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Occurrences of *E. coli* in dromedary camels in some provinces in Iraq with a molecular study of *E. coli* O157:H7

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Abstract

This study was designed to investigate Escherichia coli's occurrence in camels, especially *E. coli* O157:H7 serotype, by bacteriological and molecular assay with sequencing. Two hundred fifty fecal samples from camels were taken at the field located in Iraq in 3 provinces (Al Najaf, Karbala, and Al-Mothana). Fecal samples were initially streaked on MacConkey and EMB agar, and then bacteriological confirmation was done by using Gram stain and different biochemical tests to isolate *E. coli.* The detection of *E. coli* O157 by culturing on chrome selective media and using Polymerase Chain Reaction (PCR) on the *E. coli* isolates to determine four virulent genes associated with *E. coli* O157:H7. The results revealed that affected camels showed diarrhea and watery feces with mucous or blood. Bacteriological results showed 117 *E. coli* isolates and only four isolates diagnosed as *E. coli* O157:H7 on Chromogar™ *E. coli* O157. The four isolates possessed four virulence genes: flagellin gene (FliC), somatic gene (RfbE), Shiga toxin 1(Stx1), and Shiga toxin2 (Stx2) genes detected by PCR assay. Targeting genes RfbE from four samples were recorded in the NCBI Genbank accession numbers (LC74356, LC743562, LC743563, and LC743564). Our results based on the phylogenetic tree showed that our sequences *were* near the sequence with accession numbers (CP062774.1 and LC739728.1). Our conclusion revealed the identity of *E. coli* O157:H7 in camels, and these strains of bacteria possess four virulence genes. This indicates the importance of camels as a source of transmission of these bacteria to humans and all animals.

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Introduction

Camels are characterized by their high resistance to many pathogenic and zoonotic microorganisms and ability to adapt to extreme desert ecosystems compared to other animals in the same area (1). Bessalah (2) pointed to camel diarrhea as the main cause of economic loss associated with poor growth, medication costs, and animal death. In the Camelidae members, many types of *Escherichia coli (E. coli)* have been detected in fecal samples from diarrheic and non-diarrheic camels only, then *E. coli O157:H7* strains were isolated from sick camels were recorded; these results can provide evidence on the role of infected camels by Shiga toxin-producing *E. coli* in the human outbreaks (3). *E. coli* O157:H7 is a foodborne bacterium that can be isolated from the feces of animals and meat and meat products, dairy products, vegetables, and contaminated water. WHO recommended that developing countries be attended to detect this pathogen as a research priority (4-8). The major problem in camels was the high mortality rate in camel calves aged 1-3 months, and the causes are mainly infectious diseases and poor management practices (9). Like all other animals, Camels suffer from various signs, including diarrhea caused by different bacterial antigens, such as the *E. coli* pathogen detected in neonatal calve camels with diarrhea (10). Enterohemorrhagic Escherichia coli (EHEC) causes hemolytic uremic syndrome and hemorrhagic colitis in humans. *E. coli* O157:H7 This pathogen was first recognized

