, and oxidase were also found. The results of multiplex PCR for detection of the toxin types for *C. perfringens* revealed presence of the gene coding α toxin (*cpa*) and the gene encoding β toxin (*cpb*) (Fig. 1). The latter results indicated presence of *C. perfringens* type C.

Regarding the pathogenicity test, the mortality rate and the post-mortem lesions in different challenged groups are seen in Table 6. No clinical signs were appeared in negative control non-challenged birds, but birds challenged with *E. coli* O78 showed depression, brownish diarrhea, nasal and ocular discharge and conjunctivitis. Greenish diarrhea and poor growth were seen in chickens challenged with *S. enteritidis*, while bloody diarrhea, and ruffled feather were noticed in birds challenged with *C. perfringens*. Control negative non challenged birds showed no mortalities while chickens challenged with *E. coli* O78, *S. enteritidis*, and *C. perfringens* revealed mortality rates of 25, 15 and 35%, respectively. The necropsy findings of dead and sacrificed bird's revealed perihepatitis, pericarditis and enteritis in group challenged with *E. coli* O78; enlarged liver with necrotic foci, perihepatitis, enlarged congested kidney and enteritis in group challenged with *S. enteritidis*, as well as friable ballooned intestine with hemorrhagic mucosa and necrotic pale liver in group *C. perfringens*.

Table 7 shows the average body weight and FCR in different groups. In comparison with control negative non-challenged group, challenged groups revealed significant decrease in body weight and feed consumption with $P \leq 0.05$ that reflected on the FCR.

The re-isolation rates of bacteria from different organs in different challenged groups are present in Table 8. The re-isolation rates of *E. coli*, *Salmonella*, and *Clostridia* from visceral organs were 85, 70, and 85% in birds challenged with *E. coli* O78, *S. enteritidis* and *C. perfringens*, respectively. In a group challenged with *E. coli* O78, the highest incidence of re-isolation was from heart (88.2%), then from liver (76.4%) and intestine (70.5%). For chickens challenged with *S. enteritidis*, the highest re-isolation was from liver (92.8%), intestine (85.7%) followed by heart (64.2%), while in birds challenged with *C. perfringens*, this rate was 88.2% from the intestine and 70.5% from the liver.

Fig. 2 to 4 show the histopathological examination results in the challenged groups of birds. The different lesions in the liver, intestine and heart of group challenged with *E. coli* O78 are demonstrated (Fig. 2A to 2D). Chickens exhibited diffuse hepatocellular necrosis which was characterized by numerous sporadic cell necrosis among the hepatocytes admixed with accumulation of diffuse edema, eosinophilic debris, and a variable number of mononuclear inflammatory cells infiltration. The intestine showed severe necrosis of the intestinal villi associated with intense inflammatory cells infiltration. The myocardial muscle revealed diffuse myocarditis which was illustrated by extensive number of mononuclear inflammatory cells infiltration.

Fig. 3A to 3D show the hepatic and intestinal microscopic alterations of *S. enteritidis* challenged chickens. The hepatic parenchyma showed multifocal edematous areas accompanied by dilation of the hepatic sinusoids. Numerous clusters of basophilic septicemic bacteria were detected in the hepatic sinusoids. The hepatocytes suffered from widespread coagulative necrosis. The intestinal sections showed severe necrosis which is characterized by atrophy of intestinal villi associated with accumulation of eosinophilic and karyorrhectic debris.

Table 6: The mortality rates and post-mortem lesions in different challenged groups

Group No.	Challenge bacteria	The mortality rate	Post-mortem lesions
1	-	0 (0)	-
2	*E. coli* (O78)	5 (25)	Perihepatitis, pericarditis and enteritis
3	*S. enteritidis*	3 (15)	Enlarged liver with necrotic foci, perihepatitis, enlarged congested kidney and enteritis
4	*C. perfringens*	7 (35)	friable ballooned intestine with hemorrhagic mucosa and necrotic pale liver

Values in parenthesis indicate percentage.

