# DETECTION OF ENTEROHEMORRHAGIC *ESCHERICHIA COLI* O157 IN SHEEP AND GOATS USING FLUOROGENIC AND CHROMOGENIC CULTURE MEDIA

**ALI YAHYA SAEED** [1], **KHALID SOBHI IBRAHIM** [2]
[1] Dept. of Biology, Faculty of Science, Duhok University, Kurdistan Region-Iraq
[2] Dept. of Biology, Faculty of Science, University of Zakho, Kurdistan Region-Iraq
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## ABSTRACT

**This study was carried out for the first time to investigate the occurrence of *E. coli* O157 in sheep and goats in Duhok province, Iraq. A total of 320 samples were collected from April to July 2009 as following: 100 fecal samples from sheep, 100 fecal samples from goats, 60 samples from sheep milk and 60 samples from goat milk. *E coli* O157 was isolated from 3 (3%) sheep fecal samples and 2 (2%) from goat fecal samples, while no *E. coli* O157 was detected in the milk samples from sheep and goats. *E. coli* O157 was detected only among *E. coli* isolates which were sorbitol non fermentative but MUG positive, while no *E. coli* O157 was detected among *E. coli* isolates which were negative for both sorbitol and MUG. All *E. coli* O157 isolates were resistant to 6 among 12 antimicrobial agents used in vitro drug sensitivity test**

***KEY WORDS*: *E .coli O157, Sheep, Goats, Feces, Milk***

## INTRODUCTION

Shiga toxin producing *E. coli* (STEC) O157 is an important cause of food-borne infections. This serotype can cause hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) in children. These infections occur in all parts of the world including Middle East countries (Adwan et. Al., 2005). Epidemiological studies have shown that the principal sources of human infections are asymptomatic domestic animal carriers such as cattle, sheep and goats (Molina et. al., 2003). Although sheep and goats have been subjected to fewer epidemiological surveys than cattle, but considered more important than cattle as a source of STEC O157 and non-O157 (Mc Donough et. al., 2000). Rubini et. al. (1999) found STEC O157 in sheep milk. Hemolytic uremic syndrome recorded in Czech Republic was due to non-pasteurized goat milk (Bielaszewska et. al., 1997).

The discriminate use of antibiotics has led to the emergence of antimicrobial resistance in various isolates of bacteria and it is well documented that drug resistance could be transferred between relative bacteria, such as *E. coli* and Salmonella via resistant -factor, both in vitro and vivo (Verma, 1988). Drug resistant *E. coli* O157 may not prevent life threatening HUS in human STEC infections (Shiomi et. al.,1999). Besides having clinical consequences, resistant *E. coli* strains of animal origin may be the source of determinants of resistance for the possible transfer to human strains (Oppeggaard et.al., 2001).

*E. coli* O157 does not ferment sorbitol, and this property is used in its isolation on sorbitol containing bacteriological media (Ojeda et. al., 1995). MUG (4-methyl β-D-glucuronide) assay used in conjunction with testing for sorbitol fermentation and agglutination in *E.coli* O157 antiserum is considered a useful screening test for presumptive diagnosis of *E. coli* O157 (Ware et. al., 2000).

The aim of the present study was to investigate the presence of *E. coli* O157 in sheep and goats and to determine in vitro antibiogram of the isolates.

## MATERIALS AND METHODS

### SAMPLING

A total of 320 samples were collected from April to June 2009 from Duhok province/Kurdistan/Iraq, in which 200 fecal samples from sheep and goats (each 100 samples) and 120 milk samples from sheep and goats (each 60 samples). All samples were transported to the laboratory and pre-enriched immediately using tryptic soya broth (Oxoid) containing 20µg/ml novobiocin (oxoid) and incubated at 37°C for 24 hours.

