Epidemiological Study on *E.coli* infection in native and imported duck breeds

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Abstract
A Total of 80 ducks were examined bacteriologically,50 were diseased and freshly dead or slaughter native ducks were aseptically open and taken internal organs(liver-lungs-intestine) of each for bacteriological examination while 30 imported ducks were apparently healthy, cloacal swabs were aseptically taken from these ducks ,moreover 20 samples were collected from litter and 10 samples from drinking water.
57 isolates of *E.coli* were recovered in an incidence (27.14%) and identified biochemically and serologically.
Serological identification of the isolated strains of *E.coli* from diarrheic ducks showed that they belong 8 different "O" Serogroups which were O_{111},O_1, O_{126},O_{55},O_{119},O_{157}, O_{125}, O_{146} and 11 strain were untyped. Antibiogram pattern was applied with 10 antimicrobial agents and reveled that all isolates of *E.coli* from diarrheic ducks were sensitive to danofloxacin, enrofloxacin and levofloxacin while all strains were resistance with tobramicin and ampicillin moreover variable results were recorded with the remaining antimicrobial agents.
Experimental infection in1-week old duckling using *E.coli* serogroups isolated from ducks and litter proved its pathogenicity.
Polymerase chain reaction (PCR) for intimin (*eae*) gene of isolated *E.coli* serogroups documented that 4 serogroups(O_1,O_{126},O_{157} and O_{146}) had intimin (*eae*) gene ranged from 400-450 bp.

Key words:
E. coli, ducks, serogroups, Antibiogram, intimingene.

Introduction

Poultry meat is an important food item in most countries due to its contribution in solving the problem of animal protein shortage many efforts still acting to produce poultry meat with minimal microbial contamination and pathogen free **(Gravani and Williamson, 1991).**
Enterobacteriaece had an epidemiological interest and importance as some of which were pathogenic and many cause serious intestinal infection and food poisoning. **(Schloger *et al.*, 1990; Thorns, 2000 and Schundt, 2001)** stated that non toxigenic colonizing strains of *E.coli* distinct from the recognized pathogenic types which like entero adherent aggressive strains which involved in persistent diarrhea as a major cause of illness, further types of diarrheagenic *E.coli* may be recognized as important human pathogen. *E.coli* comprises a group of bacteria found in the intestine of humans , birds and animals**,** *E.coli* O_{157}: H_7 strain produces potent toxins and can cause food borne poisoning after ingestion of very low numbers of microorganism **(Oswaled *et al.*, 1994 and Vernozy-Rozand, 1999).**
E.coli serogroup (O_{111}) was most predominant isolate obtained from diseased chicken followed by (O_{119}) **(Riley *et al.*, 1983).** However

(Torkey *et al.*, 1995) found that *E.coli* (O_{26},O_{55}, O_{86} and O_{114}) were the most predominant. The acute infection of *E.coli* septicemia in ducks has been studied in Egypt. **(Awad *et al.*, 1973)** recorded an outbreak of *E. coli* infection among duckling and they were isolated two strains O_{86} and O_{119}. Also (**Hegazy, 1992)** isolated 3 pathogenic *E.coli* strains from three flocks of ducks which suffered from recurrent enteritis. The following *E.coli* serogroups were identified (O_{128},O_{126} and O_{114}). On the other hand, **(Chuan *et al.*, 1998)** isolated 16 strains of *E. coli* from ducks and geese that died after showing typical pericarditis, perihepatitis, peritonitis, tracheitis and salpingitis, serogrouping of isolated strains were O_1 ,O_8 and O_{60}.

The emergence and dissemination of antimicrobial resistant *E.coli* has been well documented as a serious problem worldwide (**Cohen, 2000 and Schroeder *et al*., 2002) .**

Therefore, the aim of the present work was to isolate different strains of *E.coli* from ducks and its litter then documented them by serological identification for these strains. The antimicrobial resistance of these serogroups were detected and their pathogenicity recognized by experimental infection and PCR analysis for intimin gene (*eae)* responsible for attaching and effacing lesions.