Table 7: The average body weight (g/bird) and the FCR in different groups

Group No.	Challenge bacteria	Average body weight (g/bird)					FCR
		Weeks before infection		Weeks after infection			
		W1	W2	W3	W4	W5	
1	-	160.7±3.1^{a}	422.2±10.0^{a}	853.0±19.3^{a}	1347.0±9.7a	1898.0±26.0a	1.673^{a}
2	*E. coli* (O78)	161.0±4.6^{a}	430.7±10.9^{a}	762.1±12.6^{b}	1223.8±15.0^{b}	1681.2±15.5^{b}	1.794^{b}
3	*S. enteritidis*	155.2±4.0^{a}	432.0±10.4^{a}	777.1±17.9^{b}	1223.6±14.1^{b}	1682.5±14.4^{b}	1.777 ^{b}
4	*C. perfringens*	163.25±4.4^{a}	425.2±10.0^{a}	757.5±16.8^{b}	1212.3±18.8^{b}	1676.0±20.8^{b}	1.789^{b}

W=Week; FCR=Feed conversion ratio. Mean±SE in the same column followed by the different superscripts is significantly different according to ANOVA (LSD, P≤0.05).

Table 8: The re-isolation rates of bacteria from different organs in different challenged groups

Group No.	Challenge bacteria	The total re-isolation rate	The re-isolation rate from different organs		
			Liver	Heart	Intestine
1	-	0 (0)	0 (0)	0 (0)	0 (0)
2	*E. coli* (O78)	17/20 (85)	13/17 (76.4)	15/17(88.2)	12/17 (70.5)
3	*S. enteritidis*	14/20 (70)	13/14 (92.8)	9/14 (64.2)	12/14 (85.7)
4	*C. perfringens*	17/20 (85)	12/17 (70.5)	0/14 (0)	15/17 (88.2)

Values in parenthesis indicate percentage.

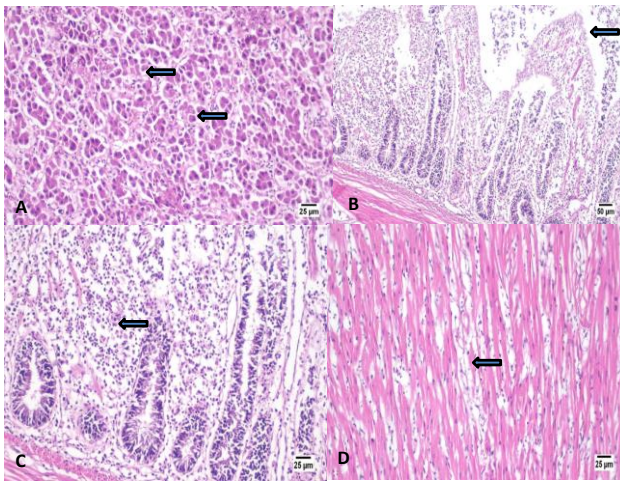

Fig. 2: A) Liver of *E. coli* challenged group showing sporadic cell necrosis (arrows); B) Intestine of *E. coli* challenged group showing severe necrosis of the intestinal villi (arrow); C) Intestine of *E. coli* challenged group showing intestinal necrosis admixed with accumulation of inflammatory cells and tissue debris in the submucosa (arrow) and Heart of *E. coli* challenged group showing myocarditis (arrow). H&E. Bar=25µm (A, B and D); 50µm (C).

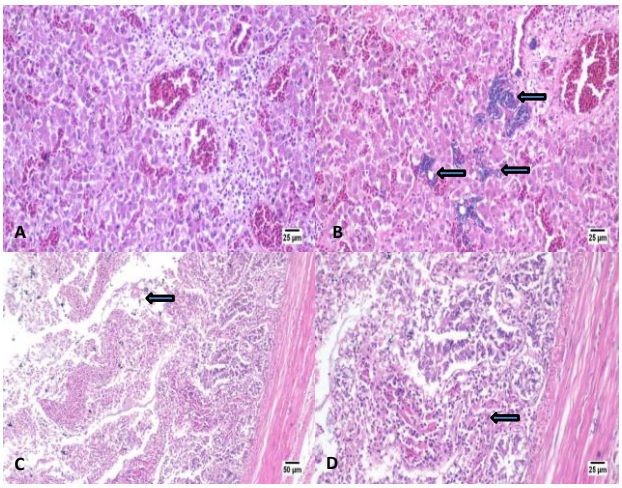

Fig. 3: A) Liver of *S. enteritidis* challenged group showing congested hepatic sinusoids, edema and few inflammatory cells infiltration; B) Liver of *S. enteritidis* challenged group showing multifocal aggregation of bacterial colonies (arrows); C) Intestine of *S. Enteritidis* challenged group showing severe necrosis of the intestinal villi, crypts and intestinal glands (arrow) and D) Intestine of *S. enteritidis* challenged group showing severe necrosis of the intestinal glands admixed with mononuclear inflammatory cells infiltration in the submucosa (arrow). H&E. Bar=25µm (A, B and D); 50µm (C).

