



Studies on the Role of Free Living Birds as a Source of Pathogenic *Escherichia coli* Infection to Chickens

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Abstract

This study investigated the prevalence of pathogenic *E. coli* in internal organs (liver, heart and spleen) of 75 broiler chickens representing 15 broiler chicken flocks showing respiratory manifestations and 60 free living birds (25 House sparrows, 15 Doves, 16 Cattle egrets and 4 white throated-King fishers) inside and in the same vicinity of previously sampled broiler chicken flocks in different localities of Kafr el-Sheikh province, Egypt from late 2013 up to early 2015. The results revealed 48 (64 %) isolates out of 75 broiler chicken samples, whereas 10 (16.67%) isolates out of 60 free living bird samples. In vitro pathogenicity test using Congo red assay showed that out of 48 isolates from broiler chicken 22 (45.8 %) showed Congo red positive, whereas out of 10 isolate from free living birds 5 (50 %) showed Congo red positive. Serotyping was carried out on 5 selected Congo red positive isolates of broiler chickens and all 10 isolates of free living birds. Three *E. coli* serogroups belong to O44, O55 and O157 strains from broiler chickens and six serogroups belong to O128, O55, O136, O127 and O164 strains from free living birds; were subjected to in vivo pathogenicity assay in one-day specific pathogen free (SPF) chicks. The pathogenicity in day-old SPF showed 100 % mortality with strain O136 (House sparrow origin), 80 % mortality with the strain O44 (broiler chicken origin), 40 % mortality with strain O55 (Dove origin), 20 % with both strains; O157 (chicken origin) and O128 (House sparrow origin), whereas other strains; O55 (chicken origin), O55 (House sparrow origin), O127 (Dove origin) and O164 (Cattle egret origin) did not caused any mortality in day-old chicks. The study suggested that free living birds may play an important role in prevalence and introduction of pathogenic strains of *E. coli* to broiler chicken farms.

Keywords: *E. coli*; Incidence; Serotyping; Pathogenicity; Broiler Chickens; Free living birds

Introduction

Egypt is home to an impressive number of free bird species reached until now to more than 481 species of birds which vary from residents to migrants (Lepage, 2015). Wild birds are usually regarded as visible indicators of diverse and healthy environments. However, from a public health perspective, this positive view is not always valid (Jones, 2005) as they can carry a wide range of viral, bacterial, fungal and protozoan pathogens harmful to poultry or other vertebrates including human, these wild birds either being themselves diseased or being apparently healthy carriers, or the host of infected vectors (Hubálek, 2004). *Escherichia coli* infection has been reported worldwide in chickens and turkeys (Kabir, 2010). Although, *E. coli* is a part of the normal flora of the intestinal tract of birds, nevertheless, virulent and sometimes lethal toxin-producing pathogenic strains do exist (Hunter 2003). It causes a variety of disease conditions in poultry including yolk sac infection, omphalitis, respiratory tract infection, swollen head syndrome, septicemia, polyserositis, coligranuloma, enteritis, cellulitis and salpingitis (Barnes and Gross, 1997). Isolation of *E. coli* from apparently healthy or diseased free living birds was reported by many authors (Awad-Allah et al., 2013 and Hassan and Bakeet, 2014). Our objective was to study the role of some species of free living birds

which live in vicinity or inside broiler chicken farms in the epidemiology of *E. coli* infection in broiler chickens.

Material and methods

Sample Collection and Preparation: Internal organs (liver, heart and spleen) were collected aseptically from 75 broiler chickens showing respiratory manifestation as well as 60 free living birds captured by mist nets (25 House sparrows, 15 Doves, 16 Cattle egrets and 4 white throated-King fishers) inside and in the same vicinity of sampled broiler chicken flocks from different localities in Kafr el-Sheikh Province, Egypt from late 2013 up to early 2015. A loopful from liver, heart and spleen of each bird were processed as one sample within a time not exceeded 6 hours after collection.

