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Occurrence of non-O157 Shiga toxin-producing *Escherichia coli* in dogs with diarrhea

Ocorrência de *Escherichia coli* não–O157 Shigatoxigênica em cachorros com diarréia

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ABSTRACT

Shiga toxigenic *Escherichia coli* (STEC) and *Attaching and effacing E. coli* (AEEC) have been associated with diarrhea illness in dogs. From January to December 2006, 92 *E. coli* isolates from 25 diarrheic dogs were analyzed, by screening for the presence of Shiga toxin-producing (stx 1 and stx 2) and intimin (eae) genes. Twelve isolates were detected by PCR to harbor the Shiga toxin genes (7 the stx 1 (7.6%); 5 the stx 2 (5.4%); and none both of them). Nine (9.8%) of the *E. coli* isolates studied were eae positive non Shiga toxin-producing. Thirteen (62.0%) isolates, carrying stx or eae gene, also showed α hemolysin production. The strains with virulence genes were also examined for resistance to 12 antimicrobial agents. Resistances to cephalothin (85.7%), streptomycin (81.0%), amoxicillin (71.4%) and gentamicin (71.4%) were predominantly observed.

Key words: *Escherichia coli*, STEC, eae gene, antimicrobial resistance.

INTRODUCTION

*Escherichia coli* is a predominant component of the intestinal microbiota of humans and other mammals. Some *E. coli* strains represent primary pathogens having an enhanced potential to cause diseases, especially diarrhea (NATARO & KAPER, 1998). An emergent pathogen, Shiga toxin-producing *E. coli* (STEC) of the serogroup O 157, designated as enterohemorrhagic *E. coli* (EHEC), has been considered to be responsible for many outbreaks of hemorrhagic colitis and hemolytic uremic syndrome (PATON & PATON, 1998). Two types of *E. coli* toxins, Stx1 and Stx 2, are known and constitute the main virulence factors in STEC strains (PATON & PATON, 1998). Domestic ruminants have been implicated as being the major reservoirs of STEC strains that cause human infections (CHAPMAN et al., 2001). However, other domestic animals like cats and dogs have also been found to carry STEC strains (BEUTIN et al., 1993; HAMMERMULLER et al., 1995; STAATS et al., 2003; BENTANCOR et al., 2007). In these cases, transmission pathways were the direct contact between humans and animal hosts.
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MATERIAL AND METHODS

Sample collection

In order to establish STEC occurrence as well as their antimicrobial susceptibility to 12 antimicrobial agents, 25 diarrheic dogs were randomly selected by order of presentation to a private clinic in the city of Ituverava, State of São Paulo, between January and December 2006. Animals of any age, sex or breed were chosen on the following clinical inclusion criteria: acute hemorrhagic or not diarrhea, inappetence, no previous antimicrobial drugs treatment, with no other gastrointestinal disease or evidence of surgical approach. Samples collected by rectal swabbing with a sterile cotton swab under veterinary supervision were placed in Stuart transport medium and taken to immediate laboratory processing.

Culture

Samples were transferred to MacConkey agar (Mac-Difco) and incubated for 24h at 37°C. At least five colonies were selected from each plate for analysis. Biochemical confirmation of the strains as *E. coli* was performed according to KONEMAN et al. (1997).

Determination of stx genes

Bacterial strains (*E. coli* isolates) grown overnight in nutrient broth (Sigma Chemical Co, St Louis, USA) at 37°C, were tested for the presence of *stx* genes (*stx* 1 and *stx* 2) using the polymerase chain reaction (PCR) protocol of ORDEN et al. (1998). DNA templates were prepared by pelleting 1ml of culture enriched by centrifugation at 12000g. The cell pellet was resuspended in 250μl of sterile distilled water and boiled for 10min at 100°C, re-centrifuged and supernatants were subjected to PCR in an Eppendorf Mastercycler (Eppendorf AG, Hamburg, Germany). The amplified DNA products were separated by electrophoresis on 1.5% agarose gel, stained with ethidium bromide and examined for detection under ultraviolet light. Reference *E. coli* strains used as controls were EDL 933 (O157:H7, *stx*1, *stx*2, eae) and DH5a (negative control), both from Dr. Tânia A. Tardeli Gomes (Department of Microbiology, Immunology and Parasitology, Escola Paulista de Medicina, São Paulo, Brasil).