### ISOLATION AND IDENTIFICATION

Samples from enrichment broth were subcultured on both chromogenic Hemorrhagic Colitis agar (Sifin, Germany) and fluorogenic medium Escherichia coli Direct agar (Sifin, Germany). Sorbitol non fermentative colonies appear as pink colour, while sorbitol

fermentative colonies in yellow colour as shown in figure (1). Sorbitol fermentative colonies were subcultured on Escherichia coli Direct (ECD) agar incubated at 37 °C for 24 hours and exposed to the ultra-violet light (Doc-print 11, France) in a darkened room. Results were recorded for 4-methylumbelliferyl-ß-d-glucuronide (MUG) positive and mug negative (i.e blue fluorescence or no blue fluorescence under UV- light respectively) as shown in figure (2).

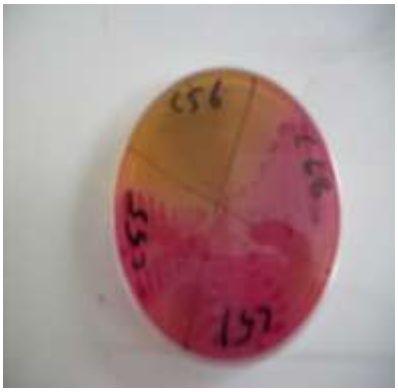

**Fig.1.** HC agar showing three non sorbitol fermentative *E. coli* (pink) and one sorbitol fermentative *E .coli* (yellow)

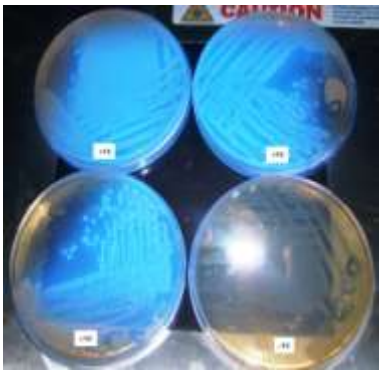

**Fig.2.** showing three MUG positive *E. coli* isolates which give blue fluorescent dye under UV light, while MUG negative *E. coli* without blue fluorescent dye

All sorbitol non fermentative and/or MUG negative colonies were submitted for the standard biochemical reactions such (IMViC) to confirm that they belong to *E. coli*. Then they were tested serologically using anti-O157 latex agglutination test (Oxoid).

## ANTIMICROBIAL SENSITIVITY TEST

The in vitro sensitivity of *E. coli* O157 isolates towards 102 different antibiotics was determined by disc diffusion method of Bauer and Kirby (1966) on Mueller-Hinton agar (Oxoid). The antimicrobial agents included Neomycin (N 30µg), Ciprofloxacin (Cip 5µg), Gentadox (GTD 40 µg), Cephalexin (CL 30 µg), Gentamycin (CN 10µg), Norfloxacin (nor 10µg), Cefaclor (CEC 30µg), Amicacin (AM 10 µg), Amoxicillin (AMX 25 µg), Clindamycin (DA), Carbencillin (PY 100 µg), Florfenicol (FL 30 µg)

## RESULTS

Sorbitol non fermentative and mug negative *E. coli* 6(6%), 5 (5%), 3 (5%)) and 0 (0%) in fecal samples of sheep, goats, milk samples of sheep and goats respectively, while sorbitol non fermentative and mug positive *E. coli* was 17 (17%), 10 (10%), 0 (0%) and 0 (0%) in fecal samples of sheep, goats, milk samples of sheep and goats respectively as shown in table (1) .

*E. coli* O157 was isolated from 3 (3%) sheep fecal samples and 2 (2%) goat fecal samples while no *E. coli* O157 was isolated from milk samples of sheep and goats as shown in table (2). All *E. coli* O157 isolates from sheep and goats were sorbitol non fermentative and mug positive, while none of sorbitol non fermentative and mug negative *E. coli* was positive with anti-O157 serum as shown in table (3)

Table1. Results of biochemical markers for E.coli O157

| Sample | Number | Source | *E.coli*[1] (%) | *E.coli*[2] (%) |
|---|---|---|---|---|
| Feces | 100 | Sheep | 6 (6%) | 17 (17%) |
| Feces | 100 | Goats | 5 (5%) | 10 (10%) |
| Milk | 60 | Sheep | 3 (5%) | 0 (0%) |
| Milk | 60 | Goats | 0 (0%) | 0 (0%) |

