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Occurrence of *Campylobacter jejuni* and *Campylobacter coli* Biotypes and Antimicrobial Susceptibility in Healthy Dogs in Southern Chile

Heriberto Fernández & Andrés Oval

**ABSTRACT**

**Background:** The thermotolerant species of *Campylobacter*, mainly *C. jejuni* and *C. coli*, are as important agents of human gastroenteritis worldwide, being a serious public health problem. They are widely distributed in the environment and the animal kingdom having as reservoirs a wide variety of animals, including dogs, which in turn can act as a source of infection for humans. The *Campylobacter* isolation rates found in dogs are heterogeneous with few available data in Latin American countries. However *Campylobacter* diarrhea in humans is a self-limited clinical process being antimicrobial treatment not always necessary, it is necessary to know their antimicrobial susceptibility/resistance profiles in clinical and reservoirs isolates, especially in *C. jejuni* and *C. coli* strains isolated from animals close to man as dogs are. The aims of this work were to determine the isolation frequency of *C. jejuni* and *C. coli* and their biotypes in healthy dogs and the susceptibility/resistance profiles of the isolated strains to six antimicrobial drugs.

**Materials, Methods & Results:** Fecal samples from 141 healthy dogs were obtained, seeded into the TEC transport medium. In the laboratory, each sample was plated out on modified Skirrow medium and incubated at 42°C for 48 h under microaerobic conditions. The isolated strains were identified to species and biotype levels through their phenotypic characteristics using the API Campy® procedure (bioMérieux, Marcy/Etoile, France) and the method described by Lior, respectively. The antimicrobial susceptibility patterns to ampicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin and tetracycline were determined by means of the E-test method, considering as the minimal inhibitory concentrations (MICs) the lowest antibiotic concentrations yielding no growth. Ampicillin resistant strains were tested for β-lactamase production with the chromogenic cephalosporin test and the disc diffusion susceptibility test for ampicillin-sulbactam. The recovery rate of *Campylobacter* was 31.2% (*C. jejuni* 22.7%, *C. coli* 8.5%). Three of the four biotypes described for *C. jejuni* and the two described for *C. coli* were found being *C. jejuni* biotype I the most frequent one (53.1%). None of the strains showed resistance to chloramphenicol, erythromycin, gentamicin and tetracycline. Resistant strains were found for ciprofloxacin (6 strains, 13.9%) and ampicillin (4 strains, 9.1%). All the ampicillin resistant strains were found to be β-lactamase producers.

**Discussion:** The *Campylobacter* isolation rate (31.2%) found is not negligible. It may provide an epidemiological insight about the risk of zoonotic infection for individuals maintaining contact with dogs, especially for children. Direct contact with pets is a risk factor for acquiring campylobacteriosis, which was also observed in Latin-American countries. Ampicillin resistance is related to β-lactamase production and was reported in different countries, including Chile. *Campylobacter* resistance to quinolones has been increasingly reported in strains of human and animal origin, especially from animals related to husbandry. The ciprofloxacin resistant strains found in this study probably could be a reflection of their spread to human pets. Because dogs have been identified as a potential source of human *Campylobacter* infections, exposure to animals carrying fluoroquinolone-resistant microorganisms could be a risk factor for acquiring this kind of strains.

**Keywords:** *Campylobacter jejuni*, *Campylobacter coli*, dogs, biotypes, antimicrobial resistance.
INTRODUCTION

The thermotolerant species of *Campylobacter*, mainly *C. jejuni* and *C. coli*, are recognized as important agents of human gastroenteritis worldwide, representing a serious public health problem in industrialized and developing countries [7,12,25].

These species are widely distributed in the environment and the animal kingdom. Their zoonotic characteristics are important in explaining their epidemiological significance, especially if their natural reservoirs are a wide variety of animals, both domestic and free-living; which in turn can act as a source of infection for humans [7,10,12,25]. The dog is one of the domestic animals recognized as *Campylobacter* reservoirs [7,10,12,15,19,25].

