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Research article

Isolation and identification of *Escherichia coli* O157:H7 from houseflies (*Musca domestica L*) at cattle barns in Al-Qadisiyah Province, Iraq

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Abstract

Recent studies identified that *Escherichia coli* O157:H7 (ECO157H7) causes hemorrhagic colitis in humans, a rare spectrum of diarrheal illness. According to the findings of epidemiological investigations, cattle serve as the principal reservoirs for this bacterium. This study was conducted to understand the incidence of ECO157H7 in houseflies (HFs), a key disease vector. HFs (n = 40) were collected from cattle barns. The HFs were cultured on MacConkey (MC) agars, and any suspected colonies were grown on Eosin Methylene Blue (EMB) agar. EMB-based metallic sheen colonies were grown on specific media, such as sorbitol Chromagar (SCA) and cefixime tellurite sorbitol-MacConkey (CT-SMAC) agar. Sorbitol non-fermenting (SNF) isolates were subjected to a commercial latex agglutination kit to identify the existence of O157 group. The results recorded 31/40 (77.5%) *E. coli* isolates identified from HF samples. The findings revealed that only 18/31 (58%) were from the SNF isolates. The identities of the isolates were further examined by targeting the *rfbO157* and *fliCH7* genes, as genetic markers in a PCR method. The PCR results reported 10/18 (55.5%) and 12/18 (66.6%) isolates that carried the *rfbO157* and *fliCH7* genes, respectively. Our research revealed that HFs represent a harbor for the pathogenic *E. coli*. The ecology and way this bacterium spreads among animals and across the environment may depend heavily on the presence of ECO157H7 in agricultural environments.

Keywords: *Escherichia coli* O157:H7, *fliCH7* gene, House fly, *rfbO157* gene

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INTRODUCTION

One of the most prevalent and important families of insects is the housefly (HF), which acts as a vector for some of the deadliest diseases that impact both humans and animals (Ali Alsudani and Lateef Al-Awsi, 2022). Animal dung, human waste, dirty animal bedding, and trash decaying in environments are rich in nutrients that enhance the growth of diverse and active microbial populations (Graczyk et al., 2001; Moon et al., 2001).

Numerous enteric bacterial microorganisms, including *Klebsiella spp., Staphylococcus aureus* (Nwankwo et al., 2019), *Vibrio cholera*, *Shigella* spp., *Campylobacter*, and ECO157H7 have been demonstrated to be mechanically spread by insects (Hald et al., 2007). Flies spread microorganisms by regurgitating plant matter and by polluting their body surfaces (feet, wings, and bodies). ECO157H7 can be transmitted between animals and environment (Moon, 2002).

The fact that *E. coli* is crucial to comprehend the bacteria because ECO157H7 might live in reservoirs, such as in cattle with no apparent disease. However, animals can frequently release ECO157H7 to environment via feces. Ruminants have extremely high ECO157H7 shedding, which suggests that they might serve as a habitat for this bacterium (Kolodziejek et al., 2022). It is important to mention that most of human *E. coli-based* infections are caused by Shiga toxin-producing *E. coli*, which were suggested to be originated from cattle (Oporto et al., 2019).

Pathogenic bacteria, such as *Salmonella*, *Campylobacter* spp., and ECO157H7 are the third highly prevalent pathogens isolated from human intestine and feces (Allerberger et al., 1996; Adwan et al., 2002). ECO157H7 can cause a fatal kidney infection, particularly in young, old, and immunocompromised patients (Mead and Griffin, 1998).

Shiga-toxin is the combination of virulence factors that are presented with attaching and inducing lesions depending on the eaeA and *hly* genes responsible of the production of adhensin and hemolysins, respectively. These are a few of the virulence factors that make ECO157H7 highly hazardous. Numerous PCR tests have been developed to differentiate between various *E. coli* strains. These assays frequently aim to pinpoint the *rfbO157* and *fliCH7* genes, which code for the O157 somatic and flagella H antigen, respectively (Gannon et al., 1997). The current study was performed to identify the prevalence of ECO157H7 in adult HFs from cattle farms.