[1] Sorbitol non fermentative and MUG negative *E. coli* isolates

[2] Sorbitol non fermentative and MUG positive *E. coli* isolates

Table 2. Results of serodiagnosis using specific antiserum

| **Sample** | **Number** | **Source** | **Positive for *E. coli* O157** |
|---|---|---|---|
| Feces | 100 | Sheep | 3 (3%) |
| Feces | 100 | Goats | 2(2%) |
| Milk | 60 | Sheep | 0(0%) |
| Milk | 60 | Goats | 0(0%) |

Table 3. Biochemical markers and serological test

| Isolates | Number of isolates | Positive (anti-O157) serologically |
|---|---|---|
| *E. coli*[1] | 14 | 0 |
| *E.coli*[2] | 27 | 5 |

[1] Sorbitol non fermentative and MUG negative *E. coli* isolates

[2] Sorbitl non fermentaive and MUG positive *E. coli* isolates

All *E. coli* O157 isolates were resistant to Cephalexin, Cefaclor, Amicacin, Amoxycillin, Clindamycin and Carbenicillin, while all of the isolates were sensitive to Ciprofloxacin, Gentadox, Gentamycin and Norfloxacin as shown in table (4).

Table 4. Frequency of antibiotic resistance among E. coli O157 strains

| Antimicrobial gagents | Resistance of E. coli O157 isolates | | | | |
|---|---|---|---|---|---|
| | G2 | G50 | Sh 76 | Sh 86 | Sh 62 |
| Neomycin (N 30µg) | I | I | I | I | I |
| Ciprofloxacin (Cip 5µg) | S | S | S | S | S |
| Gentadox (GTD 40 µg) | S | S | S | S | S |
| Cephalexin (CL 30 µg) | R | R | R | R | S |
| Gentamycin (CN 10µg) | S | S | S | S | S |
| Norfloxacin (nor 10µg) | S | S | S | S | S |
| Cefaclor (CEC 30µg) | R | R | R | R | R |
| Amicacin (AM 10 µg) | R | R | R | R | R |
| Amoxicillin (AMX 25 µg) | R | R | R | R | R |
| Clindamycin (DA) | R | R | R | R | R |
| Carbencillin (PY 100 µg) | R | R | R | R | R |
| Florfenicol (FL 30 µg) | S | S | S | S | S |

Legands: (S); sensitive, (I); Intermediate, (R);resistant

## DISCUSSION

*Escherichia coli* O157 is a main food-borne pathogen which causes hemorrhagic colitis and hemolytic uremic syndrome in human (Kuntz and Kuntz, 1999). Numerous studies have shown that healthy ruminants such as cattle, sheep and goats constitute world wide natural reservoirs of *E. coli* O157, and become the source of human infections. Documented cases of infections in humans were correlated with the consumption of unpasteurized sheep and goat milk (Novotna et. al., 2005). However, no data available in our area concerning the occurrence of this important food-borne pathogen in sheep and goats which constitute the main source of protein for people in the area and this study is the first to be carried out in the region concerning this serotype of *E coli*. A relatively frequent occurrence of the

bacteria carriers among sheep and goats and subsequently in their products may suggest that this may be a relatively important source of infection for people (Chiueh et.al., 2002). Sheep and goats of our area like other parts of the world are also carriers to these bacteria. It is hard to compare these results with others because of different methodologies used in addition to many factors which influence the results like season, age, management and diets (Jenkins et.al., 2002). The main biochemical markers used for presumptive diagnosis of *E. coli* O157 are sorbitol non fermentative and MUG negative *E. coli* (Ware et.al., 2000). In this study no sorbitol non fermentative and MUG negative *E. coli* reacted with anti-O157 sera, while all positive samples were sorbitol non fermentative and MUG positive which implies that these two biochemical markers are not reliable for presumptive identification of *E. coli* O157, since other strains of *E. coli* can produce similar biochemical profile and may be less practical particularly when tested from normal animals, food products and environmental samples because of the high background prevalence of sorbitol negative *E. coli*. (Ojeda et.al., 1995), other possibility of such isolates may be other serotypes of E. coli which cross-reacted with anti-O157 serum. Non O157 *E. coli* recorded more frequently than *E. coli* O157 in sheep in many different areas of the world and constitute major shiga toxin producing *E. coli* (Nataro and Kaper, 1998).