Isolation and identification of *Escherichia coli*: The samples were inoculated in the brain heart infusion broth (Oxoid™) and incubated at 37 °C for 18 hours. A loopful from each broth sample was streaked onto MacConkey's agar (Oxoid™). The inoculated plates were incubated at 37 °C for 24 hours. One selected pink colony from MacConkey's agar of each sample was streaked on Eosin methylene blue agar (LAB M™) and incubated at 37 °C for 24 hours. Colonial morphological characters were carefully studied and recorded after 24 hours of incubation. Colonies with the characteristic metallic sheen of *E. coli* were subcultured on

brainheart slant agar (Oxoid™) for pure culture and then characterized by using Gram's stain (Merchant and Packer., 1967) and confirmed their identity as *E. coli* by biochemical tests according to Quinn et al. (2002). These tests included triple sugar iron agar

(TSI), Methyl red, Catalase, Indole, Oxidase, Voges-Proskauer, Citrate and Urease tests.

In vitro pathogenicity testing of isolated *Escherichia coli*: *E. coli* isolates were grown on Congo red agar (Berkhoff and Vinal., 1986) and incubated at 37 °C for 24 hours and intensity of red dye binding colour was estimated as; +, ++, and +++ (Styles and Flammer., 1991).

Serotyping: Selected 15 isolates (represented by 5 Congo red positive isolates from chicken and 10 isolates from free living birds) were serotyped in animal health research institute, Dokki, Giza using: Polyvalent and monovalent diagnostic *E. coli* antisera "Denka Sieken Co. LTD" (Ewing., 1986).

In vivo pathogenicity study of *E. coli* isolates: Nine *E. coli* strains (6 strains from different free living birds and 3 from chickens) were prepared for pathogenicity assay according to Dho and Lafont.(1984). A total of 50, day-old SPF chicks were used for this study. The chicks were divided into 10 experimental groups; each group consisted of 5 chicks housed under sterile condition. The temperature of the chamber was adjusted between 34-30 °C according to advances in chicks age.

Commercial chicken starter-grower ration (containing 21 % crude protein and 3000 kcal of ME/kg) and drinking tap water was administered ad libitum to chicks. Nine groups of chicks were injected subcutaneously in the back of the neck with 0.2 ml of phosphate buffer saline (PBS) containing approx. 10^8 CFU/ml from each isolates. The last group was kept as control injected subcutaneously with 0.2 ml PBS. The virulence was assessed by mortality initially at 6 and 12 hours post-challenge and thereafter at daily intervals for up to 7 days. Clinical signs were examined every 24 hours. Birds that died before day 7 and those that survived till end of the experiment were necropsied to determine the gross pathological lesions in their organs and re-isolation was also done on liver and pericardium.

3. Results

The results of bacteriological examination revealed

that 48 out of 75 samples (64 %) from broiler chickens and 10 out of 60 samples (16.67 %) from free living birds were positive for *E. coli* based on morphological and biochemical characteristics. The highest incidence among free living birds was for both House sparrows and Doves (6.67 %) followed by 3.33 % for Cattle egret (Table 1). Out of 48 isolates from broiler chickens, 22 (45.8 %) isolates showed Congo red positive, whereas out of 10 isolate from free living birds, 5 (50 %) showed Congo red positive (Table 1). Different intensities in Congo red dye uptake among all isolates were 19(+), 7(++) and 1(+++), whereas 31 (CR-) isolates were Congo red negative (Plate 1). The serotypes of *E. coli* from broiler chickens and free living birds were illustrated in table (2 and 3). The result of in vivo pathogenicity testing in day-old SPF chicks indicated that 100 % mortality was recorded with strain O136 (House sparrow origin), 80 % mortality with the strain O44 (broiler chicken origin), 40 % mortality with strain O55 (Dove origin), 20 % mortality with strain O157 (chicken origin) and O128 (House sparrow origin), whereas strains O55 (chicken origin), O55 (House sparrow origin), O127 (Dove origin) and O164 (Cattle egret origin) did not cause any mortality in day-old chicks (Table 3). The clinical signs presented from sick birds before they died were pasty vents, depression, lameness, anorexia, ruffled feathers and weakness, also average body weight was affected compared to average body weight of control group (Plate 2). The post-mortem findings showed congested muscles (signs of septicemia), congested and swelling liver, spleen, lungs and kidney as well as pericarditis and airsacculitis (Plate 3). The cultures from liver and pericardium also were positive both for dead birds and sick birds that survived till the end of the experiment, whereas no mortalities or re-isolation was recorded in control group. The result of the pathogenicity in day-old SPF chicks not correlated with Congo red binding assay in 3 serogroups (O55 chicken origin, O136 House sparrow origin and O127 Dove origin), but correlated with the other 5 groups (Table 3).

