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**INVESTIGATION OF THREE *E. COLI* SEROGROUPS  
(APEC) ISOLATED FROM FERTILE EGGS OF  
COMMERCIAL CHICKEN IN THE SOUTHERN  
REGION OF SYRIA**

(With 6 Tables)

By

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(Received at 15/11/2011)

**التحري عن ثلاث مجموعات مصلية لبكتريا العصيات القولونية الطيرية  
المرضة المعزولة من البيض المخصب لدجاج اللحم التجاري  
في المنطقة الجنوبية من سورية**

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تم عزل بكتريا العصيات القولونية من 200 عينة بيض معد للفقس (مخصب) بعمر يوم واحد من مزارع عديدة في المنطقة الجنوبية (محافظة درعا والسويداء) من سورية. أخذت العينات من قشور البيض ومن الصفار، وبعد الزرع على المستنبتات النوعية (ماكونكي اجار - الايوزين وأزرق المتيلين) تم دراسة الصفات المزرعية للمستنبتات النامية، وكانت جميع العينات المزروعة من الصفار سلبية لنمو العصيات القولونية أما عينات القشور، وبعد إخضاع معزولاتها للاختبارات باستخدام المساطر البيوكيميائية من النوع (HiMotility) تم عزل العصيات القولونية بنسبة 94 % في منطقة درعا وبنسبة 92 % في منطقة السويداء. تم اختبار خاصية الحركة للمعزولات (كأحد عوامل الضراوة) حيث تبين أن نسبة المعزولات المتحركة من عينات درعا كانت 94.7 % ومن عينات السويداء كانت 94.6 %. تم اختبار خاصية التحلل الدموي (كأحد عوامل الضراوة) ونوع التحلل الدموي الحاصل فقد كانت نسب المعزولات المحللة للدم في عينات درعا 95.7 % وفي عينات السويداء 94.6 %. تم إخضاع المعزولات للتنميط المصلي لثلاث أنماط معروفة تنتمي للمجموعة الأنتيجينية الجسدية (O) الخاصة بالعصيات القولونية الطيرية الممرضة APEC باستخدام اختبار التراص على الشريحة وذلك للمجموعات التالية 01 - 02-078 والتي تعتبر من ضمن المجموعات الأكثر امراضيةً وشيوعاً في مزارع الدجاج وقد وجدت الأنماط الثلاثة المختبرة 01-02-078 في معزولات منطقة درعا بنسبة 74.5 % وفي معزولات منطقة السويداء 79.3 %.

**SUMMARY**

*E.coli* bacteria has been isolated from a total of two hundred one day old fertile eggs of commercial meat type chicken from poultry farms in the southern region of Syria (Daraa and Souweida) by taking samples from the eggshells and yolks. Eggs were not subjected to cleaning and sterilization. So, we obtained 94% isolates from Daraa eggshell samples and 92% isolates from Souweida but all yolk samples were found free of *E.coli* bacteria. The motility of all isolates as one of the virulence factors were tested and found that 94.6% from Souweida isolates and 94.7% from Daraa isolates were motile. The hemolysis, as another virulence factor, was tested in all isolates and we found that 94.6% of Souweida isolates were hemolytic and 76.1% of them were type beta (entirely hemolytic), but in Daraa 95.7% of the isolates were hemolytic and 72.3% of them were type beta. Isolates were serotyped by agglutination test with a specific antisera of somatic antigen to the three serogroups O1, O2 and O78 which are considered from the most prevalent and pathogenic serogroups in poultry farms. A percentage of 74.5% and 79.3% from the isolates of Daraa and Souweida, respectively were belonged to the three tested serogroups.

**Key words:** *Escherichia coli*, *Pathogenic*, *Serotyping*.

## INTRODUCTION

*Escherichia coli*, which is a part of the normal microbiota of birds (Bettelheim, 1994) in intestinal and respiratory tracts (Morris and Sojka, 1985), was forgotten as a potential pathogen. However, lesions in which *E.coli* is the primary and often the secondary agent cause economic damage due to lower corporal development, insufficient feed conversion, increasing mortality, higher cost with medicine, and condemnation of carcasses (Rocha *et al.*, 2008).

