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Differentiation Between *E. coli*s Strains Causing Diarrhea in Broiler Chicken by Using Multiplex PCR

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THIS TRIAL was to investigate the outbreak of acute diarrhea in acute diarrhea in poultry birds at Ismailia, and North Sinai for North Sinai for detection and characterization of shiga toxin *Escherichia coli* **(**STEC). Two hundred samples from natural diseased and emergency slaughtered broiler chicks were collected from poultry farms in Ismailia and North Sinai. All cases were subjected to post-mortem, bacteriological examination, hematological and biochemical analysis. *Escherichia coli (E. coli)* was isolated and identified from cloacal swabs, intestinal contents, heart blood and liver of poultry birds that died due to acute diarrhea. Phenotypic characterization was done by standard bacteriological and biochemical techniques. All the isolates were serotyped based on their somatic antigens. Virulence genes (*stx1*, *stx2*, *eaeA* and *hlyA*) were detected by multiplex PCR assay.A total of 20 *E. coli* isolates were obtained, of which O6(6), O111 (2), O55 (3), O114(2), O15(3), O125(2), and untyped (2). Out of 20 serotype, O6 carried one virulence gene of stx1 and O125 carried one virulence gene of stx 2. The other all serotype didn't carried virulence gene. The biochemical analysis of blood showed increase in AST and ALT and a significant change inprotein. Hypoalbuminemia, was observed and increase of serum uric acid, creatinine and phosphate as well as decrease in level of potassium,calcium and sodium. Blood examination revealed a significant decrease in RBcs count, hemoglobin (Hb) concentration and packed cell volume (PCV) in the affected birds indicate anemia of microcytic hypochromic.It could be concluded thatthe isolated bacterial pathogens play an important role in causing diseases in poultry and human consumer due to presence of toxin and losses in poultry farms at Ismailia and North Sinai.

Keywords: *Escherichia coli*, broiler chicken, multiplex PCR, Virulence genes.

Colibacillosis is one of the most important diseases threatening the poultry industry (Ibrahim, 1998, Ewers *et al.,* 2005 and Ayoub, 2007). Colibacillosis in chickens refers to any local or systemic infection caused entirely or partly by *E. coli* strains (Barnes *et al.,* 2003). *Escherichia coli*, strains causing systemic disease in poultry (avian colibacillosis) are termed avian pathogenic *E. coli* (APEC) (Ewers *et al.,* 2005). Traditionally, it was mostly associated with losses in broilers but, recently the incidence of the disease in layer hens has been substantially increased in many European countries (Zanella *et al.,* 2000, Vanderkerchove *et al.,* 2004 and Jordan *et al.,* 2005).

Escherichia coli, has been associated with a variety of diseases in poultry such as pericarditis, perihepatitis, airsacculitis, peritonitis, salpingitis, panophthalmitis, omphalitis, cellulites, colispticemia, coligranuloma and swollen-head syndrome (Saif *et al*., 2003).

Strains of *E. coli* predominate among the aerobic commensal flora in the gut of humans and animals. These bacteria are widespread and present wherever there is fecal contamination, causing pollution of water sources, drinking water and food. The species encompasses a variety of strains, which may be purely commensal or possess combinations of pathogenic mechanisms that enable them to cause disease in man and other animals (Greenwood *et al*., 2002). The avian intestinal tract harbors both potentially pathogenic and commensal *E. coli* strains and infections generally arise from inhalation of contaminated dust particles in poultry houses (Dziva, 2010).The versatility of *E. coli* is due to the fact that different strains have horizontallyacquired different virulence genes (Salyers and Whitt, 2002).

Even though, molecular methods for identifying specificvirulence genes are available, serotyping remains a useful toolfor epidemiologic studies. Numerous surveys have been made inmany parts of the world to determine serotypes most frequently associated with disease in poultry caused by *E.coli* (Sharada *et al*., 2001, Rosario *et al*., 2004 and Thangapandian *et al*., 2006). Enterotoxigenic *Escherichia coli* (ETEC) serotype O6 and O111 was the most common ETEC serotype identified during outbreaks occurring in the United States (Dalton *et al.*, 1999). Shiga toxin-producing Escherichia coli (STEC) are major food-borne pathogens associated with gastroenteritis and sometimes fatal haemolyticuraemic syndrome complication to human consumer (Dalton *et al*., 1999, Beatty *et al*., 2004 and Ojo *et al*., 2010).

