Distribution of *E.coli* O157:H7 in fecal and urine samples of cattle

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Summary

This study was designed to isolate of Enterohaemoragic *E.coli* O157:H7 from feces and urine in cattle. 175 samples (80 fecal and 95 urine) were collected to isolate EHEC O157:H7 during the period from March to June 2014 from Al- Hindia slaughter house from local breeding cattle, aged between 1-2 years and over 2 years, and the samples were subjected to culture on specific media Cefixime Tellurit- Sorbitol MacConkey agar at 37 ˚C for 18-24 hrs. then the isolates were identified by biochemical tests (Indol test, Potassium cyanid test) and seriologically by Latex Kit test. The results showed that 73(91.25%) out of 80 fecal samples and 39(41%) out of 95 urine samples were *E.coli* O157:H7 positive, the total of positive isolates were 64%. That reveals the important role of feces and urine samples to contaminate the environment and disseminate of infection.

Keywords: *E.coli* O157:H7, Cattle, Fecal, Urine.

Introduction

Escherichia coli O157:H7 a food borne bacteria, were considered as a very important zoonotic bacteria that induced clinical signs ranged from limited watery to severe bloody diarrhea and hemolytic colitis HC (1) some animal case developed hemolytic uremic syndrome (HUS) and kidney failure (2) and act systemically on sensitive cells in the kidneys, brain and other organs (3). Old people and children might progress to Thrombotic Thrombocytopenic Purpura TTP and Disseminated Intravascular Coagulation DIC (4) although most Enterohaemoragic *E.coli* strains produce Stxs, EHEC O157:H7 are especially virulent and are responsible for the majority of HUS cases of bacterial etiology worldwide (5). The major animal carriers are healthy domesticated ruminants, primarily cattle (3) and, to a lesser extent, sheep and possibly goats (6). Cattle and other ruminants harbor *E.coli* O157:H7 without clinical symptoms and these animals shed this pathogen with feces for long period that induced food contamination (7). Large (8) revealed that beef and dairy cattle were carried 0.5 - 2%. Feces of cattle that carry this bacteria is the main source of contamination to environment and considered the primary reservoir for this pathogen, the high STEC prevalence detected in dairy herds evidences that bovine feces might play an important role as a contamination. Source in the environment (9) this bacteria still active for several month in soil water and animal houses (10). The aim of this study was to isolate of Enterohaemoragic *E.coli* O157:H7 from feces and urine in cattle.

Materials and Methods

Samples collection: A hundred and seventy five samples were collected from cattle at the Al-Hindia slaughter house, 80 sample of feces and 95 urine. 20 gm of fecal samples were collected from the rectum with sterile gloves and put in sterile clean screwed cups, Also urine samples were collected by using sterile syringe 50cc and put in sterile 100 cc container brought to the laboratory of zoonotic disease unit in Coll. Vet. Med. Baghd. Uni. The fecal samples were processed upon arrival or within 18- 24 hrs. of collection. 3gm of each fecal sample was mixed with normal saline 7 ml, and centrifuged at 3000 rpm, the supernatant was discarded and the deposit was inoculated with Tryptic Soya broth and incubated at 37 °C for 18- 24 hrs (11). Twenty (20) ml urine sample were taken from each sample, 10 ml centrifuged at 3000rpm, the supernatant was discarded and the deposit was inoculated with Tryptic Soya broth with Cefixime Tellurit and incubated at 37°C for 18-24 hrs. (12).

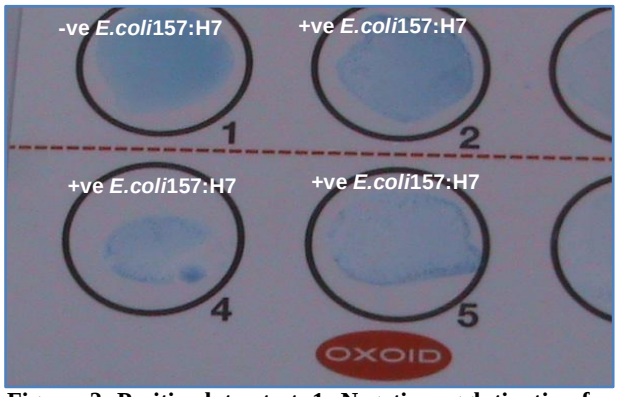
Selective enrichment and isolation of *E. coli O157:H7*: 1g of feces were placed in 10 ml of universal pre-enrichment broth (Difco, Inc., Detroit, Mich.) with a 15-mg/ml final concentration of novobiocin (Sigma-Aldrich, St. Louis, Mo.) and incubated at 42°C for 18 to 24 hrs. The tube was vortexed and a loop full sample was plated with Cefixime Tellurit-Sorbitol MacConkey agar (CT-SMAC), the

