Archives of Razi Institute, Vol. 77, No. 3 (2022) 1269-1273

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DOI: 10.22092/ARI.2022.358832.2317

*Original Article*

# Presence of *Escherichia coli* O157:H7 in Dairy Farms located in Najaf, Baghdad, Kirkuk, and Erbil, Iraq

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Received 10 May 2022; Accepted 30 May 2022

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**Abstract**

It has been approved that one of the most dangerous foodborne pathogenic bacteria is *E. coli* O157:H7, which is responsible for several infection and death cases worldwide. It is well documented that in the developing countries *E. coli* O157:H7 is considered the main causative pathogen of human gastrointestinal infections. Therefore, the current research was aimed to evaluate the prevalence of *E. coli* O157:H7 in dairy cattle's milk using a rapid method, in Iraq (Najaf, Baghdad, Kirkuk, and Erbil). Over a period of 6 months (During hot months) samples were obtained and investigated by culturing on selective media (CT-SMAC). The multiplex PCR (m-PCR) also used for milk sample direct investigation. Using biochemical tests the recorded data showed that, 2 recognized isolates were *E. coli*, while the recorded data obtained from m-PCR assay revealed that none of the isolated *E. coli* was toxigenic *E.coli* O157:H7. The results of m-PCR on the milk samples revealed that 45 milk samples contained at least one of the following genes: *O157*, *H7*, *stx1*, *stx2* genes. Also the results of the m-PCR revealed that 2 samples (raw milk) were toxigenic O157:H7 positive. In conclusion, to the best of authors' knowledge, this investigation was the first report on the prevalence of *E. coli* O157:H7 in the raw milk samples in Iraq. The results showed that the proportion of contaminated milk samples contaminated with *E. coli* O157:H7 identified in the current survey were similar to that the results of the previously published research from different dairy products across different countries in the Middle East region.

**Keywords:** Milk, *Escherichia coli* O157:H7, polymerase chain reaction

## 1. Introduction

It has been approved that one of the most dangerous foodborne pathogenic bacteria is *E. coli* O157:H7, which is responsible for several infection and death cases worldwide (1). Although the infection caused by *E. coli* O157:H7 is generally self-limiting, in immunocompromised individuals and children this bacterium can lead to life-threatening complications (2).

In infected humans with *E. coli* O157:H7 the main symptoms of the infection are as follows: 1)

gastrointestinal signs including hemorrhagic colitis (HC), and hemolytic uremic syndrome (HUS); 2) thrombotic thrombocytopenic purpura (3).

It has been well documented that 4 virulence factors of shiga toxin-producing *E. coli* (STEC) identified as follows: two phage-encoded cytotoxins, named Shiga toxin 1 and 2 which are encoded by *stx1* and *stx2*, the protein intimin which is encoded by *eae* gene, enterohaemorrhagic *E. coli* haemolysin (EHEC-HlyA) encoded by the ehxA (4). Inductions of attaching and effacing lesions are the two main identified characteristics of Enterohemorrhagic *E.coli* (EHEC) infection. Enterohemorrhagic *E.coli* has a pathogenicity island known as the locus of enterocyte effacement (LEE) (5). A translocated intimin receptor (Tir) (6) and three secreted proteins including EHEC secreted protein A (EspA), EspB, and EspD, are known as type III secretion system which is encoded by the pathogenicity island (LEE) (7).

It has been well approved that cattle are considered as the major reservoir of *E. coli* O157:H7. While in a study conducted by Hancock, Besser (8) the results showed that *E. coli* O157:H7 was also isolated from different livestock. The utilization of unpasteurized milk and undercooked contaminated meat in human nutrition has been known as an important transmission route in humans. Conventional culture, serological and molecular assays are the most important techniques have been introduced for the isolation and characterization of *E. coli* O157:H7 in samples obtained from clinical, food, and environmental specimens.

Since the traditional analysis for identification of *E. coli* O157:H7 isolate take more time compared with molecular assays, several studies have been designed based on polymerase chain reaction (PCR) for fast identification of *E. coli* O157:H7 (9). The existance of *E. coli* O157:H7 in cattle's milk has been investigated in several regions worldwide (10), but to the current knowledge, there have not been any published studies in Iraq on the identification of *E. coli* O157:H7 in meat and dairy products.

Therefore, the current investigation was prepared to evaluate the prevalence of E. coli O157:H7 in dairy cattle's milk using a rapid method, in Iraq.