in 1982 (11), As this is related to hamburgers (undercooked) consumption. It has been found that the cattle were harboring *E. coli* O157:H7, so ruminants were now recorded as a main reservoir, Shiga toxigenic *Escherichia coli* (STEC) O157:H7, was detected in other animal species (12). In addition, *E. coli* O157:H7 has been detected in fecal samples of apparently healthy cattle with diarrhea (13), camels (14), dogs (15), and zoo animals (16). Isolation of these pathogens using bacteriological methods is a big challenge, and the PCR analysis for identifying this bacterium is essential in all animals (13-17). Molecular assays are precise and rapid for confirmation as compared with cultural methods. Moreover, sequence analysis is suitable for delineating species and tracking epidemiology (18). The protein encoded by the gene RfbE on *E. coli* O157 was perosamine synthetase with monosaccharide component synthetase associated with the biosynthesis of O Ag in the bacterial LPS (19). As the bacteria bind the intestine's mucosa and grow, they secrete Shiga toxin (Stx) toxins. This toxin was a substantial virulence factor in *E. coli*. The toxin Stx2 is more toxic than Stx1 for cells of the endothelial renal artery (20). Shiga toxin-producing *E. coli* was responsible for causing diarrhea. Adamu and Collages (21) recorded that the *E. coli* STEC isolated from camel fecal samples possessed virulence genes (Stx1, Stx2, and Stx1 with Stx2). Salehi (3) isolated 2 *E. coli* O157:H7 strains from diseased camels using *E. coli* DNA microarray. The two isolates displayed positive hybridization signals for Locus of enterocyte effacement (LEE) gene probes for stx1 and stx2. Hussein and Yousif (13) detected *stx₂* and *stx₁ from E. coli* O157:H7 isolated from calves in Baghdad by conventional PCR.

This study aimed to determine the occurrence of *E. coli* isolates from diarrheic and apparent healthy camels with the bacterial and molecular assay of *E. coli* O157:H7 that separated camels in some Iraqi provinces, and study the genetic diversity and sequencing of this microorganism,

Materials and methods

Ethical approved

The research and ethical committee of Vet approves the study. Medicine College (Book No. 39 / D A on 7/12/ 2021). University of Baghdad, Iraq.

Animals

Two hundred and fifty camels aged one day to 7 years from both sexes were conducted for this study, which extended from January 2021 to December 2021. Although located in three provinces, Karbala, Najaf, and Al-Muthana, some camels suffer from diarrhea, and others without diarrhea. All other clinical signs were recorded.

Sample collection and bacteriological examination

Two hundred and fifty Fecal samples were taken from camels. The samples to be analyzed for bacteria were tagged and then placed in their sterile test tubes, which were then stored in ice boxes. They were brought to the Veterinary Medicine Laboratory. Culturing of these samples on MacConky and EMB /incubated aerobically for 24 to 48 hrs at 37°C. Gram stain and biochemical tests were done on the suspected *E coli* isolates identified by Catalase, Oxidase, Indole, MR- VP test, Simmon's Citrate, Motility, and TSI. (22). Then sub-cultured on selective Hichrome O157agar.

Molecular study

DNA of the four *E. coli* O157:H7 isolates were extracted by (Addprep bacterial genomic DNA extraction Kit) according to the manufacturer company (Addbio (Korea). Nanodrop spectrophotometers were used to estimate the purity and concentration of extracted DNA. Four primers were used (Table 1). For detecting the virulence genes, the PCR amplification mixture includes the master mix (12.5µl), two µl of DNA (template), 2µl of each F and R primers, and nuclease-free water (6.5µl) to complete the mixture to volume 25µl. The PCR reaction was done of the Thermal-Cycler utilizing (Biobase(china), the conditions optimized for the four primers: 1 cycle (94ºC/3 minutes.) for the initial denaturation, 35 cycles at (94ºC/60 sec.) for denaturation. The Annealing temp for *Stx1 and Stx2* genes were (61.5ºC/30 sec.) and for *RfbE*. *FLIC* genes were (60ºC/30 sec.) and (72ºC/60 Sec.) for an extension for each gene, then followed by one cycle [72ºC/ seven minutes] for the final extension. The PCR products were examined in electrophoresis in 2% agarose gel using a 100 DNA ladder (Qiagen, Germany). Then, visualization of DNA bands by a UV transilluminator (Clinx Science/ China).

