



Short communication

Detection of diarrheagenic *Escherichia coli* strains isolated from dogs and cats in Brazil

Juan Puño-Sarmiento^{a,1}, Leonardo Medeiros^{a,1}, Carolina Chiconi^{d,1},
Fernando Martins^{b,1}, Jacinta Pelayo^{a,1}, Sérgio Rocha^{a,1}, Jorge Blanco^{c,1},
Miguel Blanco^{c,1}, Marcelo Zanutto^{d,1}, Renata Kobayashi^{a,1},
Gerson Nakazato^{a,1,*}

^a Department of Microbiology, Center of Sciences Biological, Universidade Estadual de Londrina, Londrina, Paraná, Brazil

^b Laboratory of Bacteriology, Instituto Butantã, São Paulo, São Paulo, Brazil

^c Department of Microbiology and Parasitology, Universidad Santiago de Compostela, Lugo, Galicia, Spain

^d Department of Veterinary Clinical, Center of Agrarian Sciences, Universidade Estadual de Londrina, Londrina, Paraná, Brazil

ARTICLE INFO

Article history:

Received 13 May 2013

Received in revised form 11 July 2013

Accepted 12 July 2013

Keywords:

Escherichia coli

Diarrhea

Pets

Animals

Virulence factors

Zoonosis

ABSTRACT

Escherichia coli are gut microbiota bacteria that can cause disease in some humans and other animals, including dogs and cats that humans often keep as pets. Diarrheagenic *E. coli* (DEC) strains are classified into six categories: enteropathogenic (EPEC), enterotoxigenic (ETEC), Shiga toxin-producing (STEC), enteroinvasive (EIEC), enteroaggregative (EAEC), and diffuse-adhering *E. coli* (DAEC). In this study 144 and 163 *E. coli* colonies were isolated from the fecal samples of 50 dogs and 50 cats, respectively, with and without diarrhea from a Veterinary Hospital (clinical isolates). The virulence factors were determined using multiplex Polymerase Chain Reaction. Adherence assays, antibacterial susceptibility and serotyping (somatic or flagellar antigens) were performed on DEC isolates. We found 25 (17.4%) and 4 (2.5%) DEC strains isolated from dogs and cats, respectively. Only the EPEC and EAEC pathotypes were found in both animals. Meanwhile, genes from other pathotypes (STEC, EIEC, and ETEC) were not found in these clinical isolates. All of the DEC strains showed mannose-resistant adherence to HEP-2 and HeLa cells, and aggregative adherence was predominant in these isolates. Multiresistant strains to antimicrobials were found in most DEC strains including usual and unusual antimicrobials in veterinary practices. The serotypes of these DEC isolates were variable. The ONT serotype was predominant in these isolates. Some serotypes found in our study were described to human DEC. Here, we demonstrate that pets carry virulent DEC genes, which are mainly strains of EPECs and EAECs. The presence of these virulence factors in isolates from animals without diarrhea suggests that pets can act as a reservoir for human infection.

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Escherichia coli strains can cause several enteric disease and extra-intestinal diseases (Kaper et al., 2004). *E. coli* is a normal commensal inhabitant of the gut in humans and other warm-blooded animals. Previously, the most extensive investigations of *E. coli* infection have been described in cattle, sheep, and pigs. However, recently dogs and cats

* Corresponding author. Tel.: +55 43 3371 4396; fax: +55 43 3371 4788.

E-mail addresses: gersonnakazato@yahoo.com.br, gnakazato@uel.br (G. Nakazato).

¹ All authors contributed equally to this work.

that live in close proximity with humans could become a focus of disease transmission studies. Because the contact between humans and pets has increased, the possibility of pathogenic micro-organism transmission between these organisms is very high.

The virulence markers associated with diarrheagenic *E. coli* (DEC) fall into five categories in dogs and cats (Beutin, 1999): Enteropathogenic (EPEC), Enterotoxigenic (ETEC), Enteroinvasive (EIEC), Shiga toxin-producing (STEC), and Enteroaggregative (EAEC).

Some DEC strains induce attaching and effacing (A/E) lesions in the gut mucosa leading to diarrheal disease. EPEC strains possessing virulence markers have been isolated from healthy and diarrheic dogs and cats (Beutin, 1999; Goffaux et al., 2000; Morato et al., 2009; Nakazato et al., 2004). Cattle and other ruminants were identified as a major natural reservoir for STEC (Blanco et al., 2001), but dogs and cats can also serve as reservoirs of STEC strains (Bentancor et al., 2007). The occurrence of ETEC in pets and their role in diarrheal illness is less well defined. ETECs are well known in humans (Nataro and Kaper, 1998), calves and pigs (Holland, 1990). ETEC strains have been reported in dogs (Beutin, 1999; Olson et al., 1985) but few strains have been studied in cats. EIEC and EAEC are studied mainly in human infections. The role of these *E. coli* strains and their virulence factors in diarrhea is not well known and requires further investigation.

