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Short communication

Prevalence of Shiga toxin-producing and enteropathogenic *Escherichia coli* in slaughtered camels in Iran

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ABSTRACT

To investigate the prevalence of Shiga toxin-producing *Escherichia coli* (STEC) and enteropathogenic *E. coli* (EPEC) strains in Iranian camels, *E. coli* strains isolated from fecal samples of 125 slaughtered camels were subjected to PCR for detection of *stx1*, *stx2*, *eaeA* and *Ehly* genes. The strains determined positive were then tested for O157:H7, O111 and O26 serotypes and *bfpA* gene using PCR. In addition, the intimin subtype of *eaeA*-positive isolates was determined by PCR-RFLP and sequencing. EPEC strains, none of which belonged to the tested serogroups, were detected in 2.4% (3 out of 125) of stool samples, whereas no STEC strain was detected. The *eaeA*-positive isolates were considered as atypical EPEC due to lack of *bfpA* gene, two of which contained beta intimin subtype. To our knowledge, this is the first molecular description of EPEC strains in camels.

1. Introduction

Diarrheagenic *Escherichia coli* strains, considered as major health threats for both humans and animals, are categorized into various pathotypes, two of which are Shiga toxin-producing *E. coli* (STEC), producing two different types of Shiga toxins encoded by *stx1* and *stx2* genes, and enteropathogenic *E. coli* (EPEC), recognized as intimin-containing diarrheagenic *E. coli* strains without *stx* genes (Kaper et al., 2004).

Differentiation of intimin alleles has represented an important tool whereby STEC and EPEC strains can be typed in pathogenesis, epidemiological, and clonal studies (Jenkins et al., 2003; Blanco et al., 2006). The 5′ region of the *eaeA* gene is conserved, whereas the 3′ region is variable. The heterogeneous region of the *eaeA* gene encodes the C-terminal end (Int-280) of intimin, which is responsible for receptor binding. It has been suggested that the different intimin subtypes may be responsible for different host tissue cell tropisms (Zhang et al., 2002). PCR-RFLP, type-specific PCR and sequencing have been applied to identify different intimin subtypes (Ramachandran et al., 2003; Blanco et al., 2006). Ramachandran et al. (2003) have developed a PCR-RFLP method capable of identifying 14 intimin subtype: α1, α2, β, γ, κ, ε, η, ι, λ, θ, ζ, μ, ν and ξ.

Domestic animals including cattle, sheep and goats have been regarded as natural reservoirs of STEC and

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EPEC strains (Paton and Paton, 1998a; Hernandes et al., 2009). O157, O111 and O26 are among the most frequent serogroups of STEC and EPEC isolates and are of clinical and epidemiological importance (Kaper et al., 2004; Hernandes et al., 2009). STEC strains belonging to O157, O111 and O26 serogroups have been described to be possibly associated with calf diarrhoea (Kang et al., 2004; Lee et al., 2008). O157 and O26 serogroups have been isolated from camels but O111 serogroup has not been found (Chauhan and Kaushik, 1991; Salehi et al., 2012). In Iran, camels are mostly raised traditionally, which makes their contact with other domestic animals such as cattle, sheep and goats possible. As a result, it is likely that camels play a role in epidemiology of STEC and EPEC. There is little information available on status of STEC and EPEC infections in camels. Accordingly, the present study was conducted to investigate the prevalence of STEC and EPEC strains in fecal samples of apparently healthy camels in Tehran province, Iran, and also, to molecularly characterize the isolates determined positive.

2. Materials and methods

2.1. Sampling and isolation procedure

From June to November 2008, 125 fresh fecal samples were collected from apparently healthy camels slaughtered at Saman Goosht Asia abattoir, the only abattoir for camel slaughtering in Tehran province, Iran, and then transferred to the laboratory and examined within 12 h after sampling. Fecal samples were directly cultured on MacConkey agar (Merck, Germany) and incubated at 37 °C for 24 h. One lactose positive isolate from each plate was chosen and subcultured. The suspected isolates were confirmed as *E. coli* by conventional biochemical tests.

2.2. PCR procedure for detection of virulence genes and molecular serotyping of isolates

Total genomic DNA was extracted from overnight cultures on Luria Bertani agar (Merck, Germany) by the boiling method, as described by Zahraei Salehi et al. (2007), and 2–3 μl of the supernatant was subsequently used as the template in the PCR mixture. All *E. coli* isolates were first screened for presence of *stx1*, *stx2*, *eaeA* and *Ehly*, encoding enterohemolysin, genes (Paton and Paton, 1998b). The common primers for *stx* genes (Lin et al., 1993) were also selected because of their high sensitivity in detection of the virulence gene variants and subtypes. The strains determined positive for former genes were subsequently subjected to molecular serotyping using specific primers for O157 and O111 somatic antigen *rfb* genes (Paton and Paton, 1998b), O26 *wzx-wzy* genes (O-antigen flippase and polymerase, respectively; Durso et al., 2005) and flagellar H7 gene (*fliCH7*; Gannon et al., 1997). Specific primers for *bfpA* (encoding bundle forming pili) gene was also used to classify EPEC isolates as typical or atypical (Gunzburg et al., 1995). *E. coli* ATCC 35218, *E. coli* O157:H7 Tarbiat Modarres strain, *E. coli* O111, *E. coli* O26 and a typical EPEC strain from the microbial collection in the Department of Veterinary Microbiology, University of Tehran, were used as positive controls. In addition, negative controls (sterile water) were included in all of the PCRs.