Hematological alterations were recorded the severity of infection of *E. coli*, a significant decrease in RBcs count, Hb concentration and PCV in the affected birds indicate anemia of microcytichypochromic (Mona *et al*., 2012). Moreover, The biochemical analysis recorded increase in AST and ALT and a significant change inprotein. Hypoalbuminemia, was observed, increase of serum urea, creatinine (Mona *et al*., 2012).

Aim of the work: The specific objective of this study was planned as the following: isolation and identification of *E.coli* from different organsof broiler chicken as well as from cloacal swab, serotyping of isolated *E. coli*, detection of virulence genes of isolated *E.coli* by using of polymerase chain reaction (PCR) and determined the effect of these pathogenic organisms on blood indices with liver and kidney functions.

Material and Methods

Collection of samples

An outbreak of diarrheal disease in farms of broiler chickens at Ismailia and North Sinai in 2012 was attended. A total of 200 birds were collected (50emergency slaughter and 150 diseased). These birds were brought to the microbiology and clinical pathology labs in Ismailia and Alarish. During post-mortem examination, cloacal swabs, intestinal contents, heart blood, kidney and pieces of liver were collected aseptically for isolation and identification of causative agents.

Bacteriological screening of clinical specimens

The clinical samples (heart blood, liver, kidney, cloacal swab, intestinal contents) were immediately inoculated on 10 per cent sheep blood agar and MacConkey's agar (HiMedia, Mumbai, India) plates and incubated at 37°C for 18-24 h. Pure and a single population of bacterial colonies were recorded from all samples. Five randomly selected colonies from MacConkey's agar and 10 per cent sheep blood agar plates were picked up and subcultured on eosin methylene blue (EMB) agar (HiMedia, Mumbai, India) plates to observe the characteristic metallic sheen of *E. coli*. The well separated pure colonies were picked up on nutrient agar slants as pure culture and subjected for standard morphological and biochemical tests (Quinn, *et al.,* 1994).

Serotyping of E. coli

The 20 *E. coli* isolates were serotyped based on their somatic (O) antigens at Animal health research institute, Dokki, Egypt.

Preparation of E. coli DNA for PCR assay

Boiling method (Bansal, 1996). Bacterial pellets were washed once with 1 ml phosphate buffered saline (PBS), pH 7.4, resuspended in a same volume of cold water and incubated in a boiling water bath for 10 min. The clear supernatants obtained after a 5 min centrifugation at 12000g were used for PCR reaction.

Detection of virulence genes by multiplex PCR

A multiplex PCR was carried out using 4 sets of oligonucleotide primers for *stx* 1, *stx* 2, *eaeA* and *hlyA* genes (Table 1). The PCR protocol was followed as per the method described by Paton and Paton1 (1998) with some modifications. In brief, the multiplex PCR mixture of 25.0 µl contained 1X PCR buffer, 1.5 mM of MgCl2, each primer within the 4 primer sets at a concentration of 40 nM, 200 µM each of dNTPs, 1.0 U of *Taq*DNA polymerase and 2.0 µl of template DNA. The PCR reaction was performed in a thermal cycler (Thermo Electron, Germany) using the following standard cycling procedure: an initial denaturation at 95°C for 5 min, followed by 30 cycles of denaturation at 94°C for 45 sec, primer annealing at 59°C for 45 sec and extension at 72°C for 1 min and a final extension at 72°C for 6 min.

Amplified products were separated by agarose gel (2% agarose in 1X Tris-borate-EDTA buffer) electrophoresis at 5v/cm for 2 hr and stained with ethidium bromide (0.5 µg/ml)14. Standard molecular size marker (100 bp DNA ladder) was included in each gel. DNA fragments were observed by ultraviolet transilluminator and photographed in a gel documentation system (Alpha Imager, Germany). Amplified products were separated by agarose gel (2% agarose in 1X Tris-borate-EDTA buffer) electrophoresis at 5v/cm for 2 hr and stained with ethidium bromide (0.5 µg/ml)14. Standard molecular size marker (100 bp DNA ladder) was included in each gel. DNA fragments were observed by ultraviolet transilluminator and photographed in a gel documentation system (Alpha Imager, Germany).