Table 1: Primers used in current study

Primers		Sequence (5^- to 3^-)	Product size (bp)	Reference
RfbE	F	AAG ATT GCG CTG AAG CCT TTG	497	14
	R	CATTGGCATCGTGTGGACAG		
Stx1	F	ATAAATCGCCATTCGTTGACTAC	180	23
	R	AGAACGCCCACTGAGATCATC		
Stx2	F	GGCACTGTCTGAAACTGCTCC	255	23
	R	TCGCCAGTTATCTGACATTCTG		
FliC	F	GCGCTGTCGAGTTCTATCGAGC	625	24
	R	CAACGGTGACTTTATCGCCATTCC		

DNA Sequence

All PCR-positive samples for the amplified *RfbE* were sent to South Korea for sequence analysis, and the data obtained were analyzed using Bio Edit software (version 7.5.0.3) for multiple sequence alignment through homology with global sequences in different countries. MEGA 11.0 was used to construct the pf phylogenetic tree, and the homology sequence was identified in the NCBI -GenBank-NCBI.

Results

Identification of *E. coli* O157:H7

Culturing 250 fecal samples revealed the isolation of 117 *E. coli* (46.8%) on MacConky (pink colonies), and EMB showed a green metallic sheen. Four isolates only showed a purple color on selective CHROM agar™ for *E. coli* O157 (Figure 1). Gram stain revealed Gram-negative bacterium, and confirmation by different biochemical tests given a negative for VP, Simmon's citrate, and positive for indole and motility tests, on TSI revealed yellow with/without gas production.

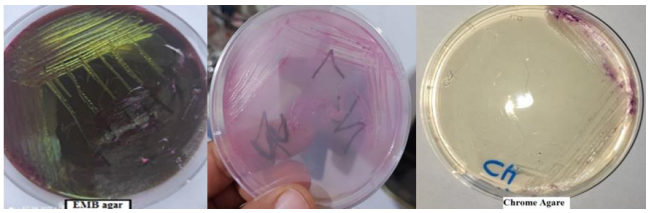

Figure 1: On the right, *E. coli* on EMB and MacConkey agar and *E. coli O157 H7 on Chrome agar*.

Percentage of infection

From 250 fecal samples of camels, 117 (46.8) samples were positive for *E. coli* bacteria, and only four isolates were diagnosed as *E. coli* O157:H7 at a percentage of 1.6% (4/250). Moreover, according to E. coli, the rate *w*as 4/117(3.41%).

Clinical study

On clinical examination, 113 of 117 camels affected with *E. coli* showed clinical signs of illness, including diarrhea (semi-solid to watery feces with yellow color), pale mucous membranes, fever, and depression. The four camels affected with *E. coli* O157: H7 showed the same signs, and only one camel had bloody diarrhea (Figure 2).

Molecular study

Out of 117 fecal samples of *E. coli*, only four isolates possessed the four genes used in this study and showed the amplified DNA of all four isolates had 497 bp for the RfbE gene (Figure 3), 625 bp for the FliC gene (Figure 4), 180 bp for Stx1(Figure 5), 255 bp for Stx2 (Figure 6).

Figure 2: Camel affected with Bloody diarrhea.

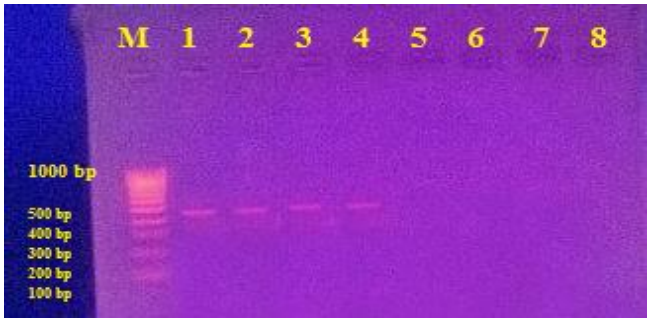

Figure 3: PCR amplicons RfbE gene with product size 497 bp.

