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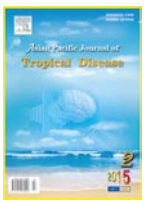

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Detection and characterization of *Escherichia coli* O157:H7 from feral pigeon in Qom province, Iran

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PEER REVIEW

Peer reviewer

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Comments

This manuscript contains helpful information with regard to an important pathogen which is responsible for a human health concern. Epidemiological knowledge in this regard can lead to take necessary measures for increasing public awareness about human health hazard resulting from pigeon fecal contamination. It is a survey appropriately carried out that deserves in my opinion to be published.
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ABSTRACT

Objective: To detect and characterize *Escherichia coli* (*E. coli*) O157:H7 in feral pigeons in Qom province, Iran.
Methods: In this survey, 290 cloacal samples were obtained from trapped feral pigeons in Qom province. Microbiological, biochemical and serological examinations were done to detect the *E. coli* O157:H7. Isolates were subjected to multiplex polymerase chain reaction for the detection of *stx1*, *stx2*, *eaeA* and *hlyA* genes.
Results: Four samples (1.38%) were positive for *E. coli* O157:H7 by using O157 and H7 antisera and only one *E. coli* O157:H7 strain isolated showed the presence of *stx1*, *stx2*, *eaeA* and *hlyA* genes.
Conclusions: The results of present survey revealed that feral pigeons in Qom province had the potential to be a reservoir of *E. coli* O157:H7. The low prevalence of *E. coli* O157:H7 can be attributed to sampling each pigeon just once and fecal culture limits, and true prevalence of the *E. coli* O157:H7 might be higher than our findings.

KEYWORDS

Pigeon, *Escherichia coli* O157:H7, Detection, Polymerase chain reaction

1. Introduction

Escherichia coli (*E. coli*) is a Gram-negative bacterium belonged to Enterobacteriacea family. Most *E. coli* strains exist normally in intestinal tract[1,2]. Some *E. coli* serotypes like *E. coli* O157:H7 are pathogenic for human and animals and the serotype O157:H7 is the major Shiga toxin-producing *E. coli* (STEC) in many parts of the world[3-5]. *E. coli* O157:H7 strains' virulence factors represented by selected genes such as the ones encoding the Shiga toxins, *stx1* and *stx2*, *eaeA* genes, responsible for the ability to attach and efface lesions and *hlyA* gene, enabling production of haemolysin[3,6-9]. The first occurrence of *E. coli* O157:H7 outbreak documented in USA in 1982, affected at least 47 persons[10]. Infections of *E. coli* O157:H7 have been reported in over 30 countries on six continents so far[1].

Although cattle are the main reservoir of *E. coli* O157:H7, the other animals such as sheep, goat, deer, dog, horse, cat,

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rat and pigeon can be a source of this bacterium[4,8,9,11,12]. First isolation of *E. coli* O157 from birds in fecal samples collected from free-ranging birds was in 1997[12].

A large number of pigeons exist around the world, which can readily be in close contact with humans and animals due to their free flight[4]. Pigeons live in many places in urban and rural areas such as parks, buildings, feed mills, *etc*.

It is believed that pigeons are a reservoir for *E. coli* O157:H7[13]. The aim of this study was to detect and characterize *E. coli* O157:H7 in feral pigeons in Qom province.

2. Materials and methods

A total of 290 pigeons were trapped within Qom province, Iran. Cloacal swabs were obtained using the BBL Culture Swab collection and transport system (Becton, Dickinson) that contained Amies liquid medium, liquid Stuart medium and Cary-Blair transport medium. Each sample was enriched in 3 mL of tryptic soy broth and incubated for 18-24 h at 37 °C. Samples from the tryptic soy broth streaked onto a Sorbitol MacConkey agar plate (containing 0.05 mg/L cefixime and 2.5 mg/L potassium tellurite) and incubated for 18-24 h at 37 °C. Isolated colonies were confirmed as *E. coli*-positive using biochemical tests: Gram staining, oxidase and IMViC .

To identify O157:H7 serotype, colorless colonies were examined by O157 and H7 antisera (MAST Co., UK) by using plate agglutination method.

DNA extraction and multiplex polymerase chain reaction (m-PCR) for *stx1*, *stx2*, *eaeA* and *hlyA* genes amplification were carried out as previously explained by Paton AW and Paton JC[3]. Bas sequence and predicted size of amplified product for each oligonucleotide primer (Cina Gen Inc, Iran) used in this study are shown in Table 1. DNA of *E. coli* ATCC 35218 was used as positive control and water was administered as negative control in m-PCR reaction.

Table 1

Primers sequence used in m-PCR (Paton AW and Paton JC, 1998).