The PCR was performed three times to ensure the repeatability of the technique and to make sure that isolates were correctly assigned to respective patterns.

TABLE 1. Oligonucleotide primers used in multiplex PCR reaction.

Sl No	Primers	Primer sequences
1	stx_1 F	5′-ATA AAT CGC CAT TCG TTG ACT AC-3′
	stx_1 R	5′- AGA ACG CCC ACT GAG ATC ATC -3′
2	stx_2 F	5′- GGC ACT GTC TGA AAC TGC TCC -3′
	stx_2R	5′- TCG CCA GTT ATC TGA CAT TCT G -3′
3	*eae*AF	5′- GAC CCG GCA CAA GCA TAA GC -3′
	*eae*AR	5′- CCA CCT GCA GCA ACA AGA GG -3′
4	*ehx*AF	5′- GCA TCA TCA AGC GTA CGT TCC -3′
	*ehx*AR	5′-AAT GAG CCA AGC TGG TTA AGC T-3′

Source: Paton and Paton1 (1998)
F: Forward
R: Reverse

Hemogram

Blood samples were collected from diseased and emergency slaughter birds. Erythrocytic count and total leucocytic count were performed using the improved Neuoberhaemocytometer with and Natt and Herrick solution as diluting fluid according to the method described by Natt and Herrick (1952). Determination of hemoglobin was performed as described by Van Kempen and Zijlstra (1961). The packed cell volume (PCV) was estimated according to Coles (1986). Blood films stained with Giemsa stain were prepared for the determination of differential leucocytic count (Jain, 2000).

Serum biochemical parameters

Serum samples were collected from diseased and emergency slaughter birds. Aspartate and alanine aminotransferase (AST and ALT) activities were determined calorimetrically according to Reitman and Frankel (1957). Total proteins and Albumin were determined according to Doumas and Biggs (1972).

Serum creatinine was determined according to Henry (1979) and uric acid (Caraway, 1963). Serum calcium was according to Sarkar and Chanhan (1967), phosphorous was measured according to Goodwin, (1970), sodium and potassium were determined by flame photometer.

Statistical analysis

The data were statistically analyzed according to Snedecor and Cochran, (1982).

Results

Epidemiological details and post mortem observations

Out of 200 birds, 50 were emergency slaughter and 20 died from 150 diseased broilers within one week time with an overall mortality and case fatality rate of 10 per cent (20/200). Prior to death, the affected birds were anorexic and emaciated, dull and depressed with ruffled feathers and showed progressive somnolence with closed eyes. Majority of the birds were shivering and huddled near the source of heat. Clinically ill birds showed profuse watery diarrhea and severe dehydration. On post- mortem, besides the generalized septicemic lesions, severe lesions of enteritis accompanied with focal necrotic lesions in the mucosa of the small intestine were prominent in majority of the cases. Spleens and livers were swollen and congested with hemorrhagic or necrotic foci.

Bacterial isolation and characterization

The bacteriological examination of heart blood, liver, kidney, cloacal swab and intestinal contents revealed the presence of Gram-negative bacilli. In biochemical tests, the isolates were identified as *E. coli*. Investigation of 250 samples collectedfrom emergency slaughter birds (200 organs) and cloacal swabs (50) revealed that *E. coli* isolates was recovered from 160 samples with overall prevalence 64%.Concerning fresh heart blood samples, 20 out of 50samples of examined fresh heart blood were *E. coli* positive with an incidence of 40%. The examined of 50 liver samples, *E. coli* were positive in 40 with a percentage of 90%. Moreover, 50 kidney samples collected from emergency slaughter broilers were investigated. Out of 50 examined kidney samples, 30 were *E. coli* positive with a prevalence of 60%. Concerning examination of small intestine of emergency slaughter broilers, 40 out of 50 samples were positive for *E. coli* with a prevalence of 80%. Finally, isolation of *E. coli* from cloacal swabs of diseased broiler. Thirty out from 50 samples of cloacal swabs revealed *E.coli* isolation with prevalence of 60%. Results of serological tests of 20 isolates from organs and cloacal swabs were illustrated in Table 2.