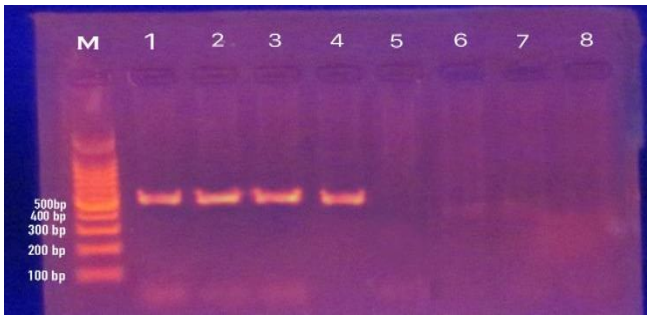

Figure 4: PCR amplicons after amplification of *FLiC* gene of product size 625pb.

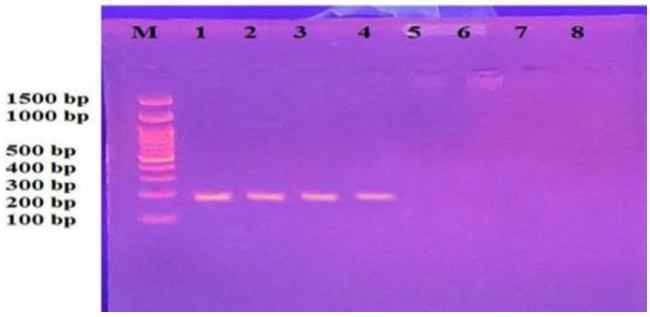

Figure 5: PCR amplicon after amplification of *Stx1 gene* with product size 180pb.

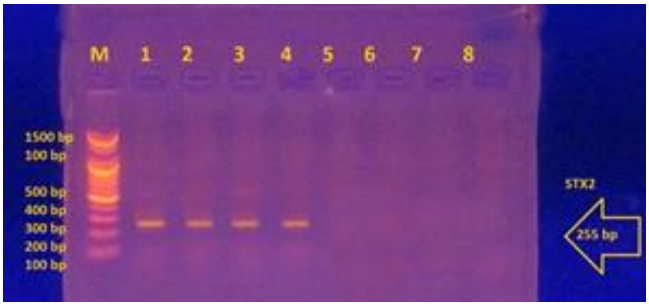

Figure 6: PCR amplicon after amplification of *Stx2gene* with product size 225pb.

Sequencing

The four samples positive to RfbE by PCR were taken for gene analysis, and the sequences of this gene were made available at NCBI-GenBank under the accession numbers: *E. coli* O157H7; LC74356.1, LC743562.1, LC743563.1 and LC743564.1, variation in taxonomic detection depended in query coverage, A phylogenetic tree (Figure 7) of *E. coli* O157H7 revealed that the four bacterium isolates investigate in the current study clustered in one clade: Clade 1 (CP103864.1 and CP038496.1) with 79% similarity between isolates, 81% similarity with anther clade two which include all our sequence isolates (LC74356.1, LC743562.1, LC743563.1 and LC743564.1) with 100% similarity.