Primer	Target	Sequence (5′-3′)	Specificity	Amplicon size
*stx1*F	*stx1*	ATA AAT CGC CAT TCG TTG ACT AC	nt 454-633	180 bp
*stx1*R	*stx1*	AGA ACG CCC ACT GAG ATC ATC	nt 633-454	
*stx2*F	*stx2*	GGC ACT GTC TGA AAC TGC TCC	nt 603-857	255 bp
*stx2*R	*stx2*	TCG CCA GTT ATC TGA CAT TCT G	nt 857-603	
*eaeA*F	*eaeA*	GAC CCG GCA CAA GCA TAA GC	nt 27-410	384 bp
*eaeA*R	*eaeA*	CCA CCT GCA GCA ACA AGA GG	nt 410-27	
*hlyA*F	*hlyA*	GCA TCA TCA AGC GTA CGT TCC	nt 70-603	534 bp
*hlyA*R	*hlyA*	AAT GAG CCA AGC TGG TTA AGC	nt 603-70	

3. Results

In this study, from 290 cloacal samples, four samples (1.38%) were positive for *E. coli* O157:H7 by using O157 and H7 antisera with plate agglutination method. From four positive samples deducted, only one sample possessed *eaeA*, *stx2*, *stx1* and *hlyA* genes with application of PCR method (Figure 1).

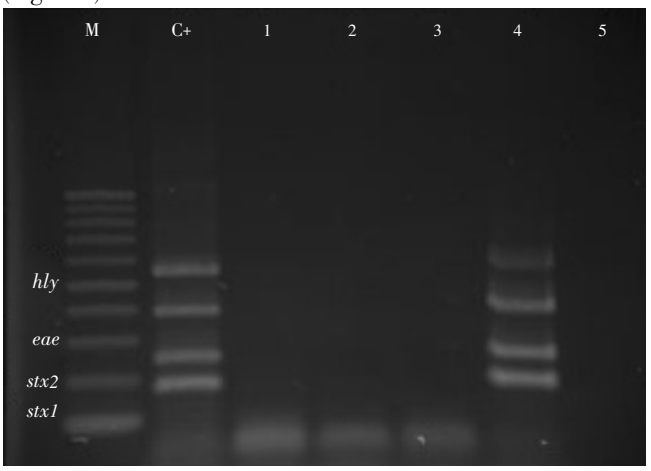

Figure 1. Multiplex PCR: *stx1* (180 bp), *stx2* (255 bp), *eae* (384 bp), *hly* (534 bp). M: Marker; C+: control positive; Lane 1: sample 1; Lane 2: sample 2; Lane 3: sample 3; Lane 4: sample 4; Lane 5: control negative.

4. Discussion

Transmission of infectious agents from birds to human being has a great importance in some disease epidemiology, since birds have the potential to spread microorganisms in public places. STEC endurance in unfavorable situation and its indirect transmission result in its ability to infect new hosts. The pathogenic strains of *E. coli* O157:H7, with virulence genes, for human have been partly isolated from pigeons[14,15].

Contact and consumption of contaminated food with *E. coli* O157:H7 can lead to human infection, so infection sources′ recognition plays an important role in decreasing the prevalence of this pathogen[13]. Several studies were carried out to determine the presence of STEC in pigeons. A same survey in Kerman, Ghanbarpour and Daneshdoost reported that five isolates (3.62%) from 154 fecal sample containing one of the *stx1* and *stx2* genes, were considered as STEC strains[14]. Two studies worked on fecal samples from chickens and pigeons in Finland and India, but no STEC strain was detected[4,15]. Morabito *et al.* isolated STEC from 70 faecal samples in 649 feral pigeons in Italy[16]. Grossmann *et al.* studied in Germany revealed that 66.9% of droppings from ornamental and racing pigeons contained Shiga-toxin-gene positive *E. coli*, while from these, 36% were positive for *stx1*, 9% for *stx2*, and 37% for *stx2f*[17]. According to Silva *et al.*, from 100 fresh fecal samples of urban pigeons, viable diarrhea genic *E. coli* was identified at an overall rate of 12.1%[11].

The difference among studies results may be related to number of samples, sampling method, number of sampling each pigeon, isolation method, *etc*. The results of present study revealed that free-living pigeons in Qom province have the potential to be a reservoir of *E.coli* O157:H7.Although, this pathogen prevalence found in this study was not considerable, the potential for pigeon to transmit *E. coli* O157:H7 to animals and human is significant due to their mobility and traveling over long distances.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Since pigeons inhabit near human living places, achieving more information about the prevalence of the *E. coli* O157:H7 occurrence in the different regions may help to promote control strategies.

Research frontiers

In this survey, the authors isolated *E. coli* O157:H7 from pigeons by using culturing and plate agglutination method to identify O157 and H7 antigens. In order to detect *stx1*, *stx2*, *eaeA* and *hlyA* genes, mPCR was performed.

Related reports

Other surveys carried out all over the world to find that *E. coli* O157:H7 in fecal samples of urban, and feral pigeons and virulence genes such as *stx1* and *stx2* were detected in these isolates.

Innovations & breakthroughs

This survey was the first investigation carried out in this region to determine the prevalence of *E. coli* O157:H7 in pigeons and their virulence gene profile including *stx1*, *stx2*, *eaeA* and *hlyA*.

Applications

Birds are involved in transmission of some agents of infectious diseases to human beings. So, detection of STEC O157:H7 in pigeons has a great importance in terms of public health, since they have a great mobility and travel over long distances.

Peer review

This manuscript contains helpful information with regard to an important pathogen which is responsible for a human health concern. Epidemiological knowledge in this regard can lead to take necessary measures for increasing public awareness about human health hazard resulting from pigeon fecal contamination. It is a survey appropriately carried out that deserves in my opinion to be published.

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