MATERIALS AND METHODS

During the period between April to June, 2022, 40 adult HFs were collected from four cattle barns distributed over different rural regions (three barns) and the cattle station (one barn) at the College of Veterinary Medicine, University of Al-Qadisiyah, Al-Qadisiyah Province, Iraq. The HFs were collected with a sterile net, placed in sterile screw tubes. The captured HFs were transported right away to the college microbiology laboratory. The HFs were sacrificed by freezing for 20mins. All obtained samples were individually placed onto nutrition broth, homogenized by vortex, left for 15mins, and then the HFs were removed using sterile forceps (Malik et al., 2007).

The nutrient broth tubes were incubated at 37°C for 24hrs. One pink colony for each sample on MC agar were sub-cultivated on EMB agar. For 24-48hrs, the EMB was incubated aerobically at 37°C to enhance the purity of isolated colonies.

In order to identify bacterial strains, cultivation, Gram staining, and biochemical testing were employed. The isolates were analyzed initially using CT–SMAC and SCA. Suspected SNF isolates were then examined to identify whether the isolates belonged to the O157 serogroup by using Remel™ Wellcolex™ E. coli O157 latex agglutination test (Markey et al., 2014).

DNA extraction

The DNA from the ECO157H7 isolates was extracted using Geneaid's PrestoTM Mini g DNA Bacteria Kit (USA). The *rfbO157* and *fliCH7* genes were determined with the aid of the specialized primers; *rfbO157* (F: CGGACATCCATGTGATATGG and R: TTGCCTATGTACAGCTAATCC) Paton and Paton (1998) and *fliCH7* (F: GCGCTGTCGAGTTCTATCGAGC and R: CAACGGTGACTTTATCGCCATTCC) Gannon et al. (1992) that targeted genetic pieces at 259bp and 625bp, respectively. The primers were purchased from Bioneer Inc. (Korea).

DNA purity and concentration measurements

The purity and concentration of the isolated DNA were determined using a Nanodrop spectrophotometer.

PCR

The GoTaq® Green Master Mix (Promega, USA) was used to prepare the PCR master mix reaction according to the kit accompanied instructions. Based on 25µl total reaction volume, 12.5µl master mix, 4µl of each primer direction, 2.5µl H_2O, and 2µl DNA were included.

The thermocycler conditions were 94˚C for 300s of one cycle initial denaturation, 35 cycles of each of (94˚C for 60s of denaturation, 57˚C for 30s of annealing, and 72˚C for 60s of extension), and 72˚C for 600s of final extension.

RESULTS

Isolation and identification *E coli*

Thirty-one samples out of 40 HFs tested positive for *E coli*, making the prevalence of *E coli* 77.5%. All *E. coli* isolates had a brilliant pink color on MC agar (Figure 1a) and were distinguished by a metallic green sheen on EMB agar (Figure 1b), which was verified by additional biochemical tests as shown in Table 1.