Material and Methods

1-Samples and cultivation.

A total of 80 ducks were examined 50 were diseased and freshly dead or slaughter native ducks were aseptically open and taken internal organs (Liver-lung-intestine) of each for bacteriological examination while 30 ducks were apparently healthy, cloacal swabs were aseptically taken from these imported ducks. Moreover 20 samples were collected from litter and 10 samples from drinking water. All samples were obtained from private farm at the Giza Governorate.

All fecal samples and internal organs were inoculated directly into nutrient broth and incubated for 24 hours at 37^{o}C then subcultured onto MacConkey's agar and EMB agar **(ICMSF, 1978).**

The inoculated plates were incubated at 37^{o}C for 24-48 hours. The isolation, purification and biochemical identification of bacterial isolates were carried out according to **(Koneman *et al.*, 1996).**

2- Serological identification.

Antisera of *Escherichia coli* were used for serological identification of somatic antigen "O" using slide agglutination test according to **(Ewing, 1986)**. The *E.coli* immune-O-sera polyvalent sera, 8 vials, monovalent sera,43 vials, were obtained from DENKA SEIKEN Co. LTD Tokyo, Japan.

3-Antibiogram pattern.

Antibiogram was applied on the different "O" serogroups of *E.coli* recovered from diarrheic ducks in vitro using disc diffusion technique according to **(Quinn *et al*., 2002)** using Mueller Hinton Agar Plates. Ten discs of chromotherapeutic agents (tobramicin, danofloxacin oxyletracyclin, cefadroxil, enrofloxacin, levofloxacin gentamicin, ampicillian , amkacin and Nalidxic acid) were used to determine sensitivity of various serogroups of *E.coli*. The results were interpretated according to **Oxoid Manual (2000).**

4-Experimental infection in 1-week duckling.

A total of (54), 1-week old duckling were used in this experiment. They were divided into (9) groups each of (6) duckling.

Each group was divided into (2) equal subgroups, each containing (3) birds .One subgroup was inoculated orally with (0.5ml) of each *E.coli* serogroup suspension containing approximately ($3x10^8$) viable organisms per 1ml and the other subgroup was injected intraperitoneally with (0.2ml) of each *E.coli* serogroups suspension Table (1). The last group was injected with sterile saline.

All infected and control birds were under observation for a period of 1-week, birds dying during this period of experiment were subject to post. Mortem and bacteriological examination in trials to re-isolate *E.coli* strains used in this experiment.

Table (1). *E.coli* serogroups dose and route of inoculation in 1-week old duckling.

Groups	Inoculated serogroups	No of inoculated I-P (0.2ml)	Ducks orally(0.5ml)
1	O_{111}	3	3
2	O_{1}	3	3
3	O_{126}	3	3
4	O_{55}	3	3
5	O_{119}	3	3
6	O_{157}	3	3
7	O_{125}	3	3
8	O_{146}	3	3
9	Sterile saline	3	3

5- Detection of virulence gene.

The isolates of *E.coli* were subjected to PCR for detection of intimine (*eae*) gene. The primer was chosen from published squences with the aid of the primer select software (DNASTAR Inc, Madison, Wis)

Primer	Accession number	Primer sequences	Position open reading frame	Size of rodut bp
Intmin	Z11541	ATATCCCGTTTATACGCTATCT	992-1031 0f *eae* A	425

The primer was synthesized by integrated DNA technologies, Inc, (Coralville, lowa). Because the EPEC and EHES genes which encode intimin have considerable heterogeneity on the 3'end, the (*eae*) a primer was made to amplify a constant 5' region of EPEC and EHEC genes **(Sophia *et al.*, 1998).**

Bacterial DNA was obtained by suspending a colony of bacteria grown over night on tryptic case soya agar in 50u of H_2O and boiling at 100^{o}C for l0 min. The samples were amplified in a gene Amp. PCR 2400 thermalcycler (Perkin-Elmer) under the following Conditions, 25 cycles beginning with denaturation at 94^{o}C, primer annealing at 50^{o}C. For 45 sec, followed by extension for 1 min. at 70^{o}C .

