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Identification of *Escherichia coli* O157 in sheep and goats using PCR technique

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Abstract: *A total of 41 sorbitol non fermentative E. coli from previous work were used in this study. Among these isolates 5 were positive serologically by anti-O157 serum. All isolates were tested by PCR technique. No E. coli O157 were detected in all isolates by PCR. The results revealed that gene based method such as PCR technique is more reliable than biochemical and serological tests for diagnosis of E. coli O157.*
Key words: *Non sorbitol fermentative E. coli, PCR*

I. Introduction

Escherichia coli O157 is an important food-borne pathogen causing hemorrhagic colitis and hemolytic uremic syndrome in humans [1]. The most studied serotype is *E. coli* O157:H7 which is named enterohemorrhagic or Verotoxin-producing *E. coli* [2]. Ruminants such as cattle, sheep and goats have implicated as a reservoir of this bacteria [3]. Identified human infections were traced to eating undercooked hamburger beef [4], drinking of contaminated water [5] and unpasteurized sheep and goat's milk [6].
Different methodologies were used for diagnosis of *E. coli* O157. Most of these methods relied on the unique biochemical markers of *E. coli* O157 like inability to ferment sorbitol or to produce β-glucuronidase [7]. Other pathotypes of *E. coli* such as Enteroinvasive *E. coli*, Enteropathogenic *E. coli* and Uropathogenic *E. coli* as well as other bacteria can also produce similar biochemical profile. Moreover, some strains of *E. coli* O157:H7 are sorbitol fermentative [8, 9].

Serological diagnosis based on the reaction with anti-O157 serum is not specific, since cross-reactions occur with other bacteria [10, 11]. Both cultural methods and serological tests can not differentiate toxigenic strains from non-toxigenic strains, therefore gene based methods such as PCR technique is necessary for diagnosis of *E. coli* O157 its virulence factors such as shiga toxins, intimin and hemolysin [12].
The objective of the present study was to apply PCR technique for diagnosis of *E. coli* O157 from all sorbitol non fermentative *E. coli* including serologically positive for *E. coli* O157 isolated from sheep and goats.

II. Materials and Methods

2.1. Bacterial strains

A total of 41 Sorbitol non fermentative *E. coli* strains from previous work were tested for *E. coli* O157 by PCR technique. Among these isolates 5 were positive by latex agglutination test using anti-O157 serum (Oxoid).

2.2. DNA extraction

DNA was prepared according to the method used by Abdulmawjood *et al* [13]. All sorbitol non fermentative *E. coli* including those gave positive with anti-O157 serum were cultured to exponential phase, and then cells were pelleted by centrifugation at 12000 rpm for 10 minutes. The cells were washed in double-distilled water, and boiled for 10 minutes at 100 ºC. After centrifugation at12000 rpm for 5 s) the supernatant was used as a course of DNA for PCR technique.

2.3. Oligonucleotide primer

The oligonucleotide primers Gi- O157-1 5′- CGA GTA CAT TGG CAT CGT G-3′ and Gi- 157-II 5′ ATT GCG CTG AAG CCT TTG-3′ (MWG-Biotech, Germany) were used which target *rfbE* gene encoding for *E. coli* O157 antigen .

2.4. PCR conditions

The PCR mixture (25 µl) contained 1 µl of each primer (10 pmol/ µl), 2.5 µl dNTP (200µM each, Roche, Mannheim, Germany), 2.5 µl 10X thermophilic buffer with 15mM MgCl2 (PE Applied Biosystem, Weiterstadt, Germany), 0.2 µl Taq polymerase (5 U/ µl, PE Applied Biosystem) and 15.3 µl molecular biology grade water. Finally, 2.5 µl DNA solution was added to each reaction mix. The PCR was carried out in a thermal cycler (Master cycler, Eppendorf, Hamburg, Germany) with the flowing program: 1X 3 min precycle at

93 °C, 30 X 15 s at 93 °C, 15 s at 60 °C and 30 s at 72 °C followed by a final extension incubation at 72 ºC for 5 min. A 10 µl aliquot of a PCR product was loaded on a 1% agarose gel containing 0.5 µg of ethidium bromide/ ml and electrophoresed at 80 V/30 min (Bioblock scientific) with Tris-borate-EDTA (TBE) buffer (pH 8.3) and a 100-1000 bp DNA ladder (Biolab, Germany) as a molecular marker. Control positive was DNA from reference strain of *E. coli* O157:H7 (EDL 933) kindly supplied by Institute of Veterinary Food Science, Justus-Liebig University, Giessen, Germany.

