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Molecular Analysis Of *E. coli O157* Isolated From Deer And Workers in Maysan AL-Reem Sanctuary

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Abstract/

Since their discovery in the early 1980s, strains of *E. coli* have been associated with a wide variety of illnesses in both animals and humans. Hemorrhagic colitis, diarrhea, and hemolytic uremic syndrome (HUS) have all been linked to *E. coli* 0157:H7 infections in humans, affecting 2-7% of individuals. our study aimed to shed the light on this correlation. A hundred samples were collected and transfer with transport media to the lab. The results showed that *E. coli* were isolated from 92% of deer feces, 40% of food, 40% of water, 70% of worker hands, and 100% of worker feces. In addition, using Hicrome agar, we find that *E. coli* O157:H7 is present in just 6% of deer feces Only two of the deer fecal samples tested positive for *E. coli* O157. In sum, our data demonstrated that pathogenic *E. coli* strain O157 is detected in deer fecal samples. More samples from different sources and in many seasons need for future work.

Keywords: Molecular Analysis , *E. coli O157*, Deer, Maysan AL-Reem Sanctuary, Hicrome agar.

INTRODUCTION

Esherichia coli is a Gram-negative, facultative anaerobic, rod-shaped, coliform bacterium of the genus Escherichia that is commonly found in the lower intestine of warm-blooded organisms [1,2,3] Enterohemorrhagic *Escherichia coli* (EHEC) strains, a subset of *Shiga* toxin-producing *E. coli* strains, have been linked to both animal and human disorders since their discovery in the early 1980s [4]. In humans, 2-7% of those infected with *E. coli* 0157:H7 experienced the hemorrhagic colitis, hemorrhagic diarrhea, and the hemolytic-uremic syndrome (HUS) [5,6,7]. This occurred in various parts of the world [8,9,9,10,11]. One of the most significant food-borne and water-borne pathogens in the world is *E. coli* serotype O157:H7, which expresses somatic

(O) antigen 157 and flagellar (H) antigen 7 [12]. Human illnesses with *E. coli* O157:H7 were linked to deer meat jerky in 1995 [13].
Children and the elderly are particularly susceptible to serious infection, making this a highly charged topic in public health and the agricultural industry [14,11, 15,16,17].
Verotoxin-producing *Escherichia coli* O157 has been detected in a wide variety of animals and environments, including cattle [18,19,20,21,22], sheep, goats, heifers, birds, deer, geese, turkey, seabirds, dogs, cats, gulls, chickens, pigs, monkeys, reptiles, llamas, and horses[10,23,24,25,26,27,28] , and flies [29]. Fish [30].The role that these animal species actually play in the epidemiology of O157 infection, however, has yet to be determined. Transmission of O157 has been observed not just through contact with contaminated food or water, but also through direct contact with animals or animal dung [31,32].
Petting zoos are fun for the whole family and have even become an integral part of young children's education. Children can learn a lot from trips to farms, both about animal care and agriculture in general. The risk of contracting serious zoonotic infections during visits to petting zoos is high due to the frequent encouragement of close contact with the animals, such as caressing and feeding the animals, especially to the major group of visitors, young children. In various countries, including Pennsylvania [9], Washington [33], Canada [34], and North Wales [35], there has been an outbreak of *Escherichia coli* O157 infections among people who visited farms during agricultural fairs, festivals, and petting zoos.

Prevention of introduction, routine testing of brought-in replacement animals, culling infected animals, and closing infected petting zoos do not appear to be feasible or effective due to the capacity of STEC 0157 to persist and multiply in the farm environment (animal feces, straw, soil, water) [34,36] and their natural occurrence in several wild animal species from which interspecies transmission to domestic animals may occur [37,38]; who conducted a longitudinal investigation at a public farm, came to a similar conclusion with pre-entry bacteriological testing of animals. White-tailed deer (Odocoileus virginianus) feces have been found to contain *Escherichia coli* O157:H7, but it is unclear whether or not this poses a direct or indirect zoonotic danger [39].
Outbreaks of gastrointestinal disorders produced by STEC have drawn attention to the danger they pose to public health because of the severity of the symptoms they can cause. In order to ensure timely diagnosis and locate the source of infection, which could aid in risk management, it is important to track the prevalence of *E. coli* in animals. The frequency in other species is not well understood in Iraq since epidemiological research into the O157 strain in animal populations has concentrated mostly on the bovine reservoir and, more recently, in horses. This research set out to quantify the frequency with which *E. coli* O157 was found in feces taken from animals housed at the Maysan AL-Reem Sanctuary, Iraq.