In *C. perfringens* challenged group, variable lesions have been noticed in the liver and intestine (Fig. 4A to 4D). The liver showed multifocal to diffuse hemorrhagic areas accompanied by disorganization of the hepatic parenchyma. The hepatocytes showed marked necrobiotic changes that showed vacuolation and several stages of nuclear necrosis that including karyorrhexis, karyolysis, and pyknosis. The intestine revealed marked atrophy of the intestinal villi accompanied by enteritis that showed expansion of the submucosal layer with edema and mononuclear inflammatory cells infiltration.

DISCUSSION

Enteric bacterial diseases are regarded as one of the most important group of diseases that affect broiler chickens and causes severe economic losses in poultry production (Hafez 2011; Abdel-Alim and Ahmed 2020). Therefore, this study is focused on isolation and characterization of some important enteric bacteria in different Egyptian governorates.

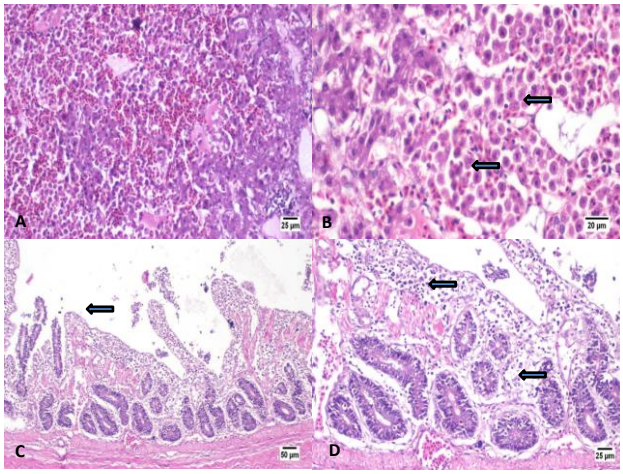

Fig. 4: A) Liver of *C. perfringens* challenged group showing hepatic hemorrhages; B) Liver of *C. perfringens* challenged group showing several stages of nuclear necrosis in the affected hepatocytes (arrows); C) Intestine of *C. perfringens* challenged group showing marked atrophy and shortening of the intestinal villi (arrow) and D) Intestine of *C. perfringens* challenged group showing expansion of the submucosa with edema and mononuclear inflammatory cell accumulation (arrows). H&E. Bar=25µm (A, B and D); 50µm (C).

Here, *E. coli* strains have been detected in 17 out of 100 samples with isolation rate of 17%. Previous Egyptian studies on *E. coli* revealed either higher isolation rates 75% (Abd El-Tawab et al. 2015a), 43.1% (Heba et al. 2012), 35% (Amer et al. 2018), 34.6% (Ellakany et al. 2019) and 27.7% (Abd El-Mongy et al. 2017), while other showed lower isolation rate 8.3% (Ahmed et al. 2017). These differences in the isolation rates may be owing to the differences in the number of samples, the age of birds, the geographic regions, the isolation methods, and previous medication. The cultural morphology, Gram staining characters and biochemical reactions of suspected *E. coli* isolates were similar to those reported by Hadiujjaman et al. (2016), Surra et al. (2018) and Ibrahim et al. (2019). Moreover, the serological identification results of this study revealed that the main serotypes of the isolated *E. coli* were O78 and O1. These findings are parallel to the studies of Rahman et al. (2004) and Ibrahim et al. (2019) who found that *E. coli* O78, O1, and O2 were the predominant serotypes of the pathogenic *E. coli* in chickens. Besides, the Egyptian study of Amer et al. (2018) declared that 25% of *E. coli* isolates were O78.

This study showed isolation of *Salmonellae* in percentage of 11 (11/100). Similar isolation rates were previously reported by Kaoud et al. (2018) and Soliman et al. (2018) who isolated *Salmonellae* from the Egyptian broiler flocks in rates of 11.33 % and 10.37%, respectively, Moreover, in Seri Lanka, Jayaweera et al. (2020) demonstrated isolation of *Salmonellae* from broiler chickens in rate of 11.6%. However, Egyptian studies of Sedeik et al. (2019) and El-Sheikh et al. (2019) showed isolation of *Salmonellae* from broiler chicks in rates of 7.5% and 16%, respectively. Regarding the colonial morphology and biochemical tests results of *Salmonella*, parallel results were also described by Akeila et al. (2013), Islam et al. (2016), Al-Mamun et al. (2017) and Mridha et al. (2020). Moreover, the serological results of the isolated *Salmonellae* showed that *S. enteritidis* and *S. kentucky* were the most among the isolates. Similar findings were reported by Moawad et al. (2017), Al-Baqir et al. (2019) and Sedeik et al. (2019) who demonstrated that *S. enteritidis* and *S. kentucky* were the most highly prevalent strains that isolated from chicken farms in Egypt. In addition, Wang et al. (2020) demonstrated variations of *Salmonella* serotypes in chicken's flocks and found that *S. enteritidis* was the most common isolated strain.