TABLE 2. Serotyping of *E. coli* isolates recovered from examined samples.

No. of isolate	Source of isolate	Serotype
1	Liver	Un typed
2	Intestine	O125
3	Cloaca	O111
4	Cloaca	O6
5	Cloaca	O6
6	heart	O55
7	liver	O114
8	intestine	O15
9	intestine	O111
10	Heart	O6
11	intestine	O55
12	heart	O15
13	liver	O125
14	Cloaca	O15
15	liver	O6
16	heart	O6
17	intestine	O55
18	heart	untyped
19	liver	O6
20	kidney	O114

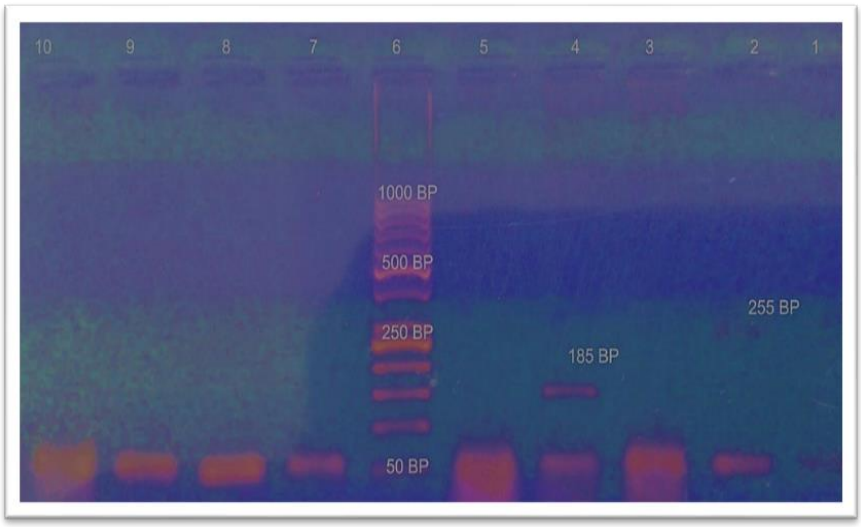

Fig. 1. Agarose gel electrophoresis of *Stx1*, *Stx2* and intim in genes from randomly selected *E. coli* (9 isolates). 6, 100 bplambda marker, Positive amplifications were presented as following, lane 2, *Stx2*gene (255bp)(O125) isolated from intestine. Lane 4, *Stx1*gene (185bp)(O6) isolated from cloaca.

Multiplex PCR for virulence genes

Multiplex PCR assay yielded amplified products of ~180 bp, ~255bp, ~384bp and ~534 bp specific for *stx* 1, *stx* 2, *eaeA* and *hlyA* genes, respectively (Fig.1). Out of 20 isolates, O6 carried one virulence gene were detected as STEC carried only *stx*1, and O125 carried one virulence gene of *stx*2.The other all serotype didn't carried virulence gene (Table 3). Table 4 revealed a significant decrease in RBcs count, hemoglobin (Hb) concentrations, PCV, MCV, MCH, McHc also TLC such decrease was very highly significant on infection ($P< 0.01$). In Table 5 there is a significant increase in ALT and AST if compared with control group $P <0.05$. Total protein and Albumin showed highly significant decrease if compared with control group. Table 6 revealed a significant increases inserum uric acid and creatinine and Phosphorous ($p<0.01$). also there is a significant decrease in serum level sodium, potassium and calcium ($p<0.01$).

TABLE 3. Virulence genes profile of *E. coli* strains isolated from poultry birds with diarrhea.