In the present study, sensitivity of *E. coli* O157 was studied in vitro against 12 antimicrobial agents. The isolates were resistant to 6 among 12 antimicrobial agents which imply that multiple drug resistant *E. coli* O157emerged in animals which due to the misuse of antimicrobial agents for treatment of animal infections. It has also been reported in Palestine (Adwan et.al., 2002; Adwan and Adwan; 2004) that 49% of human *E. coli* O157 and 55% from animals were resistant to three or more antibiotics.

The use of antibiotics for the treatment of *E. coli* O157 infections may be contraindicated because certain antibiotics induce the release and dispersion of Shiga-toxin encoding bacteriophages (Zhang et.al., 2000)), or even complicate the development of clinical situation of patients (Wong et.al., 2000).

## CONCLUSION

Based on the results, we can conclude that biochemical markers such as sorbitol fermentation and MUG assay are not reliable as biochemical markers of *E. coli* O157, since other strains of *E. coli* as well as other species of bacteria show similar pattern. Sheep and goats are carrier of *E. coli* O157 and may become source of infection to human.

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## تشخيص بكتريا ايشيريشيا كولاى **O157** في الاغنام والماعزباستعمال اوساط الزرعية المتالقة ومولدة للصبغة.

**الخلاصة**

أجريت هذه الدراسة لغرض الكشف عن تواجد بكتريا ايشيريشيا كولاى O157 في الاغنام والماعز في محافظة دهوك –العـــراق . شملـــت الدراسة 320 عينة منها 100 عينة براز الأغنام، 100 عينة براز الماعز ، 60 عينة من حليب الأغنام و 60 عينة من حليب الماعز. كان تواجد بكتريا ايشيريشيا كولاى O157 في العينات كالاتي: 3% في عينات براز الأغنام و 2% في عينات براز الماعز بينما لم تعزل هذه البكتريا مـــن عينات حليب الأغنام و الماعز . كانت جميع العزلات لبكتريا ايشيريشيا كولاي O157 غير مخمرة لسكر سوربيتول بينمـــا موجبـــة ل -4 methylumbelliferyl-β-D-glucuronide (MUG) و لم تعزل هذه الجرثومة من بين عزلات ايشيريشيا كولاى والتي كانت غـــير مخمرة لسكر سوربيتول و سالبة ل MUG . اظهرت جميع العزلات مقاومة ضد كل مـــن,Amicacin،Cephalexin, Cefaclor ، Amoxycillin، Clindamycin Carbenicillin بينما كانت جميعها حساسة ل Ciprofloxacin, Gentadox, Norfloxacin Gentamycin .

## دەستنیشنکرنا بەکتریا *E. coli* O157 ل پەزا و بزنا بکارئینانا میدیاییت جەیسوك و رەنکفەدان

**پوختە**

**فە کولینی** 320 **سامبل خوفە کرتینە ز وان** 100 **سامبل بیساتیا بەزینە ،** 100 **سامبل بیساتیا بزنانە،** 60 **سامبل شیری پەزی و** 60 **سامبل شیری بزنا.**

**بەکتریا** *E. coli* O157 **هاتە دیتن ل** 3٪ **بیساتیا بەزی،** 2٪ **بیساتیا بزنا بەلی نەهانە دیتن ل شیری بەزا و بزنا. هە می تیزین فافارتی نیکە تیفبوون بو شەکرا سوربیتول بەلی بوزە تیفبوون بو کەرەستی** MUG **. ئەف جوری بەکتریا نەهاتە دیتن دنافبەرا** *E. coli* **ئەوا نیگە تیف بو شەکرا سوربیتول و کەرەستی** MUG **.**

**هەمی تیز خوراکربوون بو** Clindamycin Carbenicillin، Amoxycillin، Amicacin، Cephalexin, Cefaclor **بە لی هەستداربوون بو** Norfloxacin، Ciprofloxacin, Gentadox, Gentamycin .