The majority of cases of human campylobacteriosis in developed countries are most probably caused by consumption of undercooked poultry, raw milk, or untreated surface water, while the remaining incidences may be assigned to a multitude of other sources (Hussain et al., 2007; Westgarth et al., 2008). There is evidence of increased risk of *Campylobacter* infection in humans associated with dog or pet ownership (Tenkate and Stafford, 2001) with studies indicating an association between *C. jejuni* and *C. upsaliensis* (Lentzsch et al., 2004) infection in humans and dogs in the same household (Westgarth et al., 2008).

Domesticated pets are known to harbor *Campylobacter* spp. in their digestive tracts, with...
incidences ranging from 1% to as much as 92% of stool samples when evaluated and characterized by either culture, polymerase chain reaction (PCR), or pulse-field gel electrophoresis (PFGE) (Hald et al., 2004; Workman et al., 2005; Acke et al., 2006).

Campylobacter is regarded as a possible cause of diarrhea in dogs and cats, although prevalence studies, experimental infections and response to antibiotic therapy have been inconclusive (Hald and Madsen, 1997; Sandberg et al., 2002). Antimicrobial therapy for dogs with clinical campylobacteriosis reduces the duration and severity of the disease, decreases microorganism shedding time, minimizing the risk of human exposure to the bacteria (Boosinger and Dillon, 1992; Modolo et al., 2003). Antimicrobial resistance studies of Campylobacter strains isolated from dogs and cats are rare. Surveillance and timely reporting of antimicrobial resistance patterns in Campylobacter spp. may provide important information to support actions directed at reducing the occurrence of resistance.

Such information is important for epidemiological purposes and could help in assessing the role of Campylobacter as a pathogen in these animals. Currently, there is limited information regarding the prevalence and antimicrobial resistance of Campylobacter in pet animals in Iran. The present study was conducted to determine the antimicrobial resistance of Campylobacter spp. isolated from dog and cat fecal samples in Fars and Isfahan provinces, Iran.

MATERIALS AND METHODS

Source of isolates

From August 2010 to February 2011, a total of 173 samples of fresh feces from pet dogs (n = 126) and cats (n = 47) were collected. The animals included in this study were randomly selected between adult (>1 year) and younger than 1 year by the owners in Fars and Isfahan provinces, Iran.

Microbiological analysis

The samples were processed immediately upon arrival using aseptic techniques. Approximately 5 g of feces were homogenized in 45 ml of Preston enrichment broth base containing Campylobacter selective supplement IV (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood. After inoculation at 42°C for 24 h in a microaerophilic condition (85% N₂, 10% CO₂, 5% O₂), 0.1 mL of the enrichment was then streaked onto Campylobacter selective agar base (HiMedia Laboratories, Mumbai, India) supplemented with an antibiotic supplement for the selective isolation of Campylobacter species (HiMedia Laboratories, Mumbai, India) and 5% (v/v) defibrinated sheep blood and incubated at 42°C for 48 h under the same condition. One presumptive Campylobacter colony from each selective agar plate was subcultured and identification of presumptive Campylobacter species was performed using standard microbiological and biochemical procedures (Bolton et al., 1992; Misawa et al., 2000). Only Campylobacter spp. isolates identified by bacteriological methods were tested by PCR. The isolates underwent genus specific PCRs for Campylobacter (Linton et al., 1996). The isolates were identified at the species level by C. jejuni, and C. coli (Denis et al., 1999), C. upsaliensis, and C. helveticus specific duplex PCR (Lawson et al., 1997). The isolates were then subjected to disc diffusion testing according to the Clinical Laboratory Standards Institute (CLSI, 2006).

Antimicrobial susceptibility testing

One strain from each Campylobacter-positive sample was selected for susceptibility tests. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method using Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) supplemented with 5% defibrinated sheep blood, according to the Clinical Laboratory Standards Institute (CLSI, 2006). The following antimicrobial impregnated disks (HiMedia Laboratories, Mumbai, India) were used: nalidixic acid (30 µg), ciprofloxacin (15 µg), erythromycin (15 µg), tetracycline (15 µg), streptomycin (30 µg), gentamicin (10 µg), amoxicillin (30 µg), ampicillin (10 µg), chloramphenicol (30 µg), and enrofloxacin (10 µg). After incubation at 42°C for 48 h in a microaerophilic atmosphere, the susceptibility of the Campylobacter spp. to each antimicrobial agent was measured and the results were interpreted in accordance with interpretive criteria provided by CLSI (2006). Staphylococcus aureus and Escherichia coli were used as quality control organisms in antimicrobial susceptibility determination. The antimicrobial agents tested in
this study are widely used to treat infections in people and in food animals in Iran.

Statistical analysis

Data were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA, USA) for analysis. Using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA), chi-square test and fisher’s exact two-tailed test analysis were performed and differences were considered significant at values of $P < 0.05$.