Avian colibacillosis is responsible for large economic losses in poultry rearing resulting in low performances, weight loss, onset of egg production and mortality. Avian pathogenic (APEC) *Escherichia coli*, the causative bacteria of colibacillosis induces various syndromes including *Yolk Sac Infection*, respiratory tract infection (*Airsacculitis*), *Acute Colisepticemia*, *Salpingitis* and *Cellulitis*, the most common forms of colibacillosis occurs among 2 to 10 week-old chickens (Aggad *et al.*, 2006).

However, *Yolk sac infection (YSI)* is a major cause of mortality of chicks during the first week post-hatching and it is an economically important disease since it increases mortality of 8 days old and causes poor weight gain. In addition, birds that survive to a *YSI* outbreak show poor carcass quality (Cortes *et al.*, 2004).

Several authors had reported earlier that contamination of fertile eggs in the nest at the broiler breeder farm is the main cause of *YSI* (Gordon and gordan, 1985; Mosqueda and Lucio, 1985; Rojo, 1987).

The rate of infection with *E.coli* bacteria (colibacillosis) is approximately 30-40% of the other diseases (Cheville and Arp, 1998).

It is worth to mention that some *E.coli* strains seem to be more aggressive than others and some serogroups (O1,O2 and O78) are more frequently associated with septicemic clinical cases (Barbour *et al.*, 1970; Cheville and Arp, 1998).

The result of hemolysis tests showed that hemolytic activities of APEC isolates correlated to the virulence of avian colibacillosis. In addition, approximately 90% of the isolates, which showed the hemolysis, harbored one or more virulence genes (Moon *et al.*, 2006).

The flagella, which are thin surface appendices, give motility to Gram positive and negative bacteria in aqueous media. Their rotating movements allow microorganisms to approach adjacent epithelial cells, crossing the mucus barrier and causing adhesion, multiplication, colonization and infection.

This study aims to investigate the three frequently strains from O serogroups (O1,O2 and O78) and to test the motility and hemolysis characteristics virulence factors.

## **MATERIALS and METHODS**

### **Samples Collection**

During the period from may 2010 to April 2011, a total of two hundred one-day-old fertile eggs of meat type chicken were collected from poultry farms in the southern region of Syria (Dara and Souweida).

Eggs were not subjected to cleaning and sterilization and transported to the laboratory after being wrapped with sterile tin foils.

### **Samples Preparation:**

Upon reaching the laboratory, samples were taken from the surface of eggs by swabs (HiMedia Laboratories Pvt. Limited, India) and incubated in nutrient broth (HiMedia Laboratories Pvt. Limited, India) overnight at 37°C.

Eggs were cleaned and sterilized then samples of yolk were taken and incubated in nutrient broth at 37°C for 48 hours.

**Cultural and Biochemical Characterization:**

The samples were cultured, using a sterilized loop, on specific solid media (MacConkey - EMB agar (OXOID)) and incubated at 37°C for 24 hours.

All bacterial colonies were selected from each sample. These colonies were isolated in pure culture for further identification.

A cultural suspension with 5 ml of physiological saline was prepared from each isolate and compared with Mcfarland standard to have a right turbidity. This material was used to inoculate HiMotility Biochemical Kit for *E.coli* (HiMedia Laboratories Pvt. Limited, India) which include the following test; Motility, Indole, Citrate, Glucoronidase, Nitrate, ONPG, Lysine, Lactose, Glucose, Sucrose and Sorbitol.

Kits were inoculated, incubated, handled, and analyzed according to the manufacturer's instructions.