plate was streaked for isolation and incubated at 37 °C for 24 hrs. the colony of *E. coli* O157:H7 appears non-sorbitol-fermenting colonies on this medium (13). Agglutination test: Non-sorbitol-fermenting colonies on this medium were tested by agglutination with *E. coli* O157 latex reagent (Oxoid).

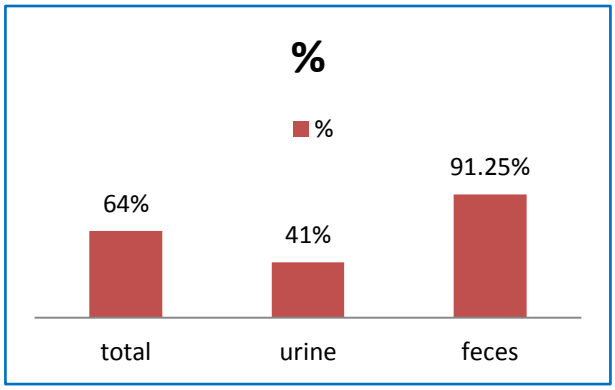
Results and Discussion

The positive isolates show a smooth, circular and colorless on SMA-CT agar at 18-24hrs post-culturing at 37 °C, (Fig. 1). In Sorbitol MacConkey agar, lactose is replaced by Sorbitol. Most strains of *E. coli* ferment Sorbitol to produce acid but *E. coli* O157:H7 could not ferment Sorbitol. This method explains that *E. coli* O157:H7 unlike 90% of *E. coli* isolates does not ferment Sorbitol rapidly. The bacterial isolated were positive for indole test and negative to potassium cyanid. Biochemical results were supported that the present isolates might be *E.coli* O157 serotype, *E.coli* O157:H7 detected by using latex kit test showed that the blue color agglutination indicated positive result for (H antigen) (Fig. 2). The result showed that 73 (91.25%) out of 80 fecal samples were *E.coli* O157:H7 positive of examined cattle in Al-Hindia slaughtering abattoir harbored *E.coli* O157:H7 in their intestinal tracts of these animal shaded this organisms with their feces (Table, 1 and Fig. 3). This result could indicate that cattle were important reservoir host of *E.coli* O157:H7 in this region of Iraq. Results revealed that the differences between two proportions were significant (P<0.001).

Figure, 1: Sorbitol agar the circular, colorless colonies of *E.coli* O157:H7.



Figure, 2: Positive latex test, 1: Negative agglutination for *E.coli* O157:H7, 2, 4 and 5 positive agglutination for *E.coli* O157:H7.



Figure, 3: The percentage of positive isolates of *E.coli* O157:H7 from feces and urine samples.

Table, 1: Positive samples for *E.coli 157:H7* in feces and urine samples

samples	No of exam samples	No of positive samples	%
feces	80	73	91.25%
urine	95	39	41%
total	175	112	64%
Chi-square	47.49		
P	<0.0001		

This result confirmed that diagnosis of the *E.coli* O157:H7 using feces are powerful than urine. The statically analysis by Chi -square show that the infection rates according to age category confirmed that the difference was significant (P=0.02) (Table, 2). Cattle more than two years age have high infection rate compared to the cattle with lower age this could be attributed to that the older cows are more exposed to pathogenic agent and this lead to increasing the opportunity of infection. The high estimate of infection rate associated with feces samples as compared with urine sample could be attributed to the pathogenic agent is usually resides in the intestines. This evidence was in agreement with observation of (14) who detected that 90 % of fecal samples

were positive *E.coli* O157:H7. Also in Pakistan (15) found that Sorbitol non-fermented *E.coli* present in 12.9% fecal samples of cattle that harbor *E.coli* O157:H7 without clinical signs might due to lack of Gb3 (Globotriaosyl ceramide Gb3) receptor on their intestinal. Also urine samples showed that 39 (41%) out of 95 urine samples were positive (Table, 1). The result of urine samples according to the age, the total infection rate for the category 1 (age ≤ 2 years) was 40% whereas the corresponding estimate for the second category (age was > 2 years) was 41.42%. The difference between two proportions was not significant (Chi-square value 0.015, P=0.90) the difference between sexes within each category was not significant (Table, 3).