This article will focus on the gastrointestinal infections caused by *E. coli* in dogs and cats and the possibility that transmission of these micro-organisms between different host species and humans can be extremely high.

2. Materials and methods

2.1. Fecal samples and bacterial strains

From January to December 2011, fecal specimens were collected from 50 dogs and 50 cats with a variety of ages, both with and without diarrhea from a Veterinary Hospital at the Universidade Estadual de Londrina (UEL). The selected pets were not treated with antibiotics. All of the animals were from the city of Londrina, in the northern state of Paraná, Brazil. Approximately 1 g of feces was collected using cotton swabs, which were then transported immediately to the laboratory in plastic containers containing Cary–Blair medium (Difco®). These samples were cultured using MacConkey agar (Difco®) plates which were then incubated at 37 °C for 24 h. These procedures were approved by the Ethical Committee for Animal Research from UEL (protocol number 24/2010). Three to four colonies from each plate were selected and examined using biochemical assays (Ewing, 1986; Toledo et al., 1982a, 1982b) to confirm the presence of *E. coli*. Approximately 307 *E. coli* isolates were examined in this study. The samples were stored in Brain Heart Infusion (BHI) (Difco®) plus 20% glycerol (Sigma®) media at –80 °C. For positive and negative controls in the PCR and adherence assays the following reference strains used: *E. coli* E2348/69 (O127:H6, *eae*+,

bfpA+, localized adherence), DEPEC 008 (O98:H28, *eae*+, localized adherence like), *E. coli* EDL 933 (O157:H7, *eae*+, *stx1*+, *stx2*+, *ehxA*+), EAEC O42 (*aggR*+, aggregative adherence), EIEC EDL 1284 (*ipaH*+), ETEC H10407 (*elt*+), ETEC B41 (*est*+), and *E. coli* K12C600 (negative control).

2.2. PCR-directed detection of virulence genes and *eae* typing

All strains were grown on Trypticase Soy Agar (Difco®) at 37 °C for 24 h. DNA was extracted by suspending five bacterial colonies in 200 µl of sterile water that was boiled for 10 min and centrifuged at 10,000 × *g* for 5 min. The supernatant was used as the template in the PCR assays. The presence of virulence genes was examined using two multiplex PCR techniques described previously (Aranda et al., 2007; Paton and Paton, 1998). Isolates positive for *eae* were typed according to previously described criteria (Blanco et al., 2006).

2.3. Adherence assays

E. coli adherence to HEP-2 and HeLa cells was detected as previously described (Cravioto et al., 1979) in 6 h of incubation. To determine the adhesion pattern previously described criteria were used (Gioppo et al., 2000; Nataro et al., 1987; Rodrigues et al., 1996; Scaletsky et al., 1999).

2.4. Antimicrobial susceptibility testing

Antimicrobial-resistance phenotypes were determined using the agar disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2012). The following antimicrobial agents were used: amoxicillin, amoxicillin-clavulanic acid, aztreonam, tetracycline, cefotaxime, imipenem, nalidixic acid, gentamicin, chloramphenicol, trimethoprim-sulfamethoxazole, ampicillin, ciprofloxacin, and streptomycin. Enrofloxacin was also tested because this antimicrobial is commonly used in veterinary clinics, and the interpretation of results was determined using CLSI M31-A3 (2008). Only isolates that exhibited some virulence factor were tested for antimicrobials. *E. coli* strain ATCC 25922 was used as quality control.

2.5. Serotyping

Isolate serotyping was performed using standard agglutination methods with antisera to somatic O (O1–O185) and flagellar H (H1–H56) antigens (Guinée et al., 1981) from the *E. coli* Reference Laboratory, Santiago de Compostela University, Lugo, Spain.

2.6. Statistical analysis

The data were expressed as the means ± SD. The bacterial groups were compared using χ^2 (Chi-square test) for independent samples; differences were considered significant at $p < 0.05$. The statistical analyses were performed using BioEstat version 5.0 software.

Download English Version:

<https://daneshyari.com/en/article/5801218>

Download Persian Version:

<https://daneshyari.com/article/5801218>

[Daneshyari.com](https://daneshyari.com)