Serogroup	No. of isolates	Stx1	Stx2	EaeA	HlyA
O6	2	+ve	-ve	-ve	-ve
O111	1	+ve	-ve	-ve	-ve
O55	1	-ve	-ve	-ve	-ve
O114	1	-ve	-ve	-ve	-ve
O15	1	-ve	-ve	-ve	-ve
O125	1	-ve	+ve	-ve	-ve
Untyped	2	-ve	-ve	-ve	-ve
Total	9	1	--	--	--

TABLE 4. Some hematological parameters in healthy and diseased birds.

Group	RBCs ($\times 10^6$µl)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	TLC ($\times 10^3$µl)
Control	2.2 ± 0.2	8.4 ± 0.2	28.9 ±0.4	131.4 ±11.8	38.2± 1.7	29.1 ±1.1	31.2 ± 0.21
Diseased	1.7±0.6*	4.8 ±0.7**	21.0±0.7**	123.5±2.2**	28.2±0.6**	22.8±0.13**	20.2 ±0.13**

* Significant at $P< 0.05$ ** highly significant at $P< 0.01$.

TABLE 5. Changes of liver function tests in serum of chicken infested with *E coli*.

Group	A S T (IU/l)	A L T (IU/l)	Total proteins(g/dl)	Albumin (g/dl)
Control	26.0 ± 0.12	15 ± 0.24	5.0 ± 0.12	2.50 ± 0.04
Diseased	46 ± 0.27*	28.8 ± 0.66**	3.6 ± 0.70**	1.00 ± 0.70**

* Significant at $P< 0.05$. ** highly significant at $P< 0.01$.

TABLE 6. Renal function in serum of chicken infested with *E coli*.

Group	Uric acid mg/dl	Creatinine mg/dl	Calcium mg/dl	Phosphorous mg/dl	Sodium mEq/l	Potassium mEq/l
Control	7.17 ± 0.7	1.5±0.6	9.00 ±0.1	6.19 ± 0.23	155±0.62	8.0± 0.13*
Diseased	9.6±0.7**	2.9±0.9*	6.1±0.12**	8.1 ± 0.80**	122±0.1*	5.8± 0.9*

* Significant at $P< 0.05$. ** highly significant at $P< 0.01$.

Discussion

Poultry production plays an important role in providing valuable proteins, poverty alleviation and economic development. Despite great potential and opportunities, poultry production is threatened by many disease outbreaks, these diseases are the major constrains for developing the poultry industry (Ewers *et al.*, 2005). During the investigation of the present outbreak of acute diarrhea in a poultry flock, the clinical symptoms and post-mortem study indicated the involvement of systemic infection by some enteric pathogens. Because of absence of group A rotavirus, *Salmonella* spp. or any other diarrhea causing parasites and isolation of pure hemorrhagic *E. coli* from heart blood as well as intestinal contents warranted for further investigation of virulence genes of *E. coli* isolates.

Typing of isolated bacteria, including *E. coli* could be achieved by phenotypic and/or genotypic protocols. The phenotypic characteristics method used for identification and characterization of *E. coli* are mainly the morphological and biochemical tests. Most of these techniques are not sufficiently sensitive to distinguish between different strains and they are affected by physiological factors (Fantasia *et al.*, 1990). Therefore, serological protocol was established to differentiate *E. coli* isolates.

Regarding the morphological tests used for identification of *E. coli*, it was found that *E. coli* isolates are G-verods appeared as pink colonies when cultured on MacConkey media, green metallic colonies on EMB medium. Nearly similar results were noted by (McClure, 2000 and Ahmed, 2011).

In general, investigation of 250 samples collected from emergency slaughter birds and cloacal swabs revealed that *E. coli* isolates was recovered from 160 samples with overall prevalence 64%. These results agreed with Ahmed (2011).

Concerning fresh heart blood samples, 20 out of 50 samples of examined fresh heart blood were *E. coli* positive with an incidence of 40%. Nearly similar results were recorded by Abhilasha and Gupta (2001) reported a lower prevalence for *E. coli* in a percentage ranged from 9.5 - 40.5%. Regarding examined liver samples, 50 samples were tested and *E. coli* were positive in 40 with a percentage of 90%. Nearly similar results were recorded by Ahmed (2011). Our were in disagree with (Abhilasha and Gupta, 2001, and Saha *et al.*,

2003) in which lower prevalence was detected. Moreover, 50 kidney samples collected from emergency slaughter broilers were investigated. Out of 50examined kidney samples, 30 were *E. coli* positive with a prevalence of 60% (Ahmed, 2011). However, (Sepehri and Zadeh, 2006) recorded higher occurrence of *E. coli* isolates from tested poultry kidney samples.

