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# Molecular characterization of Eae and $Stx_s$ genes for *E. coli* O157:H7 isolates from Calves

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## Abstract

This study aimed to detect eae and Stx genes of thirty two *E. coli* O157:H7 isolates recovered from calves. These isolates were isolated and identify by traditional methods of culturing and latex agglutination test from fecal swabs of four diarrheic calves and twenty eight apparently healthy calves. The eae and Stx genes were detected by a realtime PCR applied on the extracted bacterial DNA. The results showed that 19 isolates had stx1 gene, 10 isolates had stx2 gene and 17 isolates had eae gene. It is concluded that molecular characterization by using real time PCR is a good test for confirming infection with *E. coli* O157:H7 and detection of their eae and Stx genes .

***Keywords:*** *Real time, PCR, stx, eae, Calves, E. coli O157:H7.*

## Introduction

*Escherichia coli* regarded as one of the main cause of calf diarrhea in newborn livestock, by causing high economic losses, the prevalence of enterohemorrhagic *E.coli* (ETEC) in calves with diarrhea ranged between 70 – 75.6 % [(1)]. It has been stated that cattle may act as reservoir for *EHEC* [(2)]. Also, Osman *et al*.[(3)] reported a prevalence of O157 serotype in 63.6% of their studied calves.

*Escherichia coli* strain O157:H7 is one of the most significant bacteria that convey by the food, it mostly cause a series of clinical signs , such as diarrhea, haemorrhagic colitis and hazardous problematics in other body organs or systems. It have many virulence factors, most significant one was *Stx*s [(4)], therefore, the strain nomenclatured as shigatoxic *E.coli* (STEC) [(5)].

It infects adult cattle even the calves [(6)]; human being [(7)]; ovine [(8)]; canine [(9- 11)] and other animals , and may cause fatal disease in human being and other animals,

The most significant confirmatory tests used for detection of ETEC and STEC are PCR [(12)] or realtime PCR (RT- PCR) [(13)] which is considered as an appropriate, dependable way for confirmatory diagnosis of *E. coli* O157:H7 [(9)].

The aim of the presented study was to corroborate the *eae* and *stx* genes of virulent *E. coli* O157:H7 isolated from calves by using realtime PCR.

## Material and Method

**Source of bacteria :** A number of 32 isolates of virulent *E. coli* O157:H7 were used in this study and obtained from college of veterinary medicine / University of Fallujah and were confirmed previously by [(3)].

**Bacteriological Method:** All 32 isolates were identified as shown in a previous study [(14)] prior to the PCR test to confirm the presence of Eae, Stx1 and Stx2 genes.

Serotyping was made as mentioned in [(14)] and [(15)].

PCR assay:

This test was performed on the thirty two virulent *E. coli* O157:H7 strains, a bacterial DNA extraction was done by using Geneaid kit, USA. The assay of RT- PCR was done via using Applied Biosystems™ RapidFinder™ STEC Screening Assay in Sacace Real-Time PCR System. A twenty eight micron of water free nuclease was added to two micron of DNA. The conditions of RT- PCR were adjusted as follows: hold at

95 °C for 2 min., then denature at 95°C for 1 second and lastly, anneal/ extend at 60 °C for 20 seconds, for 40 cycles.

## Results and Discussion

The RT- PCR assay of the examined virulent isolates exposed that nineteen isolates had stx1 gene and 10 isolates had stx2 gene , while 17 isolates had eae gene and this is compatible with previous results of Yousif and Hussein, 2015 who found the same results by using conventional PCR on the same isolates (Fig. 1).

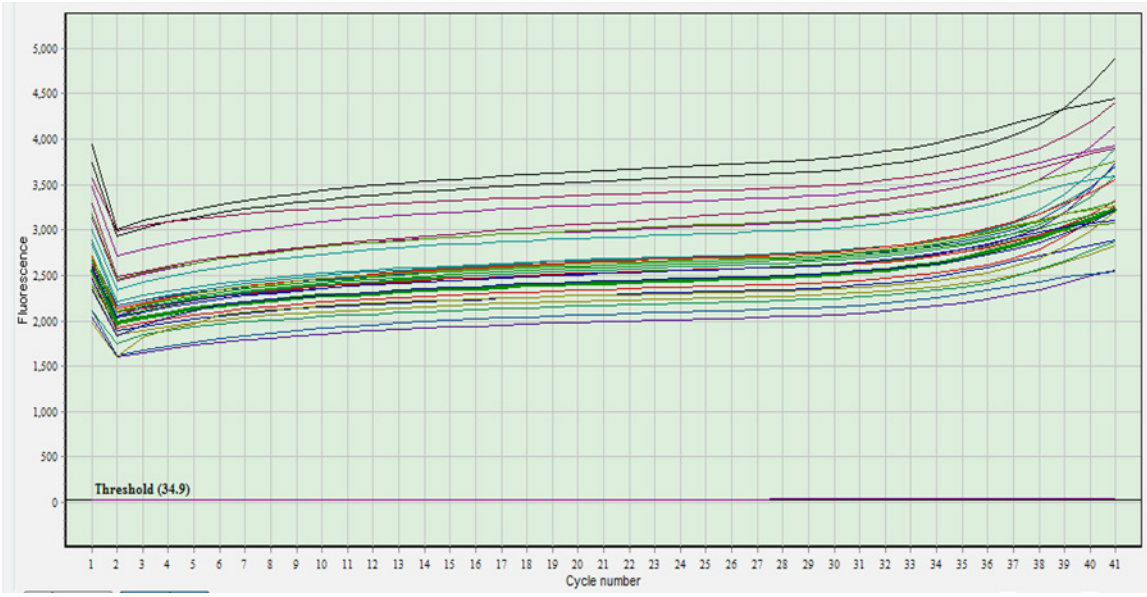


**Figure (1) Results of RT-PCR, positive samples were Cycle Threshold (CT) less than 35**

It has been reported that RT-PCR is a screening and effective method for detecting shigatoxigenic *E. coli* from fecal samples [16].

The current results were in compatible with results of Rebekka *et al*. [17] who reported that real-time PCR has a high sensitivity and accuracy for recognition of EHEC and they mentioned that RT- PCR is a quick method for detection presence or absence of EHEC.

RT- PCR method described as a "golden test" for identification of EHEC [18]. Other studies reported similar results, where Abbasi et al. [19] reported that the RT- PCR conventional form is the quicker and more accurate assay and it doesn't need any other ways for confirming the diagnosis when it used to detect the incidence of virulence factors of E. coli isolated from diarrheic children, as well as , Mondani *et al*. [20] used RT-PCR for detect STEC, they found that this test have many advantages from these: reduction the time and reducing the limitations which found in alternative traditional tests.

Also , a study done to estimate the efficacy of RT-PCR test in testing the presence or absence the virulence genes rfbO157*, Stx1*,*Stx2* from pediatric stool samples, out of fifty nine samples that positive for PCR, a twenty nine samples were +ve for *Stx* and they concluded that this test is quick way for diagnosis STEC in pediatric [21].

## Conclusions

The Real time PCR test is an exact method for specific recognition of EHEC O157:H7 and their eae and Stxs genes.

**Ethical Clearances:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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