Characterization of isolates

Isolates were confirmed as *stx*+ and tested for the accessory virulence marker eae, using the PCR protocol of CHINA et al. (1996).

O157 latex agglutination

STEC isolates were typed for O serotype O157 using the O157 Latex Agglutination test kit (Oxoid, Basingstoke, Hampshire, UK). The EDL 933 strain was used as a positive control. Strains negative to agglutination were considered non-O157 strains.

Ciência Rural, v.38, n.6, set, 2008.
Susceptibility Testing

Antimicrobial disk susceptibility tests were performed using the disk diffusion method, according to the standards of the Clinical Laboratory Standards Institute (CLSI—formerly National Committee for Clinical Laboratory Standards—NCCLS, 2002, 2003). Drug-impregnated disks (CEFAR, São Paulo, BR) were placed on the surface of the Mueller Hinton agar using a disk dispenser. The following twelve antimicrobial agents were tested: ampicillin (AMP, 10 μg); amoxicillin (AMO, 10 μg), amoxicillin/clavulanic acid (AMC, 30 μg); amikacin (AMK, 30 μg); ceftriaxone (CEF, 30 μg); streptomycin (STR, 10 μg); nalidixic acid (NAL, 30 μg); cotrimoxazole (SUT, 25 μg); ciprofloxacin (CIP, 5 μg), tetracycline (TET, 30 μg). 

Results

A total of 92 E. coli strains were isolated from 25 dogs with diarrhea. All E. coli isolates were investigated by PCR for the presence of Shiga-like toxin-producing genes (stx 1 and stx 2) and of the intimin (eae) gene. As it can be seen from table 1, 21 (22.8%) of the strains carried the stx or the eae genes. PCR showed that 7 (7.6%) of STEC strains carried only the stx 1 gene, 5 (5.4%) the stx 2 gene, and none carried both stx 1 and stx 2 genes carried only the eae gene. Twelve STEC isolates carrying stx 1 or stx 2 genes were isolated from 10 different dogs (10/25 - 40.0%) (results not shown). All STEC strains isolated were tested by the O157 latex agglutination test kit, and not one O157 isolate was detected. There existed a positive correlation between α-hemolysin production and the presence of stx or eae (13/21-62.0%).

Among the 21 isolates carrying the stx or eae genes found, the highest resistance was showed against cephalothin (85.7%), followed by those to streptomycin (81.0%), amoxicillin (71.4%) and gentamicin (71.4%); low resistance to ceftriaxone (0.0%) and to amoxicillin/clavulanic acid (14.4%) was found (Table 2). No one isolate was susceptible to all antimicrobial agents tested.

Discussion

A variety of E. coli strains including the attaching and effacing E. coli (AEEC) and the Shiga toxigenic E. coli (STEC), has been associated with diarrheic illness in dogs (BEUTIN, 1999). Occasional isolation from both healthy and diarrheic dog feces have been reported (BENTANCOR, 2006)

The occurrence of stx genes among diarrheic animals (10/25 - 40.0%) described in the present study, agree with the results reported by HAMMERMULER et al. (1995) showing 44.4% of such an outcome, as well as the presence of stx 1 and stx 2 genes but not of both together, in STEC isolates. Nevertheless, our results contrast with the report (HAMMERMULER et al., 1995) showing the predominance of the stx 2 gene among STEC isolates; in the present study, similar levels of stx 1 (7.6%) and of stx 2 (5.4%) genes were detected (Table 1).

Although the distribution of stx 1 and stx 2 genes found in diarrheic dogs agrees with that of other reports (STAATS et al., 2003; BENTANCOR et al., 2007), NAKAZATO et al. (2004) in Brazil did not find STEC strains carrying stx 1 or stx 2 genes among 146 diarrheic dogs and 36 healthy dogs analyzed. Furthermore, they did not observe a single O157:H7 strain among the animals examined.

The eae gene has been demonstrated in AEEC isolated from dogs, and attaching and effacing lesions have been found in dog intestinal tissue (BEAUDRY et al., 1996). Since the presence of the eae gene correlates with the attaching and effacing phenotype, the detection of this gene in E. coli provides sufficient evidence to indicate potential virulence (NATARO & KAPER, 1998). In the present study, four animals (16.0%) presented AEEC strains with the eae gene, in agreement with the report of NAKAZATO et al. (2004), but it was less then reported by others (BEAUDRY et al., 1996; BENTANCOR et al., 2007).