## 2. Materials and Methods

### 2.1. Sample Collection

In a period of 6 months from May to November 2021, 150 (fresh milk) samples were collected from 10 dairy farms in different regions of Najaf, Baghdad, Kirkuk, and Erbil, Iraq. All the dairy cattle used for sample collection were clinically healthy Holstein breed and the milk samples had a normal physical appearance. Mastitic samples were characterized by California Mastitis Test (CMT). A total volume of 30-40 mL milk samples were collected in sterile Corning™ Falcon 50 mL Conical centrifuge tubes. All the samples within a period of 6 h after milking were transported to the laboratory at 4ºC.

### 2.2. Culture Conditions

An isolation method based on the selective enrichment of the bacteria aggregated at the bottom of the conical centrifuge tubes containing milk samples after centrifugation of the milk was applied for the isolation of *E. coli* O157:H7, followed by the milk pellet enrichment-direct plating method (MPE-DP). Immediately after arrival at the laboratory, the conical centrifuge tubes containing milk samples were centrifuged at 3000 rpm for 20 min at 4℃. After centrifugation, the supernatant was discarded and the remaining pellet dissolved in 1 ml of the milk sample which was previously aspirated before performing centrifugation. Then this sample was added to centrifuge tubes containing 14 mL of modified Tryptone Soya Broth supplemented with 20 mg/L novobiocin (mTSB-n).

### 2.3. Multiplex-PCR Assay

PCR was applied to identify the presence of *stx1*, *stx2*, *O157* and *H7* genes in the isolated *E. coli*. The positive and negative controls were used in this study as *Escherichia coli* O157:H7 (ATCC- 43894) and sterile distilled water, respectively. The amplification conditions and reagents used in this part of this study were based on those described by Desmarchelier, Bilge (11). The primer sequences and expected size of the PCR products are tabulated in table 1.

**Table 1.** Primers sequence and the PCR product size

| Target gene | Primer sequence | Size (bp) |
|---|---|---|
| *rfb* O157 | F: 5′- CGG ACA TCC ATG TGA TAT GG -3′<br>R: 5′- TTG CCT ATG TAC AGC TAA TCC -3′ | 259 |
| *flic* H7 | F: 5′- GCG CTG TCG AGT TCT ATC GAG-3′<br>R: 5′- CAA CGG TGA CTT TAT CGC CAT TCC-3′ | 625 |
| *stx1* | F: 5′- ACA CTG GAT GAT CTC AGT GG-3′<br>R: 5′- CTG AAT CCC CCT CCA TTA TG-3′ | 614 |
| *stx2* | F: 5′- CCA TGA CAA CGG ACA GCA GTT-3′<br>R: 5′- CCT GTC AAC TGA GCA CTT TG-3′ | 779 |

The thermocycler (Eppendorf, Germany) program condition was as follows: initial incubation was performed at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 60 sec, the annealing procedure was performed at 52°C for 30 sec and the following elongation was done at 72°C for 60 sec. The final extention of product was done at 72°C for 10 min.

Agarose gel electrophoresis in TAE (tris-acetate-EDTA) buffer was used for analyzing the PCR products. Then the safe-staining was used for visualizing and documentation, the bands visualized under the UV-transilluminator. The ladder in the current assay was 100 bp DNA.

### 2.4. Direct Multiplex-PCR on Milk Samples

The enriched milk samples in N-TSB were used for direct PCR. For performing direct PCR 1 mL of enriched milk sample in N-TSB was thawed and centrifuged at 12000 rpm for 5 min. Then the sediment palate was carefully removed and dissolved in 1 mL TE buffer and gradually diluted to the 1:20 ratio in TE buffer. Then the presumptive bacterial suspension was used for DNA extraction as mentioned before. The PCR condition was similar to those mentioned above.

### 2.5. Statistical Analysis

For analyzing data the GENMOD procedure from SAS (version 27) was used. $P \leq 0.001$ was considered significantly different.

## 3. Results

From 150 milk samples, 14 non-sorbitol fermenting (NSF) colonies were isolated. However, using the biochemical assays and following enrichment and selective culture, only 2 isolates were identified as *E. Coli*. The recorded data of the molecular assays (m-PCR) using *rfb* O157 and *flic* H7 primers confirmed the presence of the serotypes other than *E. coli* O157:H7. After the second round of m-PCR with *stx1* and *stx2* genes primers, the results revealed that none of the *stx1*, *stx2* genes was presented in the milk samples.