Concerning examination of small intestine of emergency slaughter broilers, 40 out of 50 samples were positive for *E. coli* with a prevalence of 80%. Nearly similar findings werereported by Saha *et al.* (2003) and Ahmed (2011). Meanwhile, (Aphukan *et al.,* 1990 and Abhilasha & Gupta, 2001) reported lower occurrence in a percentage of 42.7%. Finally, isolation of *E. coli* from cloacal swab of diseased broiler. Thirty out from 50 samples of cloacal swabs revealed *E.coli* isolation with prevalence of 60%.

Using for serological tests and identification of randomly selected *E. coli* (20) isolates from different sources Table 2 clarified that, *E. coli* isolate was serotype O6(6), O111 (3), O55 (2), O114(2), O15(3),O125(2), and untyped (2). From the above mentioned data, it was clear that the most prevalent *E. coli* serotype isolates recovered from different sources of poultry broiler farms were O6 *E. coli* serotype, followed by O111, O15 then O114,O55, O125 and untyped. Nearly similar results were detected by Robab and Azadeh (2003) and Vandemaele, *et al.* (2003) investigated 100 APEC strains from 83 Belgian poultry farms, detecting only three serotypes O6 strains. Abd El-Salam (2004) serotyped the isolates of *E.coli* that recovered from broiler chickens. He found thatO114 (4), O55 (1), in addition to 32 untypable strains. Ibrahim (1998) showed that the predominant serotypesfrom 46 isolates of *E.coli* strains isolated from broiler chicken(2-6 weeks of age) in the Suez Canal area, Egypt were O114:K-,O78:K-, O158:K- and O125:K70. Serotypes identified included O119:K69, O126:K71, O86:K61, O55:K60, O28:K67, O111:K58, O26:K60and O127:K63. Ibrahim *et al.* (1997) recovered one hundred and ninety *E.coli* isolates from broiler chickens 2-6 weeks of age with respiratory manifestation at Suez Canal area. They performed serotyping of 46 isolates and demonstrated that serotyped O114,O78, O158, O125, O119, O126, O86, O55, O111, O26 and O127 were predominant. A total of 162 strains belonged to EPEC serogroups 026, 044, 055, 086, O111, 0119, 0125, 0126, 0127, 0128, and 0142 (Órskov and Órskov, 1984).

Regarding the occurrence of *Stx1* gene in *E. coli* isolates. Our results revealed that out of 9*E. coli* isolates recovered from various broiler samples, one isolates (O6) were positive for *Stx1* gene yielded the expected size of 185bp PCR amplification. However, PCR results were negative for *Stx1* gene in other *E. coli* isolates. Nearly similar findings wererecorded by (Osek, 2003, Kaper *et al.,* 2004, Badri *et al.* 2009 and Ahmed, 2011).

Stx2 gene in isolated *E. coli* strains showed that out of 9*E. coli* isolates (O125), one were positive for the *Stx 2* gene yielded a consistent fragment of 255 bp. While, PCR results were negative in other isolated *E. coli* isolates. These results substantiate what has been reported by (Kaper *et al*., 2004, and Leigh *et al*., 2005). Shiga toxigenic *Escherichia coli* (Stx1 and Stx 2) is an important cause of gastrointestinal disease in humans, particularly since these infections may result in life-threatening squeals such as the hemolytic-uremic syndrome (HUS) (Nataro and Kaper, 1998, Paton & Paton1, 1998, Boerlin, *et al*., 1999 and Ojo *et al*., 2010).

The present study shows a significant decrease in RBCs count, Hb concentration and PCV in the affected birds indicate anemia of microcytichy-pochromic as showed by the erythrocytic indices that were proportionally correlated with the severityof infection of *E. coli.* These resultsare in accordance with Jain (1986) and Mona *et al*. (2012).