The amplified DNA products were separated by electrophoresis on 1.5% agrose gel then stained with ethidium bromide and detected under ultraviolet light **(Sambrook *et al.*, 1989).**

This technique was done in biotechnology center for services and researches Vet. Med. Cairo University (BCSR).

Results

1-Bacteriological examination.

Out of 210 examined samples from internal organs (liver, lung and intestine) of 50 native ducks,30 fecal swabs of imported ducks,20 samples from litter and 10 water samples ,57 isolates of *E.coli* were recovered in percentage of (27.14%). As shown in table (2).

Table (2). Incidence of Enteropathogenic *E.coli* isolated from native and imported ducks.

Samples / Result	Liver	Lung	Intestine	Fecal swabs from imported ducks	Litter	Water	Total
No of samples	50	50	50	30	20	10	210
Positive (+ve)	12	8	18	11	8	0	57
Percentage (%)	24	16	36	36.6	40	0	27.14

2-Serological identification.

Serogrouping of 57 isolates of *E.coli* recovered from ducks and litters revealed 8 different "O" serogroups and "11" strains were untypable as illustrated in table 3.

Table (3). Serological identification of isolated enteropathogenic *E.coli* from ducks.

Serogroups	Liver		Lung		Intestine		Feceal swaps		Litter		Total	
	No	%	No	%	No	%	No	%	No	%	No	%
O_{111}	3	5.25	1	1.75	2	3.50	1	1.75	1	1.75	8	14
O_1	2	3.50	2	3.50	-	-	-	-	-	-	4	7
O_{126}	3	5.25	1	1.75	1	1.75	1	1.75	1	1.75	7	12.28
O_{55}	1	1.75	2	3.50	3	5.25	1	1.75	1	1.75	8	14
O_{119}	-	-	1	1.75	2	3.50	1	1.75	-	-	4	7
O_{157}	1	1.75	-	-	1	1.75	-	-	-	-	2	3.50
O_{125}	1	1.75	-	-	2	3.50	2	3.50	1	1.75	6	10.52
O_{146}	1	1.75	-	-	3	5.25	2	3.50	1	1.75	7	12.28
Untyped	-	-	1	1.75	4	7.00	3	3.25	3	5.23	11	19.25

3- Antibiogram pattern.

Antibiogram pattern of pathogenic *E.coli* recovered from ducks and litter were recorded in table 4 which showed that all tested serogroups of *E.coli* were sensitive to danofloxacine, enrofloxacin and levofloxacin while all strains were resistance with tobramicin and ampicillin, moreover, variable results were recorded with the remaining antimicrobial agents.

Table (4). Antibiogram pattern of pathogenic *E.coli* recovered from ducks.

Antimicrobial agents	Conc	O_{111}	O_1	O_{126}	O_{55}	O_{119}	O_{157}	O_{125}	O_{146}
Tobramicin	TOB10	R	R	R	R	R	R	R	R
Danofloxacin	DNF5	S	S	S	S	S	S	S	S
Oxyletracyclin	TE30	R	R	R	R	M	R	R	M
Cefadroxil	CRO30	R	S	S	R	R	R	R	R
Enrofloxacin	ENR5	S	S	S	S	S	S	S	S
Gentamicin	CN10	M	S	S	M	S	S	R	R
Levofloxacin	LEV5	S	S	S	S	S	S	S	S
Ampicillian	AMP10	R	R	R	R	R	R	R	R
Amkacin	AK30	R	M	S	M	S	R	S	R
Nalidxic acid	NA30	S	S	R	S	S	R	R	R

4- Pathogenicity test.