In addition, *C. perfringens* was isolated in rate of 39%. Nearly similar isolation rate of *C. perfringens* was recorded in the study of Osman et al. (2012) who found that out of 48 broiler chicken samples, 35.4% were *C. perfringens*. Moreover, Abd-Elall and Maysa (2014) and Helal et al. (2019) declared that the prevalence of *C. perfringens* in broilers was 38.7 and 38.3%, respectively. However, higher isolation rates of *C. perfringens* (75%) and (77.4%) were observed in the studies of El-Jakee et al. (2013) and Abd El-Hamid et al. (2015), respectively. The phenotypic characterization findings of *C. perfringens* isolates were similar to others (Das et al. 2008; Malmarugan et al. 2012; Dar et al. 2017). The results of multiplex PCR of *C. perfringens* strains confirmed presence of the gene coding α-toxin (*cpa*) and the gene encoding β-toxin (*cpb*). Although *C. perfringens* type A is the predominant worldwide biotype type that isolated from chickens (Hatakka and Pakkala 2003; Merati et al. 2017), this works proved isolation of *C. perfringens* type C. Few studies either worldwide or locally showed isolation of *C. perfringens* type C from broiler chickens (Afshari et al. 2015).

Challenged groups with *E. coli* O78, *S. enteritidis*, and *C. perfringens* should variable diarrhea, respiratory signs and poor growth rate along with mortality rates of 25, 15 and 35%, respectively. Autopsy findings showed polyserositis and enteritis, liver necrosis, congested kidney and enteritis, along with friable ballooned intestine with haemorrhagic mucosa and necrotic pale liver in groups challenged with *E. coli* O78, *S. enteritidis*, and *C. perfringens*, respectively. Similar post-mortem observations in different groups were observed by Cooper and Songer (2010), Belih et al. (2016) and El-Sawah et al. (2018).

It has been observed significant ($P \leq 0.05$) differences in the average body weight and FCR in the challenged groups when compared with non-challenged group. Our results agreed with Abd El-Tawab et al. (2015b) who demonstrated depressive effect of *E. coli* on the performance parameters of chickens, Belih et al. (2016) who found significant reduction in body weight of chicken after challenge with *Salmonella*, as well as Abd El-Hamid et al. (2017) who demonstrated decrease in body weight, feed consumption and FCR in *C. perfringens* challenged chickens.

Re-isolation rates of 85%, 70%, and 85% have been recorded in chickens challenged with *E. coli* O78, *S. enteritidis* and *C. perfringens*, respectively. These results are in partial agreement with Atta et al. (2014), Abd El-Tawab et al. (2015a) and Gast et al. (2013).

The histopathological findings of chicken's liver, intestine and heart in group challenged with *E. coli* O78 are supported by the results obtained by Abalaka et al. (2017) and Shah et al. (2019). However, the microscopic changes of the liver and intestine of *S. enteritidis* challenged

chickens revealed similar results to Saha et al. (2012) and Muna et al. (2016). The microscopic findings of the intestine and liver of *C. perfringens* challenged group correspond with other authors (Abd El-Hamid et al. 2015; Park et al. 2015).

From the previous findings, it may be concluded that many Egyptian broiler chicken's flocks showed circulation of different types of enteric bacterial pathogens, particularly *E. coli* O78, *S. enteritidis*, and *C. perfringens* type C. These organisms are responsible for problems in poultry flocks resulting from increased morbidity, mortality, loss of productivity and public health significance. Periodical surveillance studies should be conducted to detect the epidemiological situation of these infections in poultry flocks. In addition, application of biosecurity measure is essential to prevent and/or eliminate these pathogens.

Author's Contribution

Ali Z. Qandoos shared in the laboratory work, the experimental study and the collection of data, Nayera M. Alatfeehy shared in the laboratory work and experimental study and collection of data and Wafaa A. Abd El-Ghany designed the work, shared in the experimental study along with writing and submission of the manuscript. All authors approved the final version.

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