**Motility test:**

The primary Hanging Drop Method as described by Cowan (1985) was carried out to detect the motility of all isolates. Isolates which didn't show motility had been retested by using SIM agar and incubated at 37°C for 24 hours (Quinn *et al.*, 1994). In addition, results were supported with Biochemical kits which include a motility test.

**Hemolytic Test:**

The hemolysis of isolates was tested by culturing on blood agar and incubated 24h at 37°C. The isolates which showed a complete hemolysis on the blood agar had the letter "Beta", for the partial hemolysis "Alpha" and for the negative results "Gamma" (Quinn *et al.*, 1994).

**Serotyping:**

Isolates were serotyped by agglutination test with a specific antisera (Veterinary Institute *et al.*, 2009) of three different Somatic O antigens (O1,O2 and O78).

**RESULTS**

All yolk samples collected from the two regions (Daraa and Souweida) were found free from *E.coli* bacteria.

Eggshell samples had been cultured on MacConkey agar and all colonies were primary classified as *E.coli* based on the colonial characteristics (Table 1).

Table 1: Culture characteristics on MacConkey Agar

	Daraa	Souweida
Pink colonies-Lactose fermentation	100%	100%

94% of Daraa eggshell samples and 92% of Souweida's were *E.coli* isolates according to the results of Biochemical tests (using Bio. Kits) which had been applied on the two hundred samples (Table 2).

**Table 2:** *E.coli* isolates after Biochemical tests (HiMotility Kits)

	Daraa	Souweida
<i>E.coli</i> isolates after Biochemical tests (HiMotility Kits)	94%	92%

94.7% of Daraa isolates and 94.6% of Souweida were motile (Table 3).

**Table 3:** *E.coli* motility.

Percentage of <i>E.coli</i> isolates motile	Daraa	Souweida
	94.7%	94.6%

95.7% of Daraa isolates were hemolytic and 72.3% of them were type beta. 94.6% of Souweida isolates were hemolytic and 76.1% of them were type beta (Table 4).

**Table 4:** Hemolysis on blood agar.

	Daraa	Souweida
hemolytic <i>E.coli</i> isolates	95.7%	94.6%
Beta hemolysis%	72.3	76.1
Alpha hemolysis %	23.4	18.5
Gamma hemolysis%	4.3	4,5

94.7% of Daraa's isolates and 94.6% of Souweida's were motile and hemolytic at the same time.

Only 4 isolates from Daraa and 5 isolates from Souweida were motile and non-hemolytic.

Only 5 isolates from Daraa and 5 isolates from Souweida were hemolytic and non-motile (Table 5).

**Table 5:** Motility and hemolysis

	Daraa	Souweida
motile & hemolytic n (%)	85/94(90.43)	82/92(89.13)
motile & not hemolytic n	4	5
not motile & hemolytic n	5	5

The three serogroups (O1,O2 and O78) formed a percentage of 74.5 in Daraa isolates and 79.3 in Souweida's s. The percentage of O1 was 7.4 and 7.6 in Daraa and Souweida respectively.

The percentage of O2 was 21.3 and 19.6 in Daraa and Souweida respectively, while the percentage of O78 was 45.7 and 52.2 in Daraa and Souweida respectively (Table 6).

**Table 6:** Serotypes percentages

Serotypes	(O1+O2+O78)	O1	O2	O78
Daraa (%)	74.5	7.4	21.3	45.7
Souweida (%)	79.3	7.6	19.6	52.2

## DISCUSSION

In this study the isolation trials resulted in detaining of 94 and 92 *E.coli* isolates (of 200 samples) from Daraa and Souweida eggshells, respectively. All strains isolated were found in the eggshell which means that they had not crossed the eggshell barrier.

In a recent study authors had 16 *E.coli* strains isolated at 19 days of incubation obtained from inside the egg and 12 from the eggshell. Additionally, from the Yolk sac of 21 days (embryos) they found 18 *E.coli* strains of 47 isolates and 86 *E.coli* of 188 isolates from yolk sac of dead chicks (Cortes *et al.*, 2004).