Table (1): Incidence of E. coli isolates among broiler chickens and free living birds with relation to Congo red dye binding assay.

Species	No. of E. coli isolates	%	Number of positive (CR)	%	Intensity of CR positive					
					(+++)	(++)	(+)			
Chickens	48/75	64 %	22	45.8 %	1	7	14			
Free living birds	House sparrows	4/25	16.67%	5	50 %	-	-	2		
	Doves	4/15						3	3	
	Cattle egrets	2/16						-	-	-
	White throated-King fishers	0/4						-	-	-
Total	58	42.96 %	27	64.6 %	1	7	19			

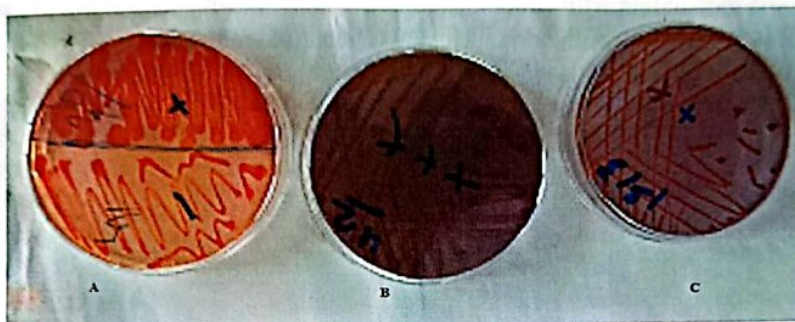


Plate (1): Congo red test showing different intensities in the dye uptake among E. coli isolates.

A- Lower half of the plate (CR -) while upper half (CR+). **B-** (CR+++). **C-** (CR++)

Table (2): Serotyping of fifteen E. coli isolates from broiler chickens and free living birds.

Serogroups	Chicken number	Free living birds		
		House sparrow number	Dove number	Cattle egret number
O44	1	-	1	-
O55	2	1	1	-
O127	-	1	1	-
O128	-	1	1	-
O136	-	1	-	-
O146	1	-	-	-
O157	1	-	-	-
O164	-	-	-	2
Total	5	4	4	2

Table (3): Correlation between in vitro pathogenicity by Congo red binding assay and in vivo pathogenicity day-old chicks with relation to serogroups.

Source of isolate	Serogroups	Congo red binding assay		Chicks pathogenicity assay	
		Intensity	Virulence	Dead %	Virulence
Broiler chicken	O44	CR+++	Pathogenic	80 %	Pathogenic
	O55	CR++	Pathogenic	0 %	Non pathogenic
	O157	CR++	Pathogenic	20 %	Pathogenic
House sparrow	O128	CR+	Pathogenic	20 %	Pathogenic
	O55	CR-	Non pathogenic	0 %	Non pathogenic
	O136	CR-	Non pathogenic	100 %	Pathogenic
Dove	O 55	CR+	Pathogenic	40 %	Pathogenic
	O127	CR+	Pathogenic	0 %	Non pathogenic
Cattle egret	O164	CR-	Non pathogenic	0 %	Non pathogenic

E. coli strains that killed >50%, 10%-50% and 0-10% of chicks were classified as virulent, moderately virulent and avirulent, respectively (Ngeleka et al., 2002, Zinnah et al., 2007).

