Assiut Vet. Med. J. Vol. 59 No. 138 July 2013

INVESTIGATION OF FIVE E. COLI SEROGROUPS (APEC) ISOLATED FROM CASES OF ARTHRITIS OF COMMERCIAL MEAT CHICKEN IN MIDDLE AND COASTAL REGIONS OF SYRIA

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Received at: 26/5/2013

Accepted: 1/7/2013

ABSTRACT

A total of 188 joint samples has been collected from commercial meat chicken with lameness from poultry farms in the middle (Hama) and coastal regions (Tartos) of Syria. These joint samples were taken from cases of arthritis and included hip, knee, hock, foot pad .Surfaces of joints were disinfected. Joint samples were cultured on (MacConkey agar - EMB agar) and identified on the basis of biochemical tests, by using HiMotility biochemical kit for E.coli. So, we obtained in total (72.9 %) isolates from infected joint samples. (74.1%) isolates from Hamaand (70.8 %) isolates from Tartos. The rates of isolation of E. coli were (20.4%) from knee joints, (14.6%) from hip joints and (13.9%) from foot joints, but the higher rate was from hock joints (88.3%). The motility of all isolates as one of the virulence factors were tested and found that (95.3%) from Hama and (95.1 %) from Tartos isolates were motile. The hemolysis, as another virulence factor, was tested in all isolates and we found that (95.7 %) of Hama and (94.6 %) of Tartos isolates were hemolytic. Isolates were serotyped by agglutination test with specific antisera of somatic antigen to five serogroups O1, O6, O8, O15 and O78 which are considered to be one of the most prevalent and pathogenic serogroups in poultry farms. The higher percentage was O1 (35.3 %) and (31.4%) from the isolates of Tartos and Hama, respectively.

Key words: *Echerichia coli, pathogenic, arthritis, serotyping*

INTRODUCTION

Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic Echerichia coli (APEC), including colisepticemia, coligranuloma, yolk sac infection, air sacculitis, salpingitis, peritonitis, arthritis and others (Sharada *et al.,* 2010). The most common forms of colibacillosis arthritis occurs among 2-10 week – old chickens (Aggad *et al.,* 2006).

Escherichia coli is a part of the non-pathogenic normal inhabitants of intestinal and respiratory tracts in birds (Jordan and Pattison, 1996), so, it was considered as a potential pathogen. However, Escherichia coli infections in various farms are responsible for significant economic losses due to lower corporal development, insufficient feed conversion, increasing mortality, and higher cost of medicine (Rocha *et al.,* 2008). Escherichia coli was the major cause of infections causing condemnation of processed chickens in Switzerland (Jacob *et al.,* 1998).

However, arthritis caused by E. coli is a major cause of lameness in chicken. E. coli has been cultured from osteomyelitis, bacterial chondronecrosis and synovitis sites in previous studies (McNamee *et al.,* 1998). Osteomyelitis may be seen in flock outbreaks of E. coli colibacillosis (Reece, 1992; Thorp, 1996) in association with septicemia, arthritis and tenosynovitis.

(Maurer *et al.,* 1998) described that many E. coli isolates commonly associated with colibacillosis in poultry belong to serogroups (O1, O6, O8, O15,O78), so these serogroups are more frequently recovered from Septicemic clinical cases (Heller and Drabkin, 1977; Cheville and Arp, 1998).

The rate of infection with E.coli bacteria (colibacillosis) is formed approximately 30-40% of the other diseases in poultry (Cheville and Arp, 1998). Serogroups (O1, O6, O8, O15, O78) are more pathogenic than other and more frequently associated with arthritis clinical cases (Barbour *et al.,* 1970; Peighambari *et al.,* 1995; Gomis *et al.,* 2001). The

previous studies have shown that hemolytic activities of APEC isolates correlated to the virulence of avian E. coli. In addition, approximately 90% of the isolates showed the hemolysis (alpha, beta, gamma) (Moon *et al.,* 2006). Also, several authors (McNamee *et al.,* 1998) had reported that E. coli which was isolated from femur of lameness birds was hemolytic (86.4%).