MATERIALS AND METHODS

SAMPLES COLLECTION

A hundred samples were collected and transfer with transport media to the lab. In details, fifty deer fecal swabs sample were collected during the study from the sanctuary animals (both sexes and different ages) using disposable swab and transport media. Twenty samples from food and ten from water of deer. Ten Swabs sample were collected from worker hands. ten samples of stool from worker in the sanctuary were collected during the study.

BIOCHEMISTRY DETECTS

Biochemically confirmed isolates preserved in glycerol broth at -20 °C were thawed in a refrigerator at 4°C overnight. Then, the isolates were sub cultured in both brain heart infusion agar and Hicrom medium. Gram stain was applied to each isolate to check the purity of our isolates.

VITEK 2 COMPACT SYSTEM

Following the manufacturer's instructions, the Vitality Index of Traditional Environmental Knowledge 2 (VITEK 2) compact system was run, and the results were compared to the relevant database in the computer connected to the VITEK 2 operator system (VITEK 2 compact test system steps in appendix)

MOLECULAR CONFIRMATION

The positive VITK 2 isolate was verified using a multiplex PCR assay with a 16s primer. Polymerase chain reaction (PCR)-based 16s rRNA molecular index of isolates and sequencing of all positive isolates as it shown in tables (1, 2) the primers sequence used and PCR condition has been used to amplified the genomic DNA isolate from *E. coli O157* [27].

PRIMERS USED IN THE STUDY 16s RNA

Table 1: The sequence of primers that used this study.

Primer	Sequence	Primer sequence	Tm (°C)	GC%	Size of Product (bp
16s RNA	**F**	**5'- AGAGTTTGATCCTGGCTCAG- 3'**	**54.3**	**50.0**	**1250bp**
	R	**5'- GGTTACCTTGTTACGACTT- 3'**	**49.4**	**42.1**	

Table 2: The optimum condition of detection

No.	Phase	Tm (°C)	Time	No. of cycle
1-	Initial Denaturation	95°C	5 min	1 cycle
2-	Denaturation -2	95°C	sec45	35 cycles
3-	Annealing	57°C	sec45	
4-	Extension-1	72°C	1min	
5-	Extension -2	72°C	5 min.	1 cycle

RESULTS AND DISCUSSION

The purpose of this investigation was to identify the most effective detection methods for *E. coli* O157 and to define the molecular characteristics of the isolates by polymerase chain reaction (PCR) and sequence analysis. The detection rate was lowest with the traditional culture

approach. The inability to identify *E. coli* O157:H7, which exhibited unusual biochemical characteristics, may be contributing factor [40].

ISOLATION AND IDENTIFICATION OF E. coli O157

All the samples were cultured on selective solid Eosin Methylene Blue (EMP) agar and HicromeTM EcoliO157:H7 agar, the positive result gave acidic reaction shown as metallic green sheen colonies in EMB as appeared in Figure 1 the positive result of O157 appear as dark purple to magenta colored moiety colony which is shown in Figure 2.

E. coli was isolated from different sources from the Maysan AL-Reem Sanctuary, Iraq which are summarized in Table 3. *E. coli* was isolated from 92% deer fecal samples, 40% food, 40% water, 70%Worker hand sample and 100%Worker fecal sample. Furthermore, the *E. coli* O157:H7 only appear in deer fecal samples with 6% and food with 5% by using Hicrome agar. Interestingly, only two samples from deer fecal showed positive to *E. coli* O157. Table (2) demonstrate the summary of the isolation. The prevalence of *E. coli* O157:H7 from industrial minced beef was 0.12% in France [41], and other French researcher reported that there was no *E. coli* O157: H7 isolation in 1,200 samples [8]. *Escherichia coli* O157:H7 strains were isolated and characterized from 22 of 174 fecal samples collected from 22 distinct kinds of petting zoo animals[42]. In Switzerland, no *E. coli* O157:H7 was detected from 400 samples [43]. Five *E. coli* O157:H7 (3.3%) were isolated from retail beef and bovine feces in Thailand, and 36 (8.7%) STEC in Spain [44]. The prevalence of STEC in North American and European cattle ranged from 0 to 10% [45]. The differences in the detection of STEC among these studies are probably due to the fact that the patterns of shedding of STEC are affected by diet, age, environmental condition, and seasonal variation [23].