In developing, as well as in industrialized countries, the *Campylobacter* isolation frequencies found in dogs are heterogeneous with few available data in Latin American countries [6,10,15,19].

In general, *Campylobacter* diarrhea in humans is a self-limited clinical process with antimicrobial treatment being not always necessary, which is recommended only when there are clinical complications [16,20,25]. However, it is necessary to know the behavior of these bacteria to antibiotics in both clinical and reservoir isolates, especially in *C. jejuni* and *C. coli* strains isolated from animals very close to man as are domestic animals and pets, including dogs [16,20,25].

The aims of this work were to determine the frequency of isolation of *C. jejuni* and *C. coli* and their biotypes in healthy dogs in the city of Valdivia (Southern Chile) establishing the susceptibility/resistance profiles of the isolates to six antimicrobial drugs.

MATERIALS AND METHODS

In this research it were studied 141 apparently healthy dogs which were randomly selected, from those submitted by their owners for vaccination at the Valdivia Health Service Antirabies Vaccination Center (Valdivia City; 39°47' Southern latitude, 73°15' Western latitude). Animals that received antibiotics up to 30 days before sampling and those without a known owner, or even having one but presenting wandering habits, were considered as stray dogs and were not admitted in the study.

A fecal sample was taken from each animal by means of a rectal swab. Each sample was identified with an appropriate code number and seeded into the TEC transport medium [5] and transported to the laboratory. Samples were plated out on modified Skirrow agar plates, which were incubated at 42°C for 48 h under microaerobic conditions [3].

The identification of the species was done with phenotypic tests using the API Campy® procedure1. The biotyping was done using the method described by Lior [14] as adapted for use in our laboratory [3].

The antimicrobial susceptibility patterns of the isolated strains to ampicillin, ciprofloxacin, chloramphenicol, erythromycin, gentamicin and tetracycline were determined by means of the E-test method1, previously used in our laboratory for testing *Arcobacter butzleri* [22]. Minimal inhibitory concentrations (MICs) were defined as the lowest antibiotic concentrations yielding no growth.

The ampicillin resistant strains were tested for beta lactamase production with the chromogenic cephalosporin test [21] and the disc diffusion susceptibility test for ampicillin-sulbactam [13].

RESULTS

As showed in Table 1, from the 141 dogs studied, 44 (31.2%) showed positive cultures for *Campylobacter*. The isolated species were *C. jejuni* [32 (22.7%)] and *C. coli* [12 (8.5%)].

All the 44 *Campylobacter* strains were amenable for biotyping. Three of the four biotypes described by Lior [14] for *C. jejuni* and the two biotypes described for *C. coli* were found (Table 1).

<table>
<thead>
<tr>
<th>ISOLATED SPECIES</th>
<th>N°</th>
<th>(%)</th>
<th>BIOTYPES</th>
<th>N°</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter jejuni</em></td>
<td>32</td>
<td>22.7</td>
<td>I</td>
<td>17</td>
<td>53.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td>14</td>
<td>43.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>III</td>
<td>1</td>
<td>3.1</td>
</tr>
<tr>
<td><em>Campylobacter coli</em></td>
<td>12</td>
<td>8.5</td>
<td>I</td>
<td>3</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>II</td>
<td>9</td>
<td>75.0</td>
</tr>
<tr>
<td>TOTAL</td>
<td>44</td>
<td>31.2</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Percentage in relation to all the samples studied; 2Percentage in relation to all *C. jejuni* isolated; 3Percentage in relation to all *C. coli* isolated.
None of the strains showed resistance to chloramphenicol, erythromycin, gentamicin and tetracycline. Resistance to ampicillin and ciprofloxacin was detected in 4 (9.1%) and 6 (13.6%) respectively. All the ampicillin resistant strains were beta lactamase producers (Table 2).