The increase in serum AST levels in this work could be due to liver damage produces by the infected bacteria. Campbell and Coles, (1986), mentioned that the increased of the activity of AST has been associated with hepatocellular damage in birds. Concerning ALT in chicken some studies reported elevation of ALT inbirds infected with bacteria (Campbell and Coles, 1986). Our result greed with Omaima (1987) and Mona *et al*., 2012) who observed a significant increase in (AST & ALT, in chicken infected with *E. coli*. The significant change in total protein and albumin in the present work could be due to liver and kidney damage which could be associated with bacterial infection. Similar findings were previously mentioned by Riley *et al.* (1983) Pai *et al.* (1984), Campbell and Coles (1986) and Ostroff *et al.* (1989).The increase in uric acid and creatinine could be dueto the effect of the micro-organisms and its Toxin onthe kidneys. Our results is completely agree with Pai*et al.,* (1984), Tzipori *et al.* (1987), Obrig *et al*. (1987) and Mona *et al.* (2012) who reported increased creatinine, urea level in case of renal disease. Hypocalcaemia, and hyperphosphatemia could be due to decrease calcium resorption by damaged renal tubules and associated with Hypoalbuminemia as reported by Campbell and Coles (1986) and Mark and Robert (1993). The decrease of potassium and sodium level inserum could be due to renal disease as reported byCampbell and Coles, (1986). Also the metabolism of calcium and phosphorus is closely linked in the body. Our result agreed with Ghanem (1986) and Campbell & Coles (1986).

Conclusion

It could be concluded that the isolated bacterial pathogens play an important role in causing diseases in poultry and human consumer due to presence of toxin and losses in poultry farms at Ismailia and North Sinai. Our findings provide the information about the involvement of STEC in diarrhea in poultry in Ismailia and north Sinai.

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التميز بين سلالات الايشريشيا كولاى المسببة للإسهال في بداري التسمين باستخدام PCR المتعدد

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في محاولة للتحقيق من تفشي الإسهال الحاد في الطيور الدواجن في الإسماعيلية، وشمال سيناء لكشف وتوصيف شيج السمية القولونية (STEC). تم جمع مائتان عينة من الفراخ المريضة طبيعيا والفراريج المذبوحة اضطراريا من مزارع الدواجن في الإسماعيلية وشمال سيناء . جميع الحالات تعرضت للتشريح والفحص البكتريولوجي وتحليل الدم والكيمياء الحيوية . تم عزل الميكروب القولوني من مسحات المذرق ومحتويات الأمعاء ودم القلب والكبد للطيورالتي نفقت بسبب الإسهال الحاد. تم تحديد وتوصيف الميكروب بالزرع البكتيري والتقنيات الكيميائية. كل المعزولات تم تصنيفها استناداً على الانتجين الجداري. تم الكشف عن الجينات السمية (stx1 stx2 وeaeA وhlyA) باستخدام PCR المتعدد. وكان تصنيف العشرين نوع من الميكروب القولوني كالاتي O6 (6)، O111 (2) ،(3) O55 ، O114 (2) ،O15(3),O125 2))، وغيرمصنفة (٢). واظهرت النتائج السمية عن ان العترة (O6) تحمل جين واحد من stx1 والعترة (O125) تحمل جين واحد من stx2 والباقي من العثرات لا يحمل أي سميات. واظهرت التحاليل الكيميائية الحيوية للدم زيادة فيAST و ALT وتغير كبير في البروتين. ولوحظ ، زيادة حمض اليوريك في مصل الدم، والكرياتينين، والفوسفات ونقص كالسيوم الدم، مع انخفاض في مستوى البوتاسيوم والصوديوم. وكشفت الفحوصات الدموية انخفاضا كبيراً في عدد كرات الدم الحمراء والهيموجلوبين والهيماتوكريت في الطيورالمتأثرة تشير إلى انيميا فقر الدم. فإنه يمكن الاستنتاج بأن مسببات الأمراض البكتيرية المعزولة تلعب دوراً هاما في التسبب في الأمراض في الدواجن والإنسان المستهلك بسبب وجود السمية والخسائر في مزارع الدواجن في الإسماعيلية وشمال سيناء.