Experimental infection in 1-week old duckling revealed that the mortality rate were 100% in both inoculated orally or intraperitoneally as shown in table (5).

Table (5). Comparative results of mortality rates and time of deaths in duckling infected with different serogroups of *E.coli* inoculated orally or injected intraperitoneally.

Tested *E.coli* serogroups	No of inoculated duck	No of mortality/ day per in inoculated orally ducks							Mortality rate	No. of Injected ducks	No of mortality/day I/P route injected ducks							Mortality rate
		1	2	3	4	5	6	7			1	2	3	4	5	6	7	
O_{111}	3	1	-	-	1	-	1	-	100%	3	-	-	2	-	-	1	-	100%
O_{1}	3	-	-	2	1	-	-	-	100%	3	1	-	-	1	-	1	-	100%
O_{126}	3	-	1	-	-	1	-	1	100%	3	-	1	-	2	-	-	-	100%
O_{55}	3	-	1	-	1	-	1	-	100%	3	-	-	1	1	-	1	-	100%
O_{119}	3	-	-	1	-	2	-	-	100%	3	-	1	-	-	1	1	-	100%
O_{157}	3	-	2	-	-	-	-	1	100%	3	-	2	-	1	-	-	-	100%
O_{125}	3	-	1	-	1	-	1	-	100%	3	-	-	1	-	2	-	-	100%
O_{146}	3	1	-	1	-	-	-	1	100%	3	-	-	1	-	-	1	1	100%
Non infected control	3	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-
Total	27	2	5	4	4	3	3	3	-	27	1	4	5	5	3	5	1	100%

5- Detection of virulence gene.

Photo 1 observed that *E.coli* serogroups (O_1,O_{126},O_{157} and O_{146}) had (*eae*) gene which their Molecular weight were 450,400,420 and 410bp respectively while the remaining serogroups have no (*eae*) gene.

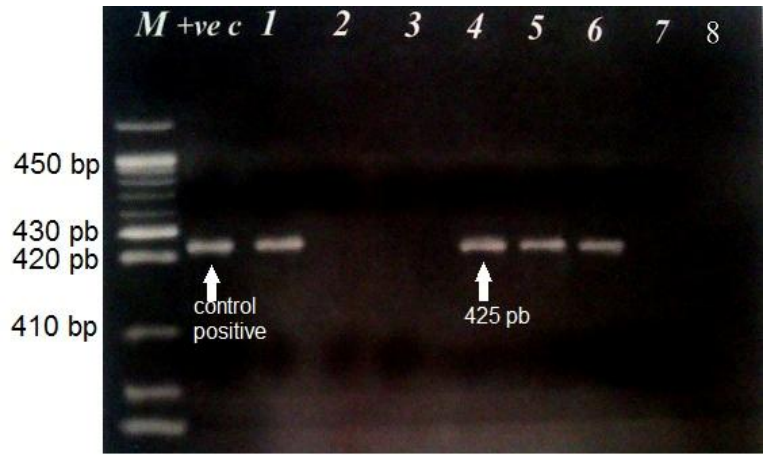

Photo (1). Agrose gel electrophoresis of reaction PCR amplified DNA of (*eae*) from (8) Serogroups of *E.coli.* lane M marker, +ve c Positive control, lanes 1,4,5 and 6 were positive for eae gene while lane 2,3,7 and 8 were negative for (*eae*) gene.