Table 2. Minimal inhibitory concentrations (MICs) of 44 Campylobacter strains isolated from healthy dog feces to six antimicrobial agents using the E-test method.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Range</th>
<th>Resistance (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>3.0</td>
<td>8.0</td>
<td>0.25 - 32.0</td>
<td>9.1*</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.38</td>
<td>1.5</td>
<td>0.047 - 6.0</td>
<td>0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.75</td>
<td>4.0</td>
<td>0.25 - 6.0</td>
<td>0</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.75</td>
<td>2.0</td>
<td>0.016 - 3.0</td>
<td>0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.032</td>
<td>0.125</td>
<td>0.047 - &gt;32</td>
<td>13.6</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.064</td>
<td>0.38</td>
<td>0.016 - 1.0</td>
<td>0</td>
</tr>
</tbody>
</table>

*beta lactamase producer strains.

DISCUSSION

The recovery rate of Campylobacter from dogs in this study (31.2%) was higher than that obtained by us (21.9%) in a previous survey in Chile [4], as well as those obtained by others in Argentina (17%) [15], Peru (25%) [11] and Brazil (27.7%) [18]. However, the present recovery rate of Campylobacter was lower than that obtained in Ireland (41.5%) [1], Barbados (46.9%) [24] and also in two other studies conducted by our group in children and animals in Chile (42.5% ; 58.3%) [8,10].

In this study, the predominant Campylobacter species was C. jejuni followed by C. coli. This distribution of species seems to be normal because, as reported in other studies, C. jejuni is always more frequent than C. coli in dogs [3,4,6-8,10,11,15].

As shown in Table 1, three of the four biotypes described by Lior [14] for C. jejuni and the two biotypes described for C. coli were found. Among C. jejuni isolates, biotype I was the most frequent one (53.1%) followed by biotypes II (43.8%) and III (3.1%). C. coli biotype II was more frequently isolated (75%) than biotype I (25%). Similar results were previously reported by our group in pet dogs but, in stray dogs, all the biotypes described by Lior [14] were found [4]. This difference could be due to the fact that stray dogs may be more exposed to environmental sources of Campylobacter than household pets are.

The Campylobacter isolation rate for asymptomatic dogs reported here is relatively high and should to be considered as not negligible; rather it may provide an epidemiological insight about the risk of zoonotic infection for individuals maintaining contact with dogs, especially for children. Evans [2] and Mahdi-Saed et al. [17] reported that direct contact with pets is a risk factor for acquiring campylobacteriosis in children, which has also observed in Latin-American countries [6-8].

In the present study, no strain was found to be resistant to chloramphenicol, tetracycline, gentamicin and erythromycin. The latter is the drug of choice to treat Campylobacter enteritis and gentamicin, the antimicrobial agent indicated to treat extra-intestinal infections due to Campylobacter [16,19,20,25].

In Brazil, Modolo et al. [19] did not find resistance for erythromycin, gentamycin, enrofloxacin and chloramphenicol, but they found high resistance to tetracycline. From the 44 strains studied, four (9.1%) were resistant to ampicillin. All of them were able to produce ß-lactamase. The presence of Campylobacter ß-lactamase producer strains is known in different countries and was reported for the first time in Chile by Fernández et al. [9].

Over the past decade, resistance to quinolones in Campylobacter has been increasingly reported in strains isolated from both human beings and animals, especially from animals related to husbandry [23]. The 13.9% of resistant strains to ciprofloxacin found in this study probably could be a reflection of the spread of these kind of strains from food-producing animals and the environment to human pets. Given that dogs have been identified as a potential source of human infections [2,7,10,12,15,17,19,25], exposure to animals carrying fluoroquinolone-resistant microorganisms could be another risk factor for acquiring fluoroquinolone-resistant Campylobacter strains.
CONCLUSION

This study provides an insight into the intestinal carriage of Campylobacter jejuni and C. coli biotypes in healthy dogs and their antimicrobial susceptibility behavior in Southern Chile. Due to the epidemiological importance of dogs as reservoirs and to the presence of Campylobacter strains resistant to ampicillin and ciprofloxacin, it is necessary to submit these pets to periodic monitoring for Campylobacter and their antimicrobial resistance.

REFERENCES