E.coli O111:B4 is predominant cause of E.coli mastitis in cattle in some areas of middean of Eupherates trough in Iraq

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Abstract

This study was included to examine (353) milk samples which were collected from normal and clinical mastitic cows from different areas in three governates in Iraq as 91 milk samples from Al-Najaf governate (Al-Bahrani state & villages of AL-Huryia township) , 128 milk samples from Al-Qadsyia governate (villages of Nufer,Al-Seeder,Sumer& Al-Saniah townships and villages of Ghamass consituency) and 134 milk samples from Babylon governate (villages of Al-Qassim township) ,and California Mastitis Test (CMT) was applied to detect the subclinical mastitis .

Out of 353 milk samples the total *E.coli* mastitis percentage (14.16%) , which included (% 31.8) of clinical mastitis and (%8.3) of subclincal mastitis.

***Echerichia coli* mastitis incidence was not influenced by type of cows breed, while the age of cows were significantly affected on *E.coli* mastitis incidence and the higher *E.coli* mastitis incidence was recorded in cows at (8 years and over)% 19.4 more than other younger aged cows.**

Cows at (1-30 days)post parturition were more susceptible to *E.coli* mastitis (% 20.14) rather than prolonged times after parturition .

***E.coli* mastitis was recorded in hindquarters of udder (right posterior , left posterior) as % 32 ,% 22 respecticvely higher than in fore quarters .**

***E.coli* was isolated and diagnosed from milk samples by using biochemical tests and API-20E system, then these *E.coli* strains were serotyped by using polyvalent sera in Central Public Health Lab. and *E.coli* O111:B4 was a predominate serotype .**

Introduction :

All mammals were colonized by ***Escherichia coli*** ,generally at birth ,and these organisms became a permanent part of the normal microflora of the gastro-intestinal tract, and certain *E.coli* strains have been associated with disease in both humans and animals (Natro & Kaper ,1998).

Mastitis remains one of the greatest problems for the dairy industry ,it is not only responsible for sever financial losses to farmers but also represents a significant threat to cattle welfare (Bradley , 2002)

Environmental mastitis caused by *Ecoli* has increased in many countries and herds at the same time as other contagious mastitis has been successfully controlled ,and the severity and outcome of *E.coli* mastitis can vary between cows in the same herd and in the same individual during different lactation stages(Peeler *et al* ., 2002) .

E.coli mastitis remains one of the most costly disease in farm animal ,and this disease affected many high producing cows in dairy herds and may cause several cases of death per year in most sever cases with economic losses to the dairy industry (Bannerman *et al* .,2004) .

Environmental mastitis caused by *E.coli* is difficult to control since the organisms are naturally found in the soil and surrounding area .

In Iraq most of mastitis studies was occurred in Baghdad governate as Al-Falluji & Robesko (1973); Abdel Al-Noor *et al.*(1977); Al-Khatib and Al-Bassam (1979); Zura(1979); Yass *et al.* (1992);Essa (1992); Al-Graibawi *et al.*(1998); Amean(2001); Al-Graibawi *et al.*(2002); Al-Taan *et al.* (2004) & Al-Dulimy (2004).

Materials and Methods :

Milk samples collection :

Milk samples were collected from 88 clinical mastitic cow and 265 normal milk samples from cows that found in AL-Najaf province (AL-Bahrani state for animal production and villages of AL- Huryia township); AL-Qadysia province (Farms and villages of Ghamass Constituency, Nufar ,Sumer, Al- Saniah and Al-Sedeer townships)and Babylon province (Farms and villages of Al-Qassim township)(Table-1).

Questionnaire paper was designed for this purpose .

Milk samples were collected in sterile tubes (2 tubes)for each sample (one for CMT and physical exam and another for bacteriological test)and a septic technique used for milk samples collection.