Discussion

Some serogroups of *E.coli* are known to play an important role as etiological agents of various diseases in birds. It was found to be associated with different diseases as septicaemia, peritonitis salpingitis, ,omphalitis, arthritis, tumor like masses in the intestine and respiratory affections. Isolation trails from different samples of ducks and litters (Table 2) revealed that (57) isolates of *E.coli* were recovered in an incidence 27.14%, the rate of *E.coli* isolation were (24%), from livers, (16%) from lungs, (36%) from intestine, feacal swab (36.6%) and (40%) from litters. These results similar those reported by **(Torkey *et al.,* 1995 and Clark, 1996)** who reported that *E.coli* was the main cause of enteritis in poultry also, **(Terzich and Vanhooser, 1993)** isolated *E.coli* with the same percent from liver of dead ducks. Moreover, **(Xingxiao *et al.,* 2009)** isolated *E.coli* (16.2 %) from ducks suffering from diarrhea. In addition **(Heba**, **2012**) isolated *E.coli* in ducks (29.8 %). While**, (Farooq *et al.*, 2009)** isolated enteropathogenic *E.coli* (4%) from ducks.

Serological identification of the isolated strains of *E.coli* from diarrheic ducks revealed(8) different "O" serogroups which were O_{111}, O_1, O_{126}, O_{55}, O_{119}, O_{157}, O_{125}, O_{146} and "11" strains were untyped as shown in Table (3), Many investigator studied serogrouping of *E.coli* isolated from poultry and ducks.

(Samad pour *et al.,* 2002) isolated *E.coli* O_{157}:H_7**,** from duck feces as well as from water samples from swimming in battle ground lake. **(Asawy and Abd El-latif 2010)** isolated entro pathogenic *E.coli* and serogrouped which were O_{26}, O_{78}, O_{86}, O_{125} and O_{157} from ducks. **(Marwah *et al.*, 2010)** isolated numerous serogroups of *E.coli* from diseases chickens from different localities in Ismailia governorate which were (O_{78}, O_{153}, O_{168}, O_{26}, O_{157}, O_{146}, O_{20}, O_{114}, O_{125} and O_{126}), while **(Knoble *et al.,* 2001)** isolated eight isolates of *E.coli* from ostriches and poultry carcasses and were serogrouped as O_2, O_7, O_8, O_9 and O_{21}. Also (**Khan *et al.*, 2005)** isolated 19 strains of *E.coli* from poultry litters and serogrouped as O_6, O_8, O_{53}, O_{56}, O_{153} and O_{174}. Moreover, **(Heba, 2012)** isolated 10 strains of E.coli from ducks and serogrouping them into O_{158}, O_{103}, O_{125} and O_{44}.

Table (4) showed that the Antibiogram pattern of the isolated pathogens from diarrheic ducks revealed variable results against different chemotherapeutic agents which *E.coli* had been used. The tested serogroups were sensitive to danofloxacin, enrofloxacin and levofloxacin. While all strains are resistance with tobramicin and ampicillin.

Moreover variable results were recorded with the remaining antimicrobial agents, these result concide with observation reported by **(Sumano *et al.*, 1998)** stated that enrofloxacin was effective in treatment of natural infection of *E.coli.* Moreover, **(Galland *et al.,* 2001 and Chroder *et al.,* 2002)** mentioned that *E.coli* O_{157}:H_7 was susceptible to ciprofloxacin and enrofloxacin. Also **(Silva *et al.,* 2009)** stated that all isolated strains of *E.coli* recovered from pigeons were sensitive to gentamycin, ceftriaxone ceflazidime and levofloxacin. While, **(Khan *et al.*, 2005)** isolated (19) fluoroquinolone resistant *E.coli* strains from poultry litter. Multiple antibacterial resistance observed in isolated serogroups. In addition **(Silva *et al.*, 2009)** mentioned that all the isolates of *E.coli* recovered from urban pigeons were resistant to ampicillin and sulbactam. The highest antimicrobial resistance frequencies among different serogroups of *E.coli* were observed tobramicin, oxytetracyclin, and ampicillin.