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 (Stenutz *et al.,* 2006).

This study aims to investigate the five frequently strains from O serogroups (O1, O6, O8, O15, O78) and to test the motility and hemolysis characteristics of virulence factors.

MATERIALS and METHODS

Samples Collection:
During the period February 2012 to March 2013, a total of 188 joint samples of arthritis cases were collected from poultry farms in the middle and coastal regions of Syria (Hama and Tartos). Joints were subjected to cleaning and disinfection of the skin, then, exudates from swollen joints or foot pads were collected by sterilized swabs.

Samples Preparation:
Upon reaching the laboratory, samples were cultured in nutrient broth (HiMedia) overnight at 37°C.

Cultural and Biochemical characterization:
The samples were cultured, using asterilized loop, on specific solid media MacConkey agar - EMB agar), then, 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) which include the following tests; Motility, Indole. Citrate, Glocoronidase, 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 at 37°C for 24 hours. The isolates which showed a complete hemolysis (Beta) on the blood agar had been recorded, as well as for the partial hemolysis (Alpha) and for the negative results (Gamma) (Quinn *et al.,* 1994).

Serotyping:
We used five Serotypes (serum) of pathogenic E.coli (O1, O6, O8, O15, O78) [DENKA SEIKEN Co.Ltd, Tokyo, Japan]) (Veterinary Institute *et al.,* 2009). Isolates were serotyped by agglutination test with specific antisera of somatic antigen to five serogroups (O1, O6, O8, O15, O78).

RESULTS

The articular region of affected hock, knee, hip and foot pad joints often appeared enlarged due to accumulated exudates distending into the joint cavity. Swelling was also observed as a result of periarticular inflammation of soft tissues and simultaneous tendovaginitis. The bacterial isolation from 188 cases of arthritis in chickens is shown in Table 1.

Table 1: Bacteriological results obtained from 188 investigated joints from chicken condemned due to arthritis.

Region	Farms involved	Number of samples	Examined Joints				Isolate of E. coli	Isolates%
			hip	Knee	hock	Foot pad		
Hama	7	116	11	17	76	12	86	74.1
Tartos	9	72	9	11	45	7	51	70.8
Total	16	188	20	28	121	19	137	72.9
%			14.6	20.4	88.3	13.9		

Collected exudates from joints had been cultured on MacConkey agar and all colonies were primary classified as E. coli based on the colonies characteristics (Table 2).

Table 2: Culture characteristics on MacConkey agar

Colonial morphology	Hama	Tartos
Pink colonies- Lactose fermentation	100%	100%

The examination of 188 joint samples collected from Hama and Tartos revealed, that 95% of Hama and 93% of Tartosjoint samples were classified as E. coli isolates according to the results of Biochemical tests (using Bio.Kits) (Table 3).

Table 3-a: E. coli isolates after Biochemical tests (HiMotility Kits)

	Hama	Tartos
E. coli isolates after Biochemical tests	95%	93%

E. coli was gram negative rod. Catalase test was positive and oxidase test was negative. Indole production and Methyl-Red test were positive. Voges-Proskauer test and Simon's citrate were negative.

Table 3-b: Results of primary biochemical tests of E. coli

Isolates	Gram	Shape	Catalase	Oxidase	Indole	MR	VP	Citrate
137	-	Rod	+	-	+	+	-	-

Table 3-c: Results of biochemical tests (HiMotility Kits)

Isolates	MOTI	IND	CIT	GLOR	NIT	ONPG	LDC	LAC	GLU	SUC	SOR
137	+	+	-	+	+	+	+	+	+	-	+

MOTI, Motility; IND, Indole; CIT, Citrate utilization; GLOR, Glocoronidase; NIT, Nitrate; ONPG,Ortho Nitrophenyl-βD-G alactopyranosidas; LDC, Lysine decarboxylase; LAC, Lactose; SUC, sucrose; GLU, Glucose; SOR, Sorbitol. negative reaction - + positive reaction

The detection of motility showed that, 95.3% of Hama and 95.1% of Tartosisolates were motile (Table 4).

Table 4: E. coli motility.