The results of the direct PCR on the enriched samples (n=150), in 45 samples different expression genes were recorded. The recorded data in the current research revealed that out of the 150 raw milk samples 31.3% were *O157* gene positive, while 92% of the isolates were not positive for the *H7*. The results showed that only 10% of the samples were positive for *O157:H7*. The results revealed that the highest spread of *E. coli* O157 was recorded in the samples obtained from Najaf and Baghdad. It is interesting that in the Erbil and Kirkuk the spread of the *E. coli* O157:H7 isolates were not detectable the results showed that there were no significant differences in the case of *E. coli* O157 contamination between Najaf and Baghdad city. The recorded data showed that the percentages of *stx2* producing bacteria were higher than that of producing *stx1* (Figure 1).

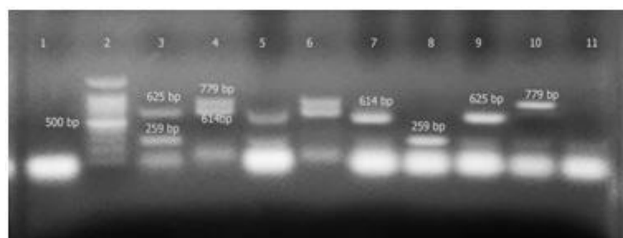


**Figure 1.** line1: negative control, line2: ladder100 bp, line3: positive control for rfbO157 (259bp) and flicH7 (625bp), line4: positive control for stx1 (614bp) and stx2 (779bp), lines 5 and 6: positive samples for all genes studied, lines 7, 8, 9, 10: positive samples for every one of the genes studied, line 11: negative sample.

## 4. Discussion

Because of the high tendency for consumption of dairy products among populations in Iraq, the current research was designed to identify the existance of *E. coli* O157: H7 in the milk samples obtained from dairy farms located in Najaf, Baghdad, Kirkuk, and Erbil province, Iraq.

The results of the current study did not differ significantly from the results of researches previously performed in different countries from raw cow's milk (12, 13). In a study previously conducted by Meshref (14) the results revealed that 6% of raw cow's milk samples evaluated in Egypt and 3% in Austria were infected with *E. coli* O157:H7. On the other hand the results of a study in the European Union revealed that of *E. coli* O157:H7 circulation vary from 1 to 13% (15). Similarities and differences in case of climate and hygiene in different regions of the globe lead to the various prevalence of *E. coli* in different countries. Although in accordance with the recorded data of the current research the presence of *E. coli* O157:H7 in milk is low, by the way, the presence of this pathogen in the milk samples has of great importance in Iraq. Similar to the results of the current study, the results of a study conducted by Rahimi, Kazemeini (16) in Iran revealed that 7% of samples were *E. coli* non-O157, 1.5% were *E. coli* O157:NM, and 0.5% samples were *E. coli* O157:H7 isolate. All the *E. coli* O157:H7/NM isolates in the study conducted by Rahimi, Kazemeini (16) were positive for *eaeA* and *stx1*, and *stx2*. While one *E. coli* O157:H7 isolate was positive for *EhlyA*. Out of the 3 *stx* positive isolates, one isolate had *stx1* and two isolates had express *stx2*. The comparison of the results obtained from different studies seems difficult due to the different methodologies, different sample sizes, and different sample types used for analyzing the samples.

The current study was performed during the hot season, which is in accordance with the findings from previous studies that reported that the peak prevalence of E. coli O157:H7 occurs in summer and early fall (17-19).

In conclusion, the current investigation was the first report on the prevalence of *E. coli* O157:H7 in the raw milk samples in Iraq. The proportion of *E. coli* O157:H7 contaminated milk identified in this research was similar to that reported previously from different dairy products across different countries in the Middle East region.

### Authors' Contribution

Study concept and design: F. N. O.
Acquisition of data: E. R. A. and K. A. A.
Analysis and interpretation of data: N. H. A. and Y. S. K.
Drafting of the manuscript: O. A. M., A. M. R. and O. K. A. A.
Critical revision of the manuscript for important intellectual content: E. R. A., F. N. O. and D. A. H.
Statistical analysis: E. M. S.
Administrative, technical, and material support: E. R. A., F. N. O. and D. A. H.

### Ethics

The study was approved by the ethics committees at Al-Ayen University, Thi-Qar, Iraq.

### Conflict of Interest

The authors declare that they have no conflict of interest.

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detection of Campylobacter jejuni, Salmonella spp., and Escherichia coli O157: H7. Mol Cell Probes. 2003;17(4):135-8.
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