The procedure for milk sample collection according to (Radostits *et al.*,2000) was used as following :

Cleaning the udder wall by brushing off any dirt or mud , then the udder was washed by a clean cloth soaked in a disinfectant solution and the udder was allowed to dry,and disinfect the teat orifice with tincture iodine solution and allowed to be dried, and when tincture iodine dried ,labeled a septic tubes to cow and quarter , then the cap of small screw–cap vials is carefully removed and held between the fingers and tube should be held at slight angle to prevent contamination of the teat orifices. Immediately, following collection, the samples were transported to the laboratory in Al-QadysiaUniversity by cooling box.

At laboratory, normal milk samples were examined by **CMT**(California Mastitis Test) (Schalm *et al.*, 1971).

Much data obtained from clinical and subclinical mastitic cow were recorded in questionnaire paper .

Culturing :

All milk samples from clinical mastitis and subclinical mastitis samples which gave a positive reaction with CMT were incubated at 37 C˚ for 24 hrs, centrifuged at 3000 rpm/15 mim ,and precipitate was cultured on blood agar and Macconky agar and incubated at 37 C˚ for 24 hrs .

Diagnosis depend on morphological character (shape ,color and size)of colony, then suspected isolates subcultured on Macconky agar .

Identification of isolates :

Diagnosis according to(Cruickshank *et al.*,1975 and Macfaddin ,2000)

1- Gram stain

2-Biochemical tests :

A-Catalase test
B- Oxidase test
C- Lactose fermentation
D- Urease test
E-Indol test
F-Methyl red test
G- Voges-Proskauer test
H- Citrate utilization test
I- Gelatin liquefaction test
L- T.S.I
M- Phenylalanine deaminase test
N- Eosin Methylene blue test

3-Api-20E system (Analytical profile index for Enterobacteriaece test) according to (Atlas, 1995) :

This test(**Api-E20 system BioMerieux**) used for diagnosis the bacterial isolates, and this test have dried material which represented the biochemical test (according to instructions of the company).

4- Serotyping of *E.coli* :

After diagnosis of bacterial isolates by gram stain , biochemical test and Api-E20 system .

E.coli isolates was cultured on slant of kligler iron agar ,and incubated 37 C˚ for 24 hrs , then the serotyping of *E.coli* isolates were performed in the Central Public Health –Baghdad Lab. , according to Spanish kit (Laboratorio de Referencia de *E.coli* LREC) .

Results:

Out of (353) milk samples which were collected from 265 normal and 88 clinical mastitic cows, and these mastitic milk samples were collected from three different places in Iraq as 91 , 128 and 134 samples from (AL-Najaf ,AI-Qadisyia and Babylon provinces) respectively(table-1) .

Table (1):Numbers of milk samples and areas of samples collection

Province	Places		Clinical mastitis	Sub clinical mastitis	Total numbers
Al-Najaf	Al-Bahrani state		14	16	91
	Villages of Al-Huryia Towinship		-	61	
Babylon	Al-Qassim Township	Al-Sadah Village	-	14	134
		Gusher Village	21	65	
		Al-Ebekher Village	13	21	
Al-Qadysia	Villages of Nufar Towinship		8	30	128
	Villages of Al.Seeder Towinship		4	11	
	Villages of Sumer Towinship		23	33	
	Villages of Al.Saniah Towinship		4	3	
	Villages of Ghamass Consituency		1	11	
Total number			88	265	353

50 *E.coli* isolates were isolated (14.16 %) as in (table -2) , *E.coli* was isolated from 28 & 22 milk samples of clinical and subclinical mastitic cases as (31.8 % , 8 .3 %) respectively (table -2) .

Table (2) :clinical ,sub clinical and total mastitis incidence .

	Milk samples	No. of positive *E.coli*	%
Clinical mastitis	88	28	31.81
Subclinical mastitis	265	22	8.30
Total No.	353	50	14.16

The results revealed that there were no significant differences between local and cross breeds of cows for their susceptability to *E.coli* strains as the causative agent of mastitis and table (3) exhibits that *E.coli* was isolated from 10 (13.3%) ,40 (14.3%) of mastitic milk samples from local and cross breed respectively .