Table, 2: Number of positive isolates for *E.coli* O157:H7 in feces samples of cattle according to the age and geneder.

	1-2 years (n=19)				More than 2 years (n=61)			
	Male (n=15)		Female (n=4)		Male (n=55)		Female (n=6)	
	+	-	+	-	+	-	+	-
Total	12		3		54		4	
	15(78.94%)				58(95.08%)			
Chi square	4.72							
P	0.02							

Table, 3: Number of positive isolates of *E. coli* O157: H7 in urine samples of cattle according to the age and gender.

	1-2 years (n=25)				More than 2 years (n=70)			
	Male (n=21)		Female (n=4)		Male (n=56)		Female (n=14)	
	+	-	+	-	+	-	+	-
Total	8		2		21		8	
	10(40%)				29(41.4%)			
Chi square	0.015							
P	0.90							

Also the association between gender and age was significant (Chi-square value 95.29, P<0.0001) this mean that the infection in female was higher than male. These results are in agreement with (15) who examined blood and urine samples from 17 calves and 19 cows and found the *E. coli* was the most frequent cause of UTI in a dairy cattle herd. Davis (16) investigated the possibility that urine plays a role in the environment contamination survival of *E.coli* O157:H7, the presence of urine-soaked bedding in a ruminant stall or dilution of that urine with rainwater could provide a growth medium for *E.coli* O157:H7.and study how that urine provided a substrate for *E.coli* O157:H7 growth have implications for understanding the on-farm ecology of this pathogen and for the safety of ruminant animal exhibits, particularly petting zoos and farms where children might inter animal pens.

Total infection rates according to age category confirmed that the difference was significant (P=0.02). Difference between males and females was not significant in first category. (Chi square value 0.047, P=0.82). Difference between males and females was significant in the second category. (Chi square value 11.49, P=0.0007). According to the age the difference between two proportions was not significant (Chi-square value 0.015, P=0.90). The difference between sexes within each category. In the first category the results showed that the difference was not significant (chi-square value 0.198, P=0.65) and the results were also not significant concerning the second category (chi square value 1.78, p=0.18). This supports the observation of (17) that isolate *E.coli O157: H7* from healthy cattle and calves and stated that Cattle are the major reservoir of E. coli serogroup O157 and frequently identified as direct or indirect sources of infection to man.

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انتشار جرثومة *E.coli* O157:H7 في عينات بول وبراز الأبقار

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الخلاصة

صمّمت هذه الدراسة لعزل وتشخيص جرثومة *E.coli* O157:H7 المعوية النزفية في الأبقار باعتبارها المصدر الرئيسي والخازن لهذه البكتريا إذ تطرحها مع البول والبراز مسببة بذلك تلوث البيئة. جمعت 175 عينة (80 عينة براز و95 عينة بول) من الأبقار لعزل جرثومة *E.coli* O157:H7 للمدّة من بداية شهر آذار إلى نهاية شهر حزيران 2014 من مجزرة الهندية من كلا الجنسين ومن سلالة محلية وتتراوح أعمار الحيوانات مابين 2-5 سنوات و زُرِعَتْ على الأوساط الزرعية الخاصة لمدة 18 - 24 ساعة وبدرجة حرارة 37 °م وبعدها عملت الفحوصات الكيموحيوية وثبت التشخيص المصلي باستعمال اختبار اللاتكس. أظهرت النتائج ان 80/73 (91.25%) عينة براز و95/39 (41%) عينة بول كانت موجبة لجرثومة *E.coli* O157:H7، النسبة الكلية للعزلات الموجبة لـ *E.coli* O157:H7 (64%) تستنتج الدراسة إن عينات براز وبول الأبقار لها دور مهم في تلوث البيئة وانتشار الاصابة.

الكلمات المفتاحية: *E.coli* O157:H7، أبقار، براز، بول.