KRAUSE et al. (2005) reported the isolation from dogs of AEEC strains resembling typical enteropathogenic E. coli (EPEC) (eae + bfpA+), confirming other published data (GOFFAUX et al., 2000; NAKAZATO et al., 2004). Dogs live in close contact with humans and direct transmission of E. coli strains is a probable occurrence. RODRIGUES et al. (2004)
demonstrated cross-infection between a dog and a child that lived in the same home in a city of the state of São Paulo, Brazil.

Hemolytic *E. coli* are a common occurrence both in healthy and in dogs with intestinal extra-intestinal infections (BEUTIN, 1999). α-hemolytic activity was predominant (52.3%), among the STEC isolates examined; however, the significance of α-hemolysin production in strains causing enteric disease in dogs and its impact on potential virulence require further investigation.

Close contact between household pets and humans provides favorable conditions for the transmission of bacteria by direct contact (petting, licking, physical injuries, etc) or through the domestic environment (contamination of food, furnishings, etc). Children are at greater risk than adults, because of their closer physical contact with dogs as well as with household environment contaminated by pets. Bacteria resistant to antimicrobials selected for use in animal pets can reach a human host and exchange their resistance genes with bacteria residing in or on the host or vice versa (GUARDABASSI et al., 2004).

Members of most antimicrobials like tetracyclines, macrolides, lincosamides, aminoglycosides, penicillins and cephalosporins have for long periods been in use both in human and veterinary medicine; the same resistance genes have been identified in bacteria from humans and pet animals (PHILLIPS et al., 2004).

NORMAND et al. (2000) reported the results of analyses of *E. coli* isolates obtained from clinical cases in companion animals (dogs and cats) in the United Kingdom between 1989 and 1997. The percentages of antimicrobial resistance described agree with those reported in the present study, except for gentamicin (2.0%) and enrofloxacin (3.0%). Although the authorization of fluoroquinolones for use in small animal veterinary practice is quite recent in Europe (mid 1990s), resistance to this antimicrobial class is appearing in pet animal bacteria (GUARDABASSI et al., 2004). In the present study the percentage of resistance to ciprofloxacin was high (19.2%); the liberalization of this compound for veterinary use in Brazil is more recent than in Europe, and could indicate its misuse in veterinary practice in Brazil.

CARATTOLI et al. (2005) analyzed 298 *E. coli* isolates from specimens of 204 dogs submitted to routine diagnostic investigation in Italy between 2001 and 2003. The reported percentages of bacterial resistance were quite similar to that found in our study for tetracycline, nalidixic acid, cotrimoxazole and fluoroquinolones but very different regarding gentamicin (8.1%) and amikacin (0.7%). To conclude, the present study showed the presence of STEC and AAEC strains in bacterial isolates from diarrheic dogs, as well as their high level of resistance to antimicrobial agents.

### REFERENCES


Ciência Rural, v.38, n.6, set, 2008.

Table 2 - Antimicrobial susceptibility testing of 21 *E. coli* strains carrying *sts* or *eae* genes (virulence factors) isolated from diarrheic dogs in Ituverava, SP, BR.

<table>
<thead>
<tr>
<th>Antimicrobial drugs</th>
<th>Resistant ( % )</th>
<th>Intermediate ( % )</th>
<th>Sensitive ( % )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>28.5</td>
<td>19.2</td>
<td>52.3</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>71.4</td>
<td>19.0</td>
<td>9.6</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>14.4</td>
<td>9.6</td>
<td>76.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>71.4</td>
<td>4.8</td>
<td>23.8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>0.0</td>
<td>33.3</td>
<td>66.7</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>85.7</td>
<td>14.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>19.2</td>
<td>28.5</td>
<td>52.3</td>
</tr>
<tr>
<td>Cotrimoxazole</td>
<td>38.2</td>
<td>28.5</td>
<td>33.3</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>71.4</td>
<td>9.6</td>
<td>19.0</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>23.8</td>
<td>38.1</td>
<td>38.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>81.0</td>
<td>14.2</td>
<td>4.8</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>38.2</td>
<td>28.5</td>
<td>33.3</td>
</tr>
</tbody>
</table>


Ciência Rural, v.38, n.6, set, 2008.