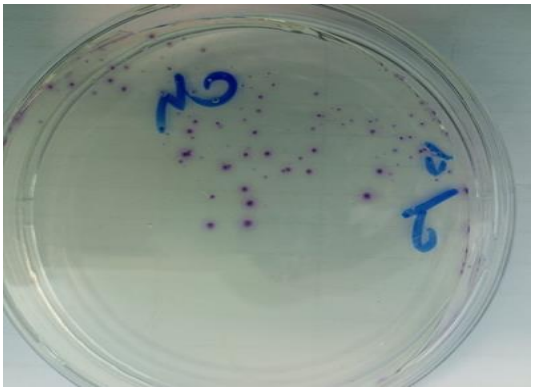

Figure 1 *E.coli* on EMP agar colonies colonies apper as metallic green sheen

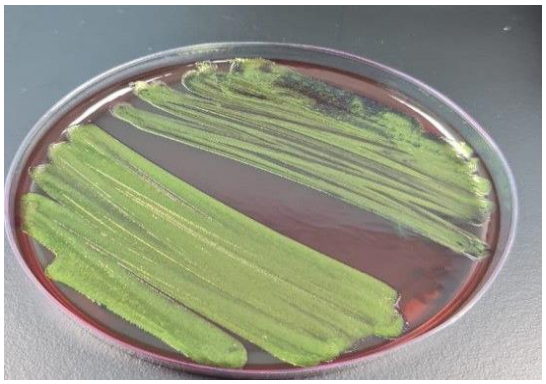

Figure 2 *E.coli O157* Hicrome agar the colonies were purple to magenta .

The study persisted from autumn to Spring The results showed that the isolation percentage of E.coli from deer fecal swab and worker faces was the highest (100%, 18/18), (100%) (5/5) respectively in autumn ,with statistical variation ($P<0.01$) from the other surrounding sources, then decreased in winter (15/18, 83.3%),(100% ,3/3) with statistical variation ($P<0.05$) from the other sources, and raised in spring (13/14, 92.8%) (2/2 . 100%) respectively, also with statistical

variation (P<0.05) from the other sources, While the *E. coli* O157 isolated only from the deer fecal sample in autumn and spring with no isolation in winter (Table 2)

Table 3: Number of positive *E. coli* O157 isolate by Hicrome agar and PCR from samples collected from different sources in the Maysan AL-Reem Sanctuary.

Type of Sample	Sample No.	No of *E.coli* Isolate in EMB	*No. of E.coliO157* isolate HiCrome	*No. E coli O157* isolate by PCR
Deer fecal sample	50	46 92%	3 6%	2 4%
Feed	20	8 40%	1 5%	0 0%
Water	10	4 40%	0 0%	0 0%
Worker hand sample	10	7 70%	0 0%	0 0%
Worker fecal sample	10	10 100%	0 0%	0 0%
Total	100	71 71%	4 4%	2 2%
P-value		<0.0001	0.67	0.72

Table 4. Season effect on the isolation percentage of *E. coli and E.coliO157* from deer and from different sample from Maysan AL-reem Sanctuary. Iraq.

Season	Type of sample	No. of samples	No. and % of *E, coli* isolates	P-value	No and % of *E. coliO157* isolates
Autumn	Deer	18	18 (100%)	<0.01	1 (5.5%)
	Feed	9	4 (44.4%%)		0
	Water	4	2 (50%)		0
	Worker swab	5	1 (20%)		0
	Worker feaces	5	5 (100%)		0
winter	Deer	18	15 (83.3%)	<0.05	0
	Feed	6	2 (66.6%)		0
	Water	3	1 (33.3%)		0
	Worker swab	3	1 (33.3%)		0
	Work feaces	3	3 (100%)		0
Spring	Deer	14	13 (92.8%)	0.05	1 (6.66%)
	Feed	5	2 (40%)		0
	Water	3	1 (33.3%)		0
	Worker swab	2	1 (50%)		0
	Worker feaces	2	2(100%)		0
Total		100	71 (71%)		0

The importance of animals as a potential source of human infection is evident in the fact that STEC strains (with similar virulence characteristics) have been isolated from human patients,

apparently healthy animals, meat, and products thereof. Majority of research on prevalence of STEC in animals and food is focused on deer since they are considered as their natural reservoir, [46] whom isolated EcoliO157 from deer and cases human infection. although there are several reports on prevalence in other animals and their products [16,47].