Table (3) : mastitis incidence in local and cross breed of cows .

Cow breed	Milk sample	No. of positive ***E.coli***	%
Local	75	10	13.3
Cross	278	40	14.3
Total	353	50	14.1

The ages of cows were affected the incidence of *E.coli* mastitis ,and table (4-3) was showed that cows less than 5 years old were more resistance to *E.coli* mastitis (7 %) than cows 5- 7 years, and 8 years and over cows 16.58 %, 19.4 % respectively.

Table (4) : Relationship between *E.coli* mastitis and age of cows .

Age /years	No.of mastitic milk samples	No.of positive *E.coli*	%
Less than 5	100	7	7
5-7	217	36	16.5
8and over	36	7	19.4

The date of calving was affected significantly on *E.coli* mastitis incidence and table (5)was explained that *E.coli* mastitis was recorded as 20.14 %, 14.2 % and 7.8 % at (1-30) days ,(31-60) days and (over than 60) days after parturition respectively .

Table (5) :Relationship between date of calving and *E.coli* mastitis

Post parturition	No. of samples	No. of positive ***E.coli***	%
1-30 days	134	27	20.1
31-60 days	91	13	14.2
Over than 60 days	128	10	7. 8

E.coli was isolated from subclinical mastitis samples 22 (31.8%), with different degrees of CMT as 3 (13.6%), 9(40.9%), 7(31.8%), 3 (13.6%) of ±, +1 , +2 and + 3 of CMT degrees respectively as showed in(table -6).

Table (6) : degrees of CMT and subclinical *E.coli* mastitis incidence .

CMT degrees	*E.coli* mastitis samples	
±	3	3 .6
+ 1	9	0.9
+ 2	7	1.8
+ 3	3	3.6

E.coli was isolated from 50 clinical and subclinical mastitic milk samples which were collected from different udder quarters and the high *E.coli* incidence were recorded in posterior quarters as show in table (7) RA 13 (26%) ,RP 16 (32%),LA 10 (20%)and LP 11 (22%) respectively **.**

Table (7) : Relationship between udder quarters and *E.coli* mastitis incidence

Udder quarters	*E.coli* mastitis samples	
Right anterior RA	13	6
Right posterior RP	16	2
Left anterior LA	10	0
Left posterior LP	11	2

4-2 –The results of biochemical tests:

E.coli strain was diagnosed and the results were elucidated in (table -8) :

Table(8): The results of biochemical tests of *E.coli*

Biochemical tes	Gram	Catalase	Oxidase	Lactose	Urease	Indol	Methyl red	Voges-prokau	Citrate	Gelatin	H2S	CO2	Phenylala--nin	EMB
E.coli	-	+	-	+	-	+	+	-	-	-	-	+	-	Metalic heen

Defenitive diagnosis :

1- Api-20E0 test :

The result of **Api-E20 test** was revealed the numerical profile(5144552) as confirmed diagnostic test for *E. coli* isolates .

2-Serotyping:-

Among 50 isolates strains of *E.coli* , 41 isolates had the somatic antigen (serogroup) determined and nine untypable strain were obtained . 16 serotypes were detected and the predominant serotype was *E.coli* O111: B4 (Table - 9) .

E.coli O111:B4 , which was isolated from severe clinical mastitis was regarded as a tool for next steps of our study . *E.coli* serotypes were illustrated according to Central Health Laboratory report .

Table (9): Serotyping of *E-coli* strains isolated from mastitic milk

	no. of *E.coli* strain	Serotype	%
1	6	0111	12
2	5	0125	10
3	5	0119	10
4	4	055	8
5	3	026	6
6	2	0128	4
7	2	08	4
8	2	05	4
9	2	0114	4
10	2	020	4
11	2	0113	4
12	2	0118	4
13	1	0126	2
14	1	076	2
15	1	086	2
16	1	0145	2
17	9	Unkown ***E.coli***?	Unkown ***E.coli***?