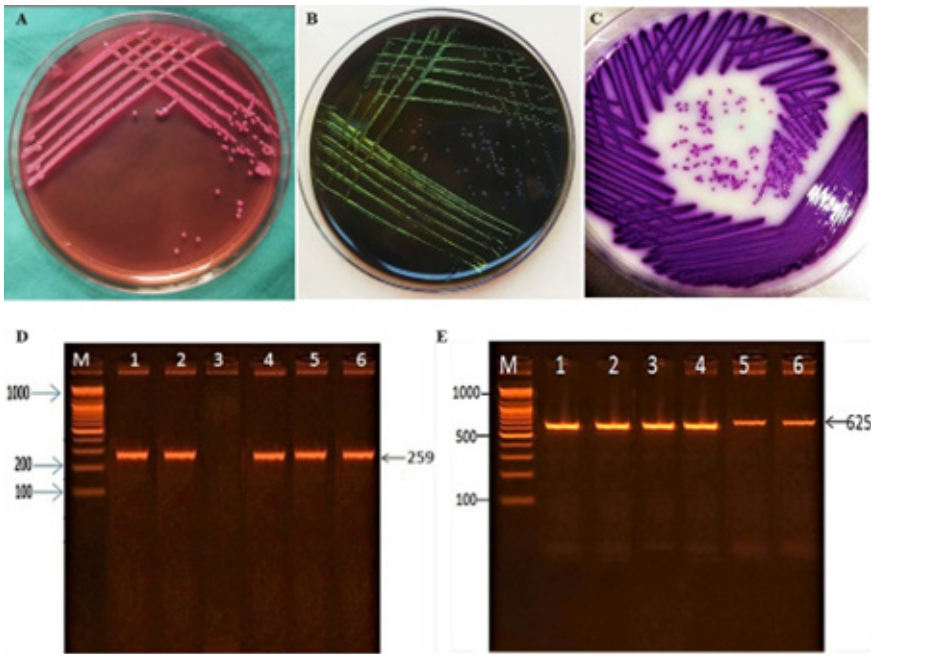


Figure 1 Isolation and identification of *E. coli* O157:H7 from houseflies. A. E. coli colonies on MacConkey agar (bright pink colonies). B. *E. coli* colonies on EMB agar (Metallic Green sheen). C. E. coli O157 colonies on CHROM agar (mauve). D. Gel Electrophoresis analysis of endpoint PCR of rfbO157 gene of *E. coli* O157:H7 at 259bp, M: marker (100-1000bp). Lanes; 1-2,4-5-6: positive samples. E. Gel Electrophoresis analysis of endpoint PCR of fliCH7 gene of E. coli at 625bp, M: marker (100-1000bp). Lanes; 1-2-3-4-5-6: positive samples

Table 1 Results of biochemical tests.

Biochemical test		Results
Indol		+
Methyl red test		+
Voges –Proskauer tests		-
Citrate utilization		-

Most non-O157 strains fermented sorbitol and produced colonies that are pink in color on CT-SMAC agar, as opposed to the colorless colonies of typical ECO157H7. SNF isolates were identified only in 18/31 (58%) isolates.

Culture on Chrome agar

On SCA, the development of mauve colonies is regarded as an indicator for ECO157H7. Only NSF isolates displayed a mauve color (Figure 1c). Both O157:H7 antigens were recognized by the Wellcolex kit. The isolates that responded well to the O157 antigen were overnight subcultured on blood agar to look for H7 antigen. The presence of O antigen was indicated by the red color of agglutination, and the existence of the H antigen was indicated by the blue color of agglutination.

Detection of *rfbO157* gene of *E. coli* O157:H7 by PCR

Our findings showed that 10/18 (55.5%) isolates had the *rfbO157* gene. Utilizing the *rfbO157* primer, the PCR method demonstrated sensitive and specific results (Figure 1d).

Detection of *fliC H7* gene of *E. coli* by PCR

The PCR technique demonstrated sensitive and specific results when utilizing *fliCH7gene* primer. Overall, 12/18 (66.6%) isolates carried the *fliCH7* gene (Figure 1e).

DISCUSSION

This study reported the presence of virulence genes in the ECO157H7 isolated from HFs, which were collected from livestock barns in Al-Qadisiyah Province, Iraq. Numerous studies on ECO157H7 isolated from diarrheic animals and kids were previously conducted. However, nothing is clearly understood regarding the connection between HFs and ECO157H7. On CT–SMAC agar, the traditional culture technique was used in this experiment. 58% of the isolates in fly samples from cattle barns did not ferment sorbitol. This rate was higher than those seen in Japan (Iwasa et al., 1999; Moriya et al., 1999) and Florida (Roxanne et al., 2017).