Studying the seasonal variation of infectious diseases has relevance to improving the understanding of host and pathogen ecology, the surveillance and prevention of infections, the prediction of epidemics, and understanding of the long-term trends in infections as a result of global climate change [48]. and Our findings may inform epidemiologists about illness prevention and risk to human health. The *E. coli* bacteria are released from human and animal feces can survive in environments such as water and soil. The colder wet climates has been shown to contribute in elevation the fecal contamination levels increased persistence of fecal bacteria, including pathogens, which impacted in waters Ans surrowndind [49,50,51].

Universal primers had been used to amplify the highly conserve region in the ribosomal locus. These primers allow the scientists to distinguish between bacteria after alignment the sequence results in the NCBI. As it displays in figure (1) the primers able to detect the 16rs sequence and shown a band in 1250pb location. 16s rRNA sequencing is a culture-independent approach for enumerating and contrasting bacterial diversity in difficult-to-study microbiomes or habitats [52]. It is widely employed for determining the genus and/or species of bacteria present in a sample.

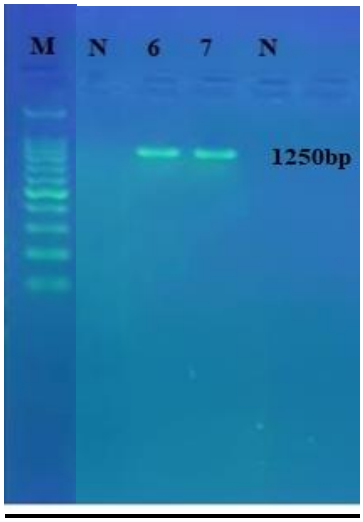

Figure 1: PCR product the band size. The product was electrophoresis on 1.5% agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

PHYLOGENIC TREE

A phylogenetic tree was constructed using nucleotide sequence data for six distinct strains of *E. coli.* The phylogenetic study relied on two 16rs gene sequences obtained from GenBank, both of which belonged to different strains of *E. coli*. The phylogenetic tree, depicted in Figure (2), was constructed using the software MEGA 6.0. This phylogenetic tree allowed us to divide *E. coli* isolates into four distinct groups. *E. coli* strains clustered into the same phylogenetic groupings as revealed by 16rs sequencing, demonstrating that PCR-based phylogenetic grouping is consistent with phylogenetic grouping found by gene sequence analysis.

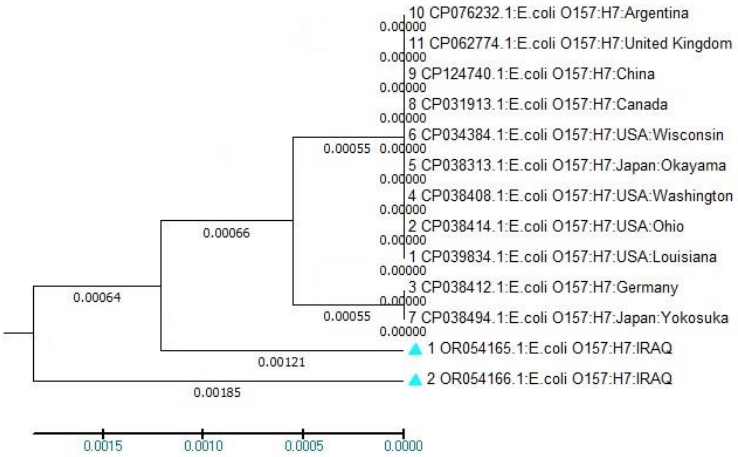

Figure 2: Phylogenetic tree of *16rs* gene of *E. coli* isolates from deer fecal and *E. coli* strains retrieved from GenBank generated using neighbor-joining method in MEGA 6.0.

CONCLUSION

In conclusion, this study is the first in Iraq to isolate in molecular level *E. coli* O157 from deer fecal samples. In addition, even though 92% of the samples showed positive to *E.coli* only 6% were positive to *E.coli* O157 strain. Finally, none of other samples which collected from different sources were positive to *E.coli* O157 strain. There is an insistent need to collect additional samples from a wide variety of sources across all four seasons and over extended time periods.

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