According to a recent study in Mexico they obtained 50% of *E.coli* isolated from fertile eggs (Rojo, 1987).

A percentage of 94.7 of Daraa isolates and 94.6 of Souweida isolates were motile, these percentages show a high level comparing with the results of other study.

In a recent study (Rocha *et al.*, 2008), motility was detected in 54.1% of the samples and higher than 36.8% that had been reported by McPeake *et al.* (2005).

By testing the hemolysis of all isolates we found that the percentage of hemolysis for the two region's isolates were approximately close even the percentage of hemolysis type, for example; 95.7% of Daraa isolates was hemolytic and that was 94.6 % for Souweida isolates.

The Beta hemolysis for Daraa isolates was 72.3% and for Souweida isolates it was 76.1%.

And according to a recent study in Korea (Moon *et al.*, 2006), it was found that 72% (48 isolates) of the APEC- isolated from birds with colibacillosis- revealed  $\alpha$  or  $\beta$  hemolysis on blood agar plates. In addition, approximately 90% of the isolates, which showed the hemolysis, harbored one or more virulence genes (Moon *et al.*, 2006), that can give a reasonable idea about the relationship between hemolysis characteristic and pathogenicity of strains.

A relationship between the two characteristics; motility and hemolysis was observed as 90.4 % of Daraa isolates and 89.1% of Souweida isolates were found motile and hemolytic.

On the other hand, only 4 isolates from Daraa and 5 isolates from Souweida were found motile and non-hemolytic, while 5 isolates from Daraa and 5 isolates from Souweida were found hemolytic and non-motile.

In conclusion, the majority of the motile isolates (85 isolates/94 for Daraa and 82 isolates/92 for Souweida) were carrying the hemolytic characteristic and showed a Beta or Alpha hemolysis on blood agar.

The results showed that the three serogroups exist in the two regions with percentages of 74.5 and 79.3 in Daraa and Souweida's samples, respectively which indicated that the two percentages were high and approximately equal.

In addition, there was no significant differences in comparing each serogroups percentage in both regions.

In Algeria, a study showed that 82% of *E.coli* strains isolated from chickens with colibacillosis belonged to the three serogroups (O1, O2 and O78) as 9%, 29% and 44%, respectively (Aggad *et al.*, 2006).

This study was repeated in the same area after 4 years in which it was found that 48% of the isolates belonged to the three serogroups (O1, O2 and O78) as 15%, 16% and 21%, respectively (Aggad *et al.*, 2010). These results agree with the results of a study in Germany. They had grouped 49.6% of *E.coli* strains isolated from colisepticemic poultry to the three serogroups (O1, O2 and O78) (Ewers *et al.*, 2004).

Other study in UK showed that the O78 serogroups represented 55% and 27% of broilers and layers colisepticemic isolates, respectively (McPeake *et al.*, 2005).

The present study indicated that the O78 serogroups exist in broilers more than in layers. There are a few researches about isolation of O serogroups from the fertile eggs, according to a study they found that the *E.coli* bacteria increased rapidly by number during the period between eggs production and post-hatching (Cortes *et al.*, 2004).

In conclusion, utilization of Biochemical Kits for detecting the characteristics of *E.coli* strains is more suitable and easier than using the standard techniques. It gives accurate results and save time although it is more expensive.

It is remarkable that the three serogroups (O1, O2 and O78) were existed in southern region of Syria with a high percentage and this isolates were holding pathogenic characteristics like hemolysis and motility. These results confirms the results of other research that these three serogroups are - even in Syria- considered from the most prevalent and pathogenic serogroups.

A more detailed study is needed in order to determine the relationship between different strains isolated in the southern and in other regions of Syria and to establish the pathogenic characteristics possessed by those strains. Ultimately, identification of potential

virulence traits may allow for their use as specific markers for the diagnosis of pathogenic strains.

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