These findings showed that pathogenic *E. coli* were commonly found in cattle barns. Cattle are temporarily colonized with ECO157H7 before shedding the pathogen (Thuan et al., 2022). The ECO157H7 that infects cattle has unknown origins, and data are limited about its ecology in the environment (Ashenafi et al., 2020). The present study findings further showed that HFs unintentionally come into contact with other organisms and spread infections. The obtained results in the present study also emphasized that HFs contain bacterial microorganisms (Jirapattharasate et al., 2018), which can be disseminated via different methods, such as physical contact. Despite the suggestion that HF is blamed for the spread of the bacterium, limited information was set up to support this suggestion. Sasaki et al. (2000) hypothesized that HF regurgitation and excretion contribute to the spread of ECO157H7 and increase the pathogen transmissibility. Furthermore, Kobayashi et al. (1999) found that ECO157H7 is likely to multiply in the gastrointestinal tract of HF, suggesting that HF may not be merely mechanical carriers of this bacterial pathogen. This hypothesis is highly supported by the large amounts of ECO157H7 bacteria found in our samples. One of the many ways, this bacterium could spread in the environment through insects contacted with animal dung and manure, especially HFs.

In cattle facilities and surrounding areas, supportive habitats (feces and manure) ensure free movement and feeding regurgitation behaviors of HFs, which presents a potential transmission route for ECO157H7 (Alam and Zurek, 2004).

An earlier laboratory-based study showed that HF may spread ECO157H7 for several days after being swallowed, and that the bacteria can continue to be infective in the fly feces (Kobayashi et al., 1999). It was reported that the ECO157H7 infectious dose is probably fewer than 100 cells (Marouani-Gadri et al., 2010).

The pathogen can be spread to people by the consumption of contaminated food, especially milk (non-pasteurized or contaminated post pasteurization) and improperly cooked ground beef (typically found in beef burgers). The peak summertime period for ECO157H7 infections in humans is May through September. This is linked to increased ECO157H7 shedding by cattle and incredibly high HF populations on cattle ranches and other operations related to animal production (Alam and Zurek, 2004).

Accurate identification of ECO157H7 from HFs can be made by culture-based isolation and presumptive identification may be helpful (Pava-Ripoli et al., 2015). Thus, the PCR targeting this bacterium can be highly specific when the isolation and identification of ECO157H7 is performed.

The frequency of isolates from HFs by detecting both *rfbO157* and *fliCH7* genes was assessed in this investigation since the use of both *rfbO157* and *fliCH7* is particular to ECO157H7 (Bilge et al., 1996). The formation of the flagellin-encoding H7 antigen (encoded by *fliCH7*), a crucial feature of ECO157H7, is necessary for the pathogen to bind to epithelial cells. These genes were chosen for screening because of their precision as genetic targets (Machado et al., 2000). The *rfbo157* gene, which codes for the enzyme perosamine synthetase needed to produce the O157 antigen, was detected in this study using PCR.

Hemolytic-uremic syndrome is primarily brought on by Shiga-toxin, which are generated by all enterohemorrhagic *E. coli* strains and are frequently recovered from people. These strains of *E. coli* produce O157 antigen (Tarr, 1995).

Our findings imply that testing only HF may be more efficient and accurate for identifying ECO157H7 on dairy farms. The present study reported that the ECO157H7 strain was found in HFs due to its contamination by cow dung. It was discovered in a prior Baghdad study that livestock acts as a ECO157H7 reservoir (Yousif and Hussein, 2015). According to a different study, ECO157H7-related human isolates are considered the most common isolates (Khudor et al., 2012). This proves that HFs were the most proper way for ECO157H7 transmission from dairy farms to humans.

CONCLUSIONS

Monitoring fly populations in neighbor farms is necessary for effective management of flies and fly-borne pathogens. According to the results of our investigation, HFs are critical for the transmission of virulent ECO157H7 among individual cattle and, potentially, to the nearby agricultural and urban environment over summer time.

The development of more thorough and risk assessment methods as well as intervention methods for ECO157H7 and its links to houseflies ensure an efficient control system of the HFs and the pathogen.

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AUTHOR CONTRIBUTIONS

All authors have participated equally in Lab-procedures, data-analysis, and writing and revising this manuscript.

CONFLICT OF INTEREST

The authors reveal no conflict of interest in this study.

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