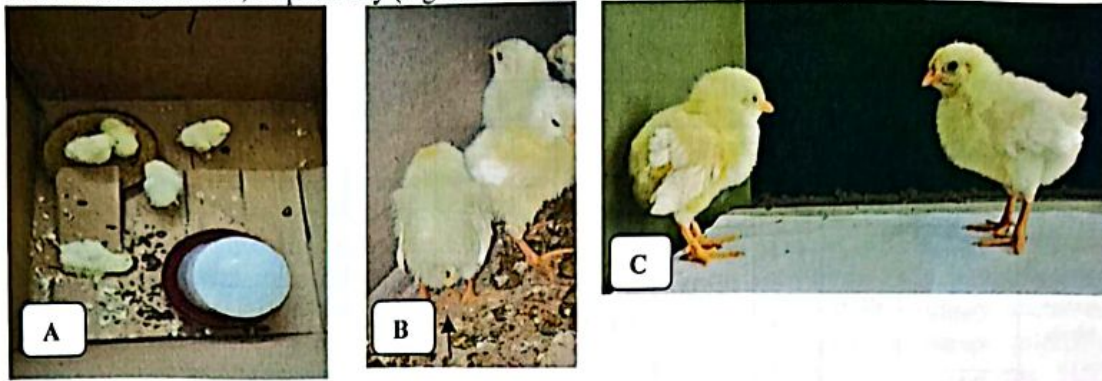


Plate (2): Clinical signs in SPF chicks post inoculation with selected *E. coli* strains

- A- Baby chicks after 48 hours post inoculation with strain O136 (House sparrow origin) showing mortality, ruffling and anorexia
- B- Three days old baby chicks showing weakness and arrow refer to pasty vents.
- C- Six day old baby chick post inoculation with strain O55 (Dove origin) showing loos of body weight and abnormal feathering (left) in comparison with normal chick from control group (right).

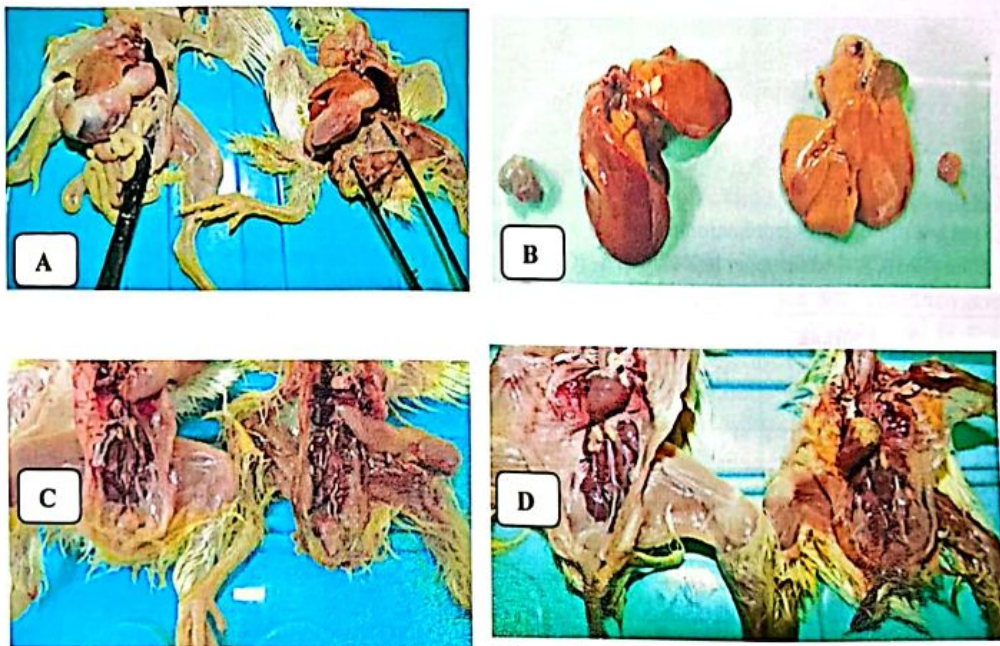


Plate (3): Postmortem gross lesions in SPF chicks post

inoculation with selected *E. coli* strains

- A- Three day old chick showing general congestion, swollen kidney and arrow refer to hemorrhagic bursa (right) in comparison with normal control (left).
- B- Five day old chick arrows (right) refer to airsacculitis, congested lung and pericarditis in comparison with normal control (left).
- C- Three day old chick showing abdominal airsacculitis (right) in comparison with normal control (left).
- D- Three day old chick's liver and spleen showing congestion and enlarged (left) in comparison with normal control (right).