RESULTS

In this study, 61 of 173 fecal samples (35.3%) were found to be contaminated with Campylobacter. The most prevalent Campylobacter isolated from canine samples was C. upsaliensis (52.1%), followed by C. jejuni (37.5%) and C. coli (10.4%). The most prevalence Campylobacter species isolated from cat samples was C. helveticus (61.5%); the remaining isolates were C. jejuni (30.8%) and C. upsaliensis (7.7%).

The resistance prevalence of Campylobacter isolates to 10 antimicrobial agents tested in this study is shown in Table 1. More than half of the Campylobacter isolates (62.3%; $n=38$) were resistant to one or more antimicrobial agent. Thirteen strains (21.3%) were resistant to one single antibiotic and 16 strains (26.2%) showed resistance to two antimicrobial agents. Multiresistance which was defined as resistance to three or more of the tested drugs was found in 16.4% of Campylobacter strains. Resistance to ciprofloxacin was the most common finding (41.0%), followed by resistance to tetracycline (39.3%), and nalidixic acid (34.4%). All of the Campylobacter spp. isolates were susceptible to chloramphenicol, and erythromycin.

Table 1. Antimicrobial resistance of Campylobacter strains isolated from fecal samples from dogs and cats

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Total No. of Campylobacter spp. $(N = 61)$</th>
<th>C. upsaliensis $(N = 26)$</th>
<th>C. jejuni $(N = 22)$</th>
<th>C. helveticus $(N = 8)$</th>
<th>C. coli $(N = 5)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>3 (4.9%)</td>
<td>2 (7.7%)</td>
<td>1 (4.5%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>6 (9.8%)</td>
<td>4 (15.4%)</td>
<td>1 (4.5%)</td>
<td>0 (0.0%)</td>
<td>1 (20.0%)</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>25 (41.0%)</td>
<td>10 (38.5%)</td>
<td>10 (45.5%)</td>
<td>3 (37.5%)</td>
<td>2 (40.0%)</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>10 (16.5%)</td>
<td>4 (15.4%)</td>
<td>3 (13.6%)</td>
<td>3 (37.5%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1 (1.6%)</td>
<td>0 (0.0%)</td>
<td>1 (4.5%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>21 (34.4%)</td>
<td>10 (38.5%)</td>
<td>21 (62.1%)</td>
<td>3 (37.5%)</td>
<td>2 (40.0%)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>3 (4.9%)</td>
<td>2 (7.7%)</td>
<td>1 (4.5%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>24 (39.3%)</td>
<td>9 (34.6%)</td>
<td>9 (40.9%)</td>
<td>4 (50.0%)</td>
<td>2 (40.0%)</td>
</tr>
</tbody>
</table>

DISCUSSION

Antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments, since drug resistance in zoonotic microbes has harassed therapeutic intervention in humans; antimicrobial resistance in food-borne pathogens has become a public health issue. Therapeutic, prophylactic and not at least the use of anti-infectives in animals for growth promotion have raised questions about the development of resistant microbes in animals. Some studies showed that approximately 6% of human enteric campylobacteriosis is transmitted from pets (Tenkate and Stafford, 2001) and that these animals represent potential sources of the spread of antimicrobial resistance due to their close contact with humans (Guardabassi et al., 2004). Direct evidence of the transmission of fluoroquinolone resistant C. jejuni between humans and pets living in the same households has also been shown (Damborg et al., 2004). Alarmingly, similar to other reports (Sandberg et al., 2002; Modolo et al., 2003; Rossi et al., 2008; Cokal et al. 2009) high resistance rates were observed for ciprofloxacin, nalidixic acid, tetracycline, and enrofloxacin. During the past
decade, fluoroquinolones have been the principal agents in the prophylaxis and treatment of enteric infections. Unfortunately, there has been a rapid emergence of quinolone resistance amongst Campylobacter spp. isolates all around the world (Cokal et al., 2009). All of the Campylobacter isolates were susceptible to chloramphenicol, and erythromycin, and only one to three isolates were resistance to gentamicin, amoxicillin, and streptomycin in Campylobacter isolates (Table 1). These results are comparable to those reported by other investigators (Sandberg et al., 2002; Modolo et al., 2003; Rossi et al., 2008). Erythromycin and chloramphenicol, use in treating dogs and cats, but were not able to eliminate the passive carrier status of some treated dogs (Monfort et al., 1990; Boosinger and Dillon, 1992; Burnens et al., 1992).

However, some studies have reported high resistance to the chloramphenicol when considering strains isolated from humans and dogs (Modolo et al., 1991). These data probably reflect the extensive use of these antibiotics in dogs and cats in Iran. Also, the results show that erythromycin, and gentamicin can be recommended for the treatment of campylobacteriosis in dogs and cats; although the in vivo effectiveness has not been tested; this opens the possibility of their therapeutic use in human medicine. To reduce resistance rates in these pathogens, surveillance, research and the use of alternatives to antimicrobial treatment like vaccination are recommended.

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REFERENCES