III. Results

Table 1 shows the results of serological and PCR for identification of *E. coli* O157 in which 3 (13.04%) and 2 (13.33%) were positive serologically for *E. coli* O157 from fecal samples of sheep and goats respectively, while No *E. coli* O157 was detected in the milk samples of sheep. No *E. coli* O157 was detected among all sorbitol non fermentative *E. coli* including those serologically positive for O157 by PCR technique (Fig.1)

Source	Sorbitol non fermentative *E. coli*	Serological test (anti-O157serum)	PCR specific for O157
Sheep feces	23	3 (13.04%)	0
Goats feces	15	2 (13.33%)	0
Sheep milk	3	0	0

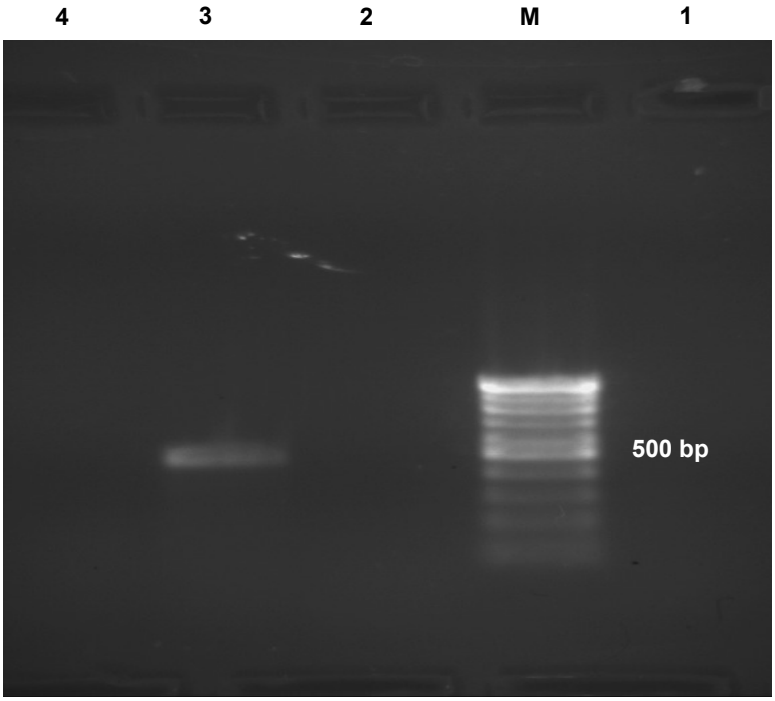

Fig.1. Results of PCR assay for detection of *rfbE* (501 bp) gene in *E. coli* O157 isolates. Lane 1 and 2, samples; M, 1 kb DNA marker; lane 3, control positive *E. coli* O157:H7(EDL 933); lane 4, cotrol negative.

IV. Discussion

There are many papers reporting the colonization of the gastroenteritis tract of both large and small ruminants with O157 and non O157 *E. coli* [14]. The direct relationship between *E. coli* O157 shed by small ruminants or the presence of the bacteria in their products on the one hand and human infections on the other has been, however, demonstrated only spoardically [15] . A relatively frequent occurance of the bacteria carriers among sheep [16] and the shiga toxin producing *E. coli* (STEC) found in sheep and goats products [17, 18, 19] suggest that this may be a relatively importnat source of infection for people living in regions with a high density of sheep and goat herds with many opportunity for contacts between poeple and small ruminants, where products of animal origin are processed by traditional methods and constitute a significant input of the local population diet per consumer basket. Published data on the prevalence of *E. coli* O157 in ruminant generally and small ruminants particulary are lacking in our region. Few studies carried carried out in human in Iraq referred

to the presence of Enterohemorragic *E. coli* O157:H7 among diarrheic children [20] but these studies dependent on the serological tests which are not reliable because of common cross-reaction with other bacteria. Our results showed that no *E. coli* O157 was detected among all sorbitol non fermentative *E. coli* including those isolates positive serologically with anti-O157. These results confirmed that the diagnosis of *E. coli* O157 can not be depend on the biochemical markers which share with many other bacteria [21] and the serologically positive isolates may be non O157 serogroups which cross-react with anti-O157 serum. The spectrum of STEC non-O157 serogroups was greater in sheep and goats than STEC O157 in different developed countries [22, 23, 24), while data on STEC non-O157 in small ruminants in the middle East countries are however missing.

V. Conclusion

Gene based method such as PCR technique is more reliable than the biochemical and serological methds for daignosis of *E. coli* O157.

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