Discussion

Avian pathogenic *E. coli* is considered as one of the principal causes of morbidity and mortality, associated with heavy economic losses to the poultry industry by its association with various

disease conditions, either as primary pathogen or a secondary pathogen (Barnes and Gross, 1997). The present study revealed isolation of 48 (64%) *E. coli* strains from 75 samples of diseased broiler chickens, whereas 10 (16.67 %) *E. coli* stains from

60 samples of apparently healthy free living birds (Table 1). Incidence of *E. coli* infection was higher in broiler chickens, it may be attributed to presence of several other pathogens, like Newcastle disease virus (NDV), infectious bronchitis virus (IBV) and *Mycoplasma gallisepticum* (MG), both wildtype and vaccine strains, in addition to unfavorable housing climate (ammonia or dust) which renders the respiratory system more susceptible to APEC infections through deciliation of the upper respiratory tract (Goren, 1981; Nakamura et al. 1994; Barnes and Gross, 1997 and Villegas, 1998). Isolation of *E. coli* from free living birds found in the vicinity of chicken farms, agree with many authors who have documented isolation of *E. coli* from free living birds found near poultry farms with variation in prevalence rates attributed to the species of wild bird examined, type of sample, localities and bird feeding habits (Hideki and Sinikka, 2002; Soad and Wafaa, 2003; El-Sheshtawy and Moursi, 2005; Rogers, 2006; Hedawy and El-Shorbagy, 2006; Awad-Alla et al 2009; 2010 and 2013). The highest incidence among free living birds was 6.67 % for both House sparrows and Doves followed by 3.33 % for Cattle egrets. These higher prevalence of *E. coli* in house sparrows and Doves may result from the urban habits of those birds which are usually found feeding on grains in feed storage facilities and garbage dumps as was previously supported by Vilela et al. (2012). In vitro virulence test depend on uptake of Congo-red showed that out of 48 isolates from broiler chicken 22 isolates (45.8 %) showed Congo red positive, whereas out of 10 isolate from free living birds 5 (50%) showed Congo red positive (Table 1), these results are in accordance with the findings of many scientists who advocated the use of Congo red dye with the objective of distinguishing between pathogenic and non-pathogenic *E. coli* (Berkhoff and Vinal., 1985; Styles and Flammer, 1991). However, most common avian pathogenic *E. coli* belong to serogroups: O78, O1, O2, O15 and O55 (Kabir, 2010), the study revealed isolation of *E. coli* serogroups O44, O55, O146 and O157 from diseased broiler chicken that agree with the findings of Roshdy et al. (2012) who isolated *E. coli* from internal organs of chicken belong to serogroups: O44 and O164, Kalin et al. (2012) who isolated serogroups O157 from liver and cecum samples of broiler chickens and Abd El Tawab et al. (2014) who isolated O55 from internal organs of broiler chicken. Isolation of *E. coli* serogroups O55, O127