2.3. PCR-RFLP and DNA sequencing for intimin subtyping

To determine intimin subtypes, all *eaeA*$^+$ strains were subjected to a typing PCR for the *eaeA* gene using one forward (EaeVF) and three reverse (EaeVR, EaeIotaVR, EaeZetaVR) primers according to Ramachandran et al. (2003). PCR was carried out in a total volume of 50 μl containing 3 μl of prepared DNA template, 1 μM of each oligonucleotide primer, 1.5 mM magnesium chloride, 0.2 mM dNTP mix, 5 μl 10× buffer, 2 U *Taq* DNA polymerase (Fermentas, Lithuania) and PCR-grade water up to 50 μl. The cycling conditions consisted of 36 cycles of 60 s at 94 °C, 60 s at 42 °C and 60 s at 72 °C, with a final extension step of six minutes at 72 °C. The quality of the products was assessed by electrophoresis and staining of the amplified products in agarose gels. The 834–876 bp specific products were subsequently subjected to restriction fragment length polymorphism (RFLP) analysis and were digested by *Alu*I and *Rsa*I (Fermentas, Germany) separately; subtypes were determined by comparing the digestion bands with the described patterns (Ramachandran et al., 2003). For definitive confirmation of the intimin subtypes, the PCR products of positive samples were also directly sequenced by Applied Biosystems 3730xl DNA analyzer (Macrogene, South Korea).

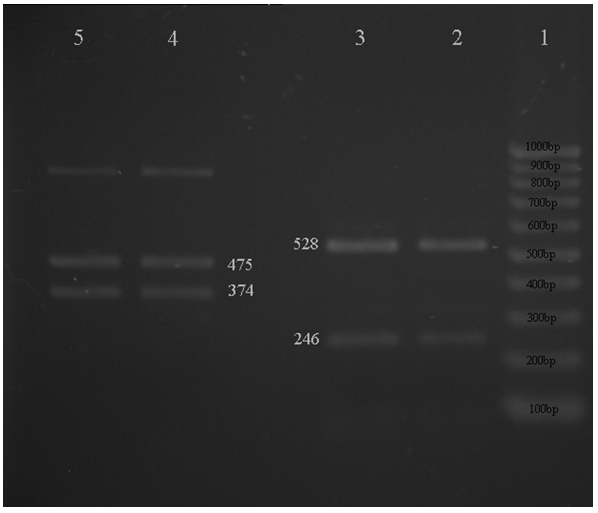

Fig. 1. RFLP pattern of intimin gene in two eaeA$^+$ isolates.100 bp DNA ladder (lane1); 528 bp and 246 bp fragments produced by digestion with *RsaI* enzyme (lane 2 and 3); 475 bp and 374 bp fragments produced by digestion with *AluI* enzyme (lane 4 and 5).

3. Results

In all PCRs, control strains produced the correspondent amplicons and no band was observed for negative controls. None of the 86 *E.coli* strains, isolated from fecal specimens of 125 slaughtered camels, was positive for *stx1* or *stx2*. Three isolates not belonging to any of tested O157:H7, O111 or O26 *E.coli* serotypes were only positive for *eaeA* gene.

The results of PCR-RFLP revealed two of the *eaeA*$^+$ isolates possessed β intimin subtype (Fig. 1), and the third one did not produce *eaeA* amplicon, and so was regarded as untypable subtype. The sequence analysis of the two *eaeA* amplicons, with an approximate length of 850 bp, confirmed the results of PCR-RFLP and further showed that they belong to β1 intimin subtype. It was demonstrated by amino acid sequence alignment that the predicted amino acid sequences of intimin, from studied camel strains were identical to those from *E.coli* isolates of rabbit (262/262; GenBank accession no. U60002), cattle (262/262; AJ275113), pig (191/191; AJ745900) and non-human primate (191/191; AJ715824) origin and were almost identical (more than 99%) to those isolated from humans (202/203; EU499354) and goats (260/262; AF253560; Fig. 2).

4. Discussion

Pathogenic *E. coli* strains belonging to STEC and EPEC pathotypes have frequently been isolated from diarrheic animals; therefore, it is essential that all possible sources of infection be identified.

In the current study, neither STEC nor *E. coli* O157:H7 was found, which agrees with findings of Moore et al.