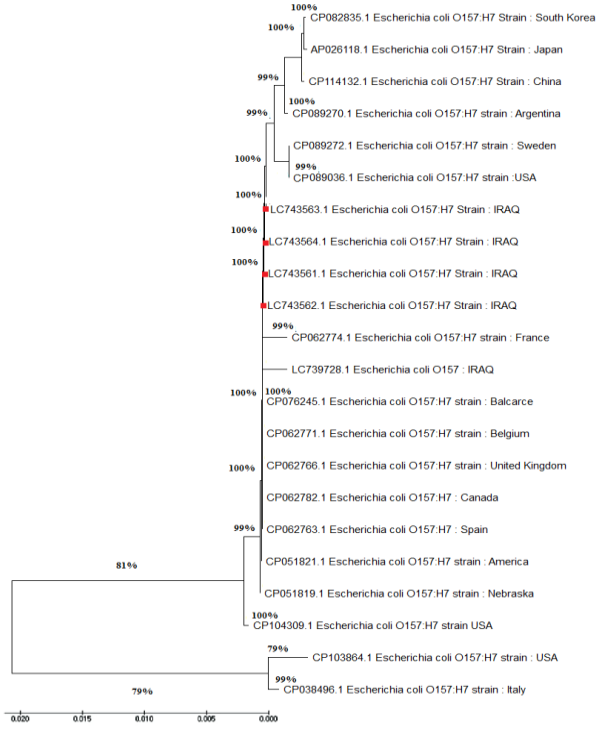

Figure 7: Phylogenetic tree of RfbE gene of *E. coli* O157H7 with different global isolates.

Discussion

Even though colibacillosis leads to severe clinical signs in the camel, including economic losses for farmers, there is minimal information about the virulence factors expressed by pathogenic *E. coli*. For example, there is a poor understanding of the etiology of colibacillosis in camel herds. Several studies explained the isolation of *E. coli* from camels. Therefore, this study was conducted to illuminate the virulence genes of *E. coli* O157:H7 strains isolated from the feces of diarrheic and healthy camels. This study showed the isolation of 117 isolates of *E. coli* from 250 fecal samples from camels 46.8%. These results were incompatible with a survey by Al-Ruwaili (25), who isolated *E. coli* in a high percentage of 58.2% in diarrheic camels. Besalah (2) observed 53 isolates 65.4% of *E. coli* from camels in herds with diarrhea. This study showed the isolation of 4 (1.6%) *E. coli* O157:H7 isolates from total 250 fecal samples with confirmation of 4 genes (RfbE, O157, Stx1, Stx2, and FliC) in these isolates. This Percentage of 1.6% was lower than the study by Admau (21), who found the percentage of STEC isolated from camels was 3.8% Maiduguri and Bosilevac (2) recorded a prevalence of 10.7% in Riyadh, Saudi Arabia. In contrast, El-Sayed (26) didn't detect *E. coli* O157 among four hundred fecal samples from camels collected from Somalia, Egypt, Djibouti, Sudan, and Kenya; they explained that this occurred due to the life pattern of camels in the desert that minimizes contact of animals with camels and therefore minimize the transmission of this bacteria.

Also, Moore *(*27) in the United Arab Emirates failed to diagnose *E. coli* in the feces of camel calves. Najim (28) studied the survival of *E. coli* O157 in produced cheese locally in Baghdad province and demonstrated a high percentage of infection, 45.84%. This difference in the infection rate is due to differences in management and living conditions. Also, an increased number of infections are in dairy products or slaughterhouses due to direct transmission between carcass or from workers, as well as rapid transmission in food products such as meat and dairy products, transmission in food products such as meat and dairy, so this required searching for new and better methods for accurate diagnosis of this essential bacteria.

The essential clinical sign that appeared on camels was diarrhea, which occurred due to the Shiga toxin produced by *E. coli* O157:H7 in the intestine and acts on sensitive cells of organs. STEC colonizes the epithelium of the intestine, particularly in the mucosa adjacent to the anorectal junction (29). In addition, a study by Al-Taii (30) concluded that *E. coli* O157:H7 caused changes in kidney and liver function in an experimental study on rabbits more severe at fifteen- and thirty-days post-infection.

Our study revealed that the four isolates of *E. coli* O157:H7 possessed four genes, fliC, Rfb, stx1, and stx2, by PCR assay. This is compatible with many studies on these bacteria; Yousif (31) diagnosed *E. coli* O157:H7 using RT

PCR, detected flic and Rfb genes and concluded the importance of these genes on the pathogenesis of this bacteria in dogs. Another Hassan (30) study detected rfb and Flic genes in *E. coli* O157:H7 isolates from diarrheic calves. Also, Sallam (6) isolated from the meat of camels 24 isolates as *E. coli* O157:H7 by Multiplex PCR and revealed the existence of four genes. Also, these results were similar to other studies, Adamu (21) recorded (stx1, stx2, and mixed) in isolates from camel fecal Salehi (3) isolated two *E. coli* O157:H7 strains possess stx1 and stx2. Hussein and Yousif (13) also detected stx_2 and stx_1 in *E. coli* O157:H7 isolates from calves in Baghdad. On the other hand, stx_2 was shown to be the most frequently identified in human isolates with a percentage 45.5% (21). The Diagnostic methods using molecular assay are more accurate and faster than the traditional bacterial culturing methods for diagnosing different bacteria (18).