Reference: Central public Health laboratory /Ministry of Health

Discussion

E.coli mastitis percentage was 14.16% which regarded as a high percentage when compared with previous studies like Al-Khatib and Al-Bassam (1979) ,Zura (1979) and Essa (1992) as 7.2% ,8.8% and 9.8 % respectively in Iraq and 8.4% in Brazil (Correa and Marin ,2002) ,but it is almost close to some previous studies as 12.63% by Yass *et al* (1992) and 15.2% by Al-Graibawi *et al*(1998) as well as 13.04% in Netherlands and 13% in Turkey (Dopfer *et al.*,1999 , Turutoglu and Mndul,2002).

Mastitis is a multifactorial disease and many factors are contributed to increase the disease occurrence like ,bad mangement ,cow susceptibility and pathogen which is normaly inhibitant in intestine of cow and normally shedding

to contaminate the surruonding envirnonment (food,water ,milking machine and milker hands)all these factor are facilitate *E.coli* reaching to teat canal and cause *E.coli* mastitis .

Seventy four percentage (74%) of cows in our study were breeding in small seperated groups that decreased the possbility of contamination and transmission of infection ,therefore many studies recorded higher percentage of *E.coli* mastitis than our study like in Iraq 22.5% (Al-Graibawi *et al.*(2002) and 23.5% by Bradley *et al.*(2007) in U.K.because these studies were carried out in higher stock densities that resulting a greater pasture,water, bed,worker and milking machine contamination with easer access of *E.coli* to udder through their teat orifice during calving and early lactation and thus increase of *E.coli* mastitis incidence .

Results were revealed that 31.8% of *E.coli* mastitis are clinical but subclinical cases were 8.3% , and 40.9% of sub clinical mastitis under Grade +1 in CMT test as indication of first step of udder inflammation.

These results were in agreement with Essa(1992);Bradley and Green (2001) and this may be due to the udders were highly exposure to environmental pathogen (*E.coli*) ,that increase the udder exposure to *E.coli* infections at lactation is less than ten days, thus most of *E.coli* strains were isolated from milk which contain clots or blood without severe symptoms as indicated by Bean *et al*.(2004).

Early lactation stage and cows at(5-7) years old were significantly affected by *E.coli* mastitis ,this is in agreement with most of *E.coli* mastitis studies which inovolved stages of lactation and the age of the cows (Sordillo *et al.*,1997 & Haas *et al.*,2002).

High *E.coli* mastitis percentage around calving and in early stage of lactation was occurring due to previous mammary gland infection during dry off period can become sever after calving and also the bovine immune system is less capable of battling pathogens during the periparturient period and early lactation due to physical condition as increase level of progesterone , besides of gestation and calving stresses , all these factors may lead to immunosuppressant state which facilitated *E.coli* infection of the udder .

Cows (5-7 years) old had a high *E.coli* mastitis percentage since that cows were have peak of milk producing at this age, therefore higher milk content which considerd as lines of defense mechanism in mammary gland were lost and low numbers of somatic cell counts are estimated during this age average(Ynte *et al* .,2003), as well as sphincter muscles of teat relaxed due to contineous functioning that led not to permsis *E.coli* penetration only ,but also easily secretion of some drops of milk during laying down that make an excellent substrate for *E.coli* growth around teat with extreme penetration to udder .

The results were also showed that posteior quarters of udder are more susceptible for *E.coli* infection than anterior (especially right quarters)and these results were in agreement with Essa(1992) which was attributed to normal laying down of cow and that caused attachment of the posterior quarters with bed and also posterior quarters can contaminated by feces on hindlegs and tail of cow (as sources of *E.coli*).