and O128 from both House sparrows and doves are in agreement with those of Awadallah et al. (2013) who isolated serogroups O128 and O55 from cloacal swabs of apparently healthy sparrows as well as O127 from Doves, whereas Knöbl et al. (2011) reported isolation of *E. coli* belonged to serogroups O128 from liver of dead psittacine birds. According to available literature it is the first report of isolation of *E. coli* serogroups O136, O44 and O164 from internal organs (liver, heart, spleen) of House sparrows, Doves and Cattle egrets respectively. However, Makino et al. (2000) reported the isolation of *E. coli* O136 from faecal samples of seagulls in Japan, whereas Hassan and Bakeet (2014) reported the isolation of O44 from affected internal organs of pigeons in Egypt. The correlation among the pathogenic capacity of *E. coli* strains and its capacity of absorption of Congo red was not perfect in this study, since strain O136 from House sparrow that did not absorb the Congo red presented an elevated mortality rate (100%) in the pathogenicity test in vivo. This deficient correlation among those tests was also previously observed by Corbett et al. (1987) and Yoder (1989). Experimental infection in day-old SPF chicks demonstrated that most virulent strains were serogroups O136 from House sparrows and O44 from broiler chickens (caused 100 and 80 % mortality respectively), followed by moderately virulent strain O44 from Dove (caused 40 % mortality), O157 from broiler chicken and O128 from House sparrow caused 20 %, whereas O55 from chicken, O55 from House sparrow, O127 from Dove origin and O164 from Cattle egret were avirulent caused no mortality (Table 3). The clinical observations of inoculated chicks were in accordance with the findings of many authors who used one day old chicks for in vivo virulence assays of *E. coli* strains and recorded a variable degree of virulence ranging from high to moderate. This may be due to difference in route of inoculation and bacterial load in the inoculum (Dho and Lafont, 1984; Ngeleka et al., 2002; Best et al., 2003; Raji et al., 2003 and Zinnah et al., 2007). Based on the in vivo pathogenicity tests an important epidemiologic relation could be established among the House sparrows strains (O136 and O128) and Dove strain (O55) with pathogenic stains affecting broiler chickens, however in order to confirm this relation, molecular studies analyzing the phylogenetic profiles of isolates of both species are necessary (Moulin-Schouleur et al., 2007; Kobayashi et al., 2009 and Vilela et al., 2012).

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الملخص العربي

في هذه الدراسة تم تقصي معدل انتشار ميكروب الإشيريشيا كولاي الممرض في الأعضاء الداخلية مثل " الكبد و القلب و الطحال " لعدد 75 طائر بداري التسمين " يمثلوا عدد 15 مزرعة لبداري التسمين تعاني من اعراض تنفسية " و عدد 60 طيور طليقة هي " 25 عصافير المنازل و 15 يمام و 16 ابو قردان و صائد السمك " من داخل و في محيط مزارع بداري التسمين في اكثر من موقع في محافظة كفر الشيخ في الفترة من اخر 2013 حتى اول 2015 و اظهرت النتائج ان نسبة انتشار ميكروب الإشيريشيا كولاي في بداري التسمين يمثل 64 % (75/48) بينما في الطيور الطليقة يمثل 16,67 % (60/10) و بدراسة عوامل الضراوة لهذه العترات باستخدام اختبار الكونغو الأحمر اظهرت 45.8 % (48/22) من العترات المعزولة من بداري التسمين ايجابية بينما 50 % (10/5) فقط من العترات المعزولة من الطيور الطليقة كانت ايجابية لاختبار الكونغو الأحمر. تم اختيار 5 عترات ايجابية اختبار الكونغو الأحمر من بداري التسمين للتصنيف السيرولوجي الذي قسمهم الى ثلاث مجموعات O44,O55,O157 بينما العشرة عترات من الطيور الطليقة قسمت الى ستة مجموعات سيرولوجية هي O55,O128,O136,O127,O164,O55. عند دراسة ضراوة هذه العترات بحقتها في كتاكيت خالية من مسببات المرضية عمر يوم كانت نسبة النفاق 100 % عند استخدام عترة O136 "المعزولة من العصافير المنزلية " بينما كانت نسبة النفاق 80 % عند استخدام عترة O44 " المعزولة من بداري التسمين " وكانت نسبة النفاق 40 % عند استخدام عترة O55 " المعزولة من اليمام " و كانت 20 % عند استخدام عترة O157 " المعزولة من بداري التسمين " و كذلك O128 "المعزولة من العصافير المنزلية " بينما لم يظهر اي نفاق عند استخدام عترة O55 و O127 و O164 " المعزولة من بداري التسمين و العصافير المنزلية و اليمام و ابو قردان على الترتيب لذلك من هذه الدراسة يتضح ان الطيور الطليقة قد تلعب دور مهم في انتشار عترات ضارة من ميكروب الإشيريشيا كولاي الى مزارع بداري التسمين.

الكلمات الدالة: (إشيريشيا كولاي) - معدل انتشار - التصنيف السيرولوجي - الضراوة - بداري التسمين - الطيور الطليقة