Percentage of E. coli isolates motile	Hama	Tartos
	95.3%	95.1%

Hemolysis test showed that, 95.7% of Hama isolates were hemolytic and 71.3% of them were type beta, while 94.6% of Tartos isolates were hemolytic and 75.1% were type beta (Table 5).

Table 5: Hemolysis on blood agar.

	Hama	Tartos
Hemolytic E. coli isolates	95.7%	94.6%
Beta Hemolysis%	71.3	75.1
Alpha Hemolysis%	24.4	19.5
Gamma Hemolysis%	4.3	5.4

88.4% of Hama and 80.4% of Tartos isolates were motile and hemolytic at the same time.
Only 5 isolates from Hama and 5 isolates from Tartos were motile and non-hemolytic.
Only 5 isolates from Hama and 5 isolates from Tartos were hemolytic and non motile (Table 6).

Table 6: Motility and hemolysis.

	Hama	Tartos
motile and hemolytic n(%)	76/86(88.4)	41/51(80.4)
motile and non-hemolytic n	5	5
hemolytic and non motile n	5	5

The five serogroups (O1, O6, O8, O15 and O78) formed a difference percentage in Hama and in Tartosisolates. O-serotype of pathogenic E. coli was O1(45 strains), O6(43), O8(5), O15(32), O78(12) (Table7).

Table 7: O-serotype of pathogenic E. coli

Isolates of E. coli		O-serotype of pathogenic E. coli				
		O1	O6	O15	O8	O78
Hama	86	27(31.4%)	25(29.06%)	21(24.4%)	4(4.7%)	9(10.5%)
Tartos	51	18(35.3%)	18(35.3 %)	11(21.6%)	1(1.2 %)	3(5.9 %)
Total	137	45(32.8%)	43(31.4%)	32(23.4%)	5(3.6%)	12(8.8%)

DISCUSSION

In this study, the isolation trials resulted in detaining of 86 and 51 E. coli isolates (of 188 samples) from Hama and Tartos arthritis cases, respectively.

The results of this study showed that the most affected joints were hock and knee joints (88.3%, 20.4%) respectively, this result is consistent with (Mutalib *et al.,* 1996). On the other hand, (McNamee *et al.,* 1998) reported that (13.6%) of hip joints was affected, this result is similar with our's(14.6%).

A percentage of 95.3% of Hama and 95.1% of Tartos isolates were motile, these percentages show a high level comparing with the results of other studies. In a recent study (Rocha *et al.,* 2008), motility was detected in 54.1% of the samples and was36.8% higher than that had been reported by (McPeake *et al.,* 2005).

By testing the hemolysis of all isolates we found that percentage of hemolysis for the two regions isolates were approximately close even the percentage of hemolysis type, for example; 95.7% of Hama isolates was hemolytic and that was 94.6% for Tartos isolates.

The Beta hemolysis for Hama isolates was 71.3% and for Tartos isolates was 75.1%. 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 α or β 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). So, this result gives a reasonable idea about the relationship between hemolysis characteristic and pathogenicity of strains.

The relationship between the two characteristics, motility and hemolysis were observed as 88.4% of Hama isolates and 80.4% of Tartos isolates which were found motile and hemolytic.

On the other hand, only 5 isolates from Hama and 5 isolates from Tartos were found motile and non-hemolytic, while 5 isolates from Hama and 5 isolates from Tartos were found hemolytic and non-motile.

In conclusion, the majority of the motile isolates (76 isolates/86 for Hama and 41 isolates/51 for Tartos) were carrying the hemolytic characteristic and showed a Beta or Alpha hemolysis on blood agar.