All sequence isolates were found in one node and branch length with no distance scale (100% identity); among other global sequence isolates, our results were found near sequences with accession numbers (CP062774.1 and LC739728.1) from France and Iraq, respectively. It was different from other sequences isolated in different countries.

Conclusion

the study showed that occurrences of *E. coli* O157:H7 strains in camels acted as a source of infection that transmitted infection to humans. Furthermore, *E. coli* O157:H7 was detected in fecal collected from camels. PCR assay revealed possession of this bacteria's four virulence genes and the presence of 4 combinations of virulence factors that increase isolates' pathogenicity. Therefore, isolating this micro-organism in Iraq will be essential for researchers to investigate future prevalence studies.

Acknowledgments

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Conflict of interest

There is no conflict of interest, and the authors declare

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حدوث الإيشريكيا القولونية في الإبل العربية في بعض محافظات العراق مع دراسة الجزيئية للإيشريكيا القولونية

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الخلاصة

صممت هذه الدراسة لمعرفة حدوث الايشيريكيا القولونية مع دراسة بكتيرية وجزيئية للنمط المصلي الايشيريكيا ومعرفه التسلسل الجيني التي تم عزلها من الإبل تم أخذ ٢٥٠ عينة براز من الإبل في الحقول الواقعة في ٣ محافظات (النجف وكربلاء والمثنى) في العراق. تم زرع عينات البراز في البداية على أكار المكونكي ثم التأكيد البكتريولوجي باستخدام صبغة كرام وثم الزرع على الأوساط الانتقائية مثل وسط الأيوسين والمثلين الأزرق وثم استخدام الاختبارات كيميائية حيوية مختلفة لعزل الإيشريكيا القولونية. ثم تم الكشف عن بكتريا الايشيريكيا القولونية الممرضة عن طريق الزرع على وسط انتقائي للكروم واستخدام تفاعل البلمرة المتسلسل على جميع العزلات لتحديد ٤ جينات ضراوة مرتبطة بالاشريكيا القولونية الممرضة .أظهرت النتائج أن بعض الإبل المصابة ظهرت عليها علامات الإسهال والبراز المائي مع المخاط أو رقائق الدم. كما وأظهرت النتائج عزل ١١٧ عزلة من الايشيريكيا القولونية وتم تشخيص ٤ عزلات فقط على أنها الايشيريكيا القولونية الممرضة على أجار الكروم. كانت عزلات الايشيريكيا القولونية الأربعة تمتلك جينات *RfbE* و*FliC* و *Stx1* و *Stx2* بواسطة اختبار تفاعل السلسلة المتبلمرة وسجل جين RfbE للعزلات الأربعة في المركز الوطني لمعلومات التقانة الحيوية (بنك الجينات) وأعطيت الأرقام التعريفية LC743561 و LC743562 و LC743563 و LC743564. وجدت نتائجنا المستندة إلى شجرة النشوء والتطور أن تسلسلات الإيشريكيا القولونية كانت قريبة من التسلسل مع رقم المدخل CP062774.1 و LC739728.1 . استنتجنا من هذه الدراسة وجود الإيشريكيا القولونية في الإبل وتمتلك هذه السلالات ٤ جينات ضراوة، وهذا يشير إلى أهمية الإبل كمصدر لانتقال هذا العامل الممرض إلى الإنسان والحيوانات الأخرى.

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