The wide spread uses of antimicrobial agents promoted the increasing frequency of *E.coli* multidrug resistance isolates which facilitate the spread of resistant plasmid to other bacteria ,and may complicate treatment of certain urinary tract and enteric infection in humans**, (Guerra *et al.*, 2003)**. Also, **(Ewers *et al.*, 2009)** proved multi-resistant against several antibiotics including beta-lactains tetracyclines and sulfonamides upon the isolated strains of *E.coli* from Mallard ducks, in addition they reported that Mallard ducks have therefore to

be considered as an important reservoir for zoonotic *E.coli* strains, thus serving as substantial non-point source especially of strains capable of causing extra- intestinal diseases.

Experimental infection in 1-week old duckling using *E.coli* serogroups isolated from ducks and litter proved its pathogenicity.

The result shown in Table (5) illustrated a mortality rate were 100% in both inoculated orally or intraperitonially. These findings were confirmed by **(Alid, 1990 and Stordeur *et al.*, 2004)** who reported that *E.coli* isolated from chicken, ducks and turkeys suffering from colibacillosis were lethal for 1-day-old chicks, they were also able to reproduce clinical signs and lesions of colibacillosis, with bacteremia and septicaemia in SPF chickens, also (**Ewers *et al.*, 2009)** proved pathogenic potential of *E.coli* isolated from Mallard ducks via inoculation in chickens.

Fourth category indicated the presence of intimin (*eae*) gene responsible for attaching and effacing lesion in the intestinal mucosa of host by using PCR for this (*eae*) gene. We observed that *E.coli* serogroups(O_1,O_{126},O_{157} and O_{146}) had (*eae*) gene which their Molecular Weight were 450,400,420 and 410 bp. respectively. These result were similar to those previously reported by **(Chin *et al.*, 1996 and Sophia *et al.*, 1998)**. We identified enterotoxigenic attaching and effacing *E.coli* serovars by amplifying genes heat stable, enterotoxin and intimin (450) bp. These results agree with the results obtained by **(Harley, 1998 and Samadpour *et al.*, 2002)** using polymerase chain upon *E.coli* O_{157}:H_7 isolated from duck feces for detection of virulence gene (Stx, *eaeA* and Hly)**.**

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دراسة عن وبائية عدوي الميكروب القولوني في سلالات البط المحلي و المستورد

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الملخص العربى

تم الفحص البكتريولوجي لعدد أجمالى 80 طائروتم أخذعينات من عدد50 طائر من البط المحلي و كانت الطيور مريضة و تم ذبحها أو حديثة النفوق و قد تم أخذ عينات من أحشائها (الكبد - الرئة - الأمعاء) بالإضافة إلي فحص 30 مسحة شرجية من البط المستورد و أيضا تم فحص 20 عينة من الفرشة و 10 عينات من مياه الشرب و تم عزل 57 عترة من المبكروب القولوني بنسبة 27.14% و تم إجراء الاختبارات الكيمائية و السيرولوجية للعترات المعزولة و كانت نتائج الاختبارات السيرولوجية أن العترات تقع تحت 8 مجموعات سيرولوجية و هي O_{1},O_{111}, O_{126},O_{55},O_{119},O_{157}, O_{125}, O_{146} و لم يتم تصنيف 11 عترة و تم إجراء اختبار الحساسية للعترات المعزولة باستخدام 10 مضادات حيوية مختلفة و كانت جميع العترات حساسة لدانوفلوكساسين و الإنروفلوكساسين و كانت جميع العترات مقاومة للتوبراميسين و الأمبسيلين و كانت النتائج متباينة مع باقي المضادات الحيوية و تم إجراء العدوي التجريبية للعترات المعزولة باختبارها في كتاكيت البط عمر أسبوع و أثبتت ضراوة العترات المعزولة كما تم إجراء اختبار تفاعل البلمرة المتسلسل لتحديد الجين المسئول عن الضراوة (*eae*). وقد تم تحديد الجين في أربع مجموعات سيرولوجية و هي O_{1}, O_{126} O_{157} و , O_{146} و الوزن الجزيئي يتراوح بين 400-450.