The results showed that the five serogroups exist in the two regions with different percentages. The most predominant serotype was O1(45 strains) accounting for 32.8% and O6(43), O15(32), O8(5), O78(12). (Gomis *et al.,* 2001) reported that E. coli of serogroups O1, O2, O78 isolated from avian colibacillosis predominantly in Canada. (Sojka and Carnaghan, 1961; Dho-Moulin and Fairbrother, 1999) described that many E. coli isolates commonly associated with colibacillosis in poultry belong to serogroups O1, O2, O78. The serotypes of E. coli in the present study were in similar with (Sojka and Carnaghan, 1961; Dho-Moulinand Fairbrother, 1999; Gomis *et al.,* 2001). On the other hand, (Sharada

et al., 2010) reported that serotyping of isolates revealed redominantly O11, O79, O111 accounting for 26.15%, but O15, O78 were with very low percentage. So, we should more examine serotypes of isolated E. coli.

The serotype of E. coli O8 was isolated from 5 isolates and (Raji *et al.,* 2003) reported that O8strain showed 100% mortality in 1 day-old chickens and was the most pathogenic serotype.
In this study, it was found that several serotypes were determined. This report is the first report of E. coli serotyping isolated from poultry with lameness and arthritis in Syria.

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 five serogroups (O1, O6, O8, O15, O78) were existed in Hama and Tartos regions of Syria with a high percentage and this isolates were holding pathogenic characteristics like hemolysis and motility. Our results were similar to the previous reports, so, these five serogroups are considered the most prevalent and pathogenic serogroups in Syria.

We need a more detailed study in order to determine the relationship between different strains which isolated in the middle, coast and other regions of Syria. So, we need to establish the pathogenic characteristics. Ultimately, identification of potential virulence traits may allow for it as specific markers for the diagnosis of pathogenic strains.

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التحري عن خمس مجموعات مصلية لجراثيم الإشريكية القولونية الطيرية الممرضة المعزولة من حالات التهاب مفاصل دجاج اللحم التجاري في المنطقتين الوسطى والساحلية من سورية

نوال القرواني ، عزام كردي

تم جمع ١٨٨عينة تتضمن مفاصل من دجاج اللحم التجاري المصاب بالعرج بأعمار مختلفة من مزارع المنطقتين الوسطى والساحلية من سورية (حماه ، طرطوس). أخذت العينات من مفاصل العرقوب والركبة والورك والقدم، وبعد تطهير أسطح المفاصل تم الزرع على المستنبتات النوعية (أجار ماكونكي، أجار الأيوزين وأزرق الميتيلين) ودراسة الصفات المورفولوجية للمستعمرات النامية وإخضاع المعزولات للاختبارات البيوكيميائية. كانت العينات المزروعة من المفاصل المصابة إيجابية لنمو جراثيم الإشريكية القولونية بنسبة ٧٢.٩%، وبلغت نسبة عزل الإشريكية القولونية من المفاصل في منطقة حماه ٧٤.١% و ٧٠.٨% في منطقة طرطوس. تم عزل هذه الجراثيم من مفصل الركبة بنسبة ٢٠.٤%، في حين كانت نسبة عزلها من مفصل الورك ١٤.٦%، وكان العزل من مفصل القدم بنسبة ١٣.٩%، وكانت أعلى نسبة عزل لهذه الجراثيم من مفصل العرقوب ٨٨.٣%. تم اختبار خاصية الحركة للمعزولات (كأحد عوامل الضراوة) حيث تبين أن نسبة المعزولات المتحركة في منطقة حماه ٩٥.٣%و ٩٥.١% في منطقة طرطوس. تم اختبار خاصية التحلل الدموي (كأحد عوامل الضراوة أيضا) ونوع التحلل الدموي الحاصل، حيث كانت نسب المعزولات المحللة للدم في العينات ٩٥.٧% في منطقة حماه و ٩٤.٦% في منطقة طرطوس. تم إخضاع المعزولات للتصنيف المصلي باستخدام اختبار التراص على الشريحة حيث تم التعرف على خمس أنماط تنتمي للمجموعة المستضدية الجسدية (O) الخاصة بالإشريكية القولونية الطيرية وهي O1-O6-O8-O15-O78 والتي تعد من ضمن المجموعات الأكثر إمراضية وشيوعا في مزارع الدجاج وقد وجدت أعلى نسبة من الأنماط الخمسة المختبرة في معزولات منطقة طرطوس O1 بنسبة ٣٥.٣% وبنسبة ٣١.٤% في منطقة حماه .