Breed of cows had no significant influence on *E.coli* mastitis infection of our study,incontrast of Essa (1992) that he was reported a local breed had been more resistance to coliform infection than cross bred ,but in agreement of the fact that was established by Heringstad *et al.* (2000) who indicated that mastitis resistance was developing in quarters with moderate(300000 cells/ml) or high(400000 cells/ml) of SCC , and this traits could be variance between individuals in the same breed ,therefore genetic selection of resistance cow for mastitis may be carried out .

E. coli serotyping :

The results showed that out of 50 E.coli isolates , 41 isolates were O-serogrups ,16 isolates have different O-serogroups and 9 untypable isolates This indicated that E.coli mastitis is not caused by specific pathogenic strains .Many researchers had similar studies which reported a wide range of ***E.coli*** serotypes in cattle (Wells *et al.*,1991;Wilson *et al.*,1992;Wieler *et al.*,1996;Miyao *et al.*,1998 and Holland *et al.*,1999).

E.coli O111:B4 was the predominant *E.coli* serogroup , and this *E.coli* serotype was isolated from healthy and diarrheic calves by Holland *et al* (1999)and from mastitic cows in Brazil by Correa and Marin (2002).

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O111:B4جرثومة الاشيريشيا القولونية ذات الضرب المصلي المسبب الشائع للالتهاب الضرع القولوني في الأبقار في بعض مناطق حوض الفرات الأوسط في العراق

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الخلاصة

تضمنت الدراسة فحص (٣٥٣) عينة حليب من أبقار مصابة بالتهاب الضرع السريري وأخرى ظاهريا سليمة من مناطق مختلفة لثلاثة محافظات في العراق تمثلت ب٩١ نموذج من محافظة النجف (محطة تربية البحراني والقرى التابعة لناحية الحرية)، و ١٢٨نموذج من محافظة القادسية (القرى التابعة لنواحي سومرونفر والسديروالسنية والقرى التابعة لقضاء غماس) و١٣٤ نموذج من محافظة بابل (القرى التابعة لناحية القاسم)، للتحري عن جرثومة الاشيريشيا القولونية كمسبب لالتهاب الضرع في الأبقار، وتم استخدم اختبار California CMT(Mastitis Test) لفحص عينات الحليب من الأبقارالسليمة ظاهريا،و كانت نسبة الإصابة الكلية بالتهاب الضرع الذي سببته جرثومة الاشيريشيا القولونية هي (14.16%) . ولم يظهر لسلالة الأبقار تأثيرا معنوياً على نسبة حدوث حالات التهاب الضرع في الأبقار .

كان لأعمار الأبقار تأثيرا معنوياً على نسبة حدوث الإصابة بالتهاب الضرع الذي تسببه جراثيم الاشيريشيا القولونية ، حيث كانت الأبقار بعمر (٨ سنة فما فوق) الأكثر حساسية للإصابة بهذا الالتهاب (19.4%)مقارنة مع بقية الأبقار الأقل عمراً ، في حين أظهرت الأبقار التي مضت على ولادتها (١– ٣٠ يوماً)حساسية أكثر للإصابة بالتهاب الضرع الذي تسببه جراثيم الاشيريشيا القولونية (20.14%)مقارنة مع بقية الأبقار التي مضت على ولادتها فترات زمنية أطول .

سجلت حالات التهاب الضرع في الأرباع الخلفية (الأيمن الخلفي ،الأيسر الخلفي) وبنسبة٣٢% ، ٢٢% على التوالي كنسبة إصابة أكثر مما عليه في الأرباع الأمامية للضرع .

شملت الدراسة عزل جرثومة الاشيريشيا القولونية من عينات الحليب وتشخيصها حيث استخدمت الفحوصات البايوكيميائية ونظام التشخيص الخاص بجراثيم العائلة المعوية API-20E system وبعد التشخيص الدقيق لجرثومة الاشيريشيا القولونية تم تنميط عزلات الاشيريشيا القولونية في مختبر الصحة المركزي مصلياً وكان الضرب المصلي *E.coli*O111:B4 هو السائد .