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Antimicrobial Resistance of *Campylobacter* Species Isolated from Fecal Samples from Cats and Dogs in Iran

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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. In this study the antimicrobial resistance of *Campylobacter* spp. isolated from 173 samples of fresh feces from pet dogs (n = 126) and cats (n = 47) were investigated . Isolates were tested for susceptibility to antimicrobial drugs by the Kirby-Bauer disk diffusion method using Mueller-Hinton agar according to the Clinical Laboratory Standards Institute. In this study, 61 of 173 fecal samples (35.3%) were found to be contaminated with *Campylobacter*. *Campylobacter* spp. were isolated from 48 dogs (38.1%) and from 13 cats (27.7%). Susceptibilities of 61 *Campylobacter* isolates were determined for 10 antimicrobial drugs. Most of the *Campylobacter* isolates (62.3%; n=38) were resistant to one or more antimicrobial agents. Resistance to ciprofloxacin was the most common finding (41.0%), followed by resistance to tetracycline (39.3%), and nalidixic acid (34.4%). None of the dogs and cats isolates was resistant to chloramphenicol, and erythromycin. To reduce resistance rates in these pathogens, surveillance, research and the use of alternatives to antimicrobial treatment like vaccination are recommended.

Key words: Antimicrobial resistance, Dogs, *Campylobacter*, Cats.

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

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incidences ranging from11% 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% N_2, 10% CO_2, 5% O_2), 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

Antimicrobial agent	Total No. of *Campylobacter* spp. (N = 61)	*C. upsaliensis* (N = 26)	*C. jejuni* (N = 22)	*C. helveticus* (N = 8)	*C. coli* (N = 5)
Amoxicillin	3 (4.9%)	2 (7.7%)	1 (4.5%)	0 (0.0%)	0 (0.0%)
Ampicillin	6 (9.8%)	4 (15.4%)	1(4.5%)	0 (0.0%)	1 (20.0%)
Chloramphenicol	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ciprofloxacin	25 (41.0%)	10 (38.5%)	10 (45.5%)	3 (37.5%)	2 (40.0%)
Enrofloxacin	10 (16.5%)	4 (15.4%)	3 (13.6%)	3 (37.5%)	0 (0.0%)
Erythromycin	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Gentamicin	1 (1.6%)	0 (0.0%)	1 (4.5%)	0 (0.0%)	0 (0.0%)
Nalidixic acid	21 (34.4%)	10 (38.5%)	6 (23.1%)	3 (37.5%)	2 (40.0%)
Streptomycin	3 (4.9%)	2 (7.7%)	1 (4.5%)	0 (0.0%)	0 (0.0%)
Tetracycline	24 (39.3%)	9 (34.6%)	9 (40.9%)	4 (50.0%)	2 (40.0%)

DISCUSSION

Antimicrobial resistance has always been a major concern for nosocomial infections in hospital environments, since drug resistance in zoonotic microbes has harassed therapeutical 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

1. Acke E, P Whyte, Jones BR, McGill K, Collins JD, Fanning S., Prevalence of thermophilic *Campylobacter* species in cats and dogs in two animal shelters in Ireland. *Vet. Rec.,* 2006; **158**: 51–54.
2. Bolton FJ, Wareing DR, Skirrow MB, Hutchinson DN., Identification and biotyping of *Campylobacter*. In: Board GR, Jones D, Skinner FA (Eds.), Identification Methods in Applied and Environmental Microbiology. Society for Applied Microbiology, Technical Series 29, Blackwell Scientific Publications, Oxford, 1992; 151-161.
3. Boosinger TR, Dillon AR., *Campylobacter jejuni* infections in dogs and the effect of erythromycin and tetracycline therapy on fecal shedding. *J. Am. Anim. Hosp. Assoc.,* 1992; **28**: 33-38.
4. Burnens AP, Angeloz-Wick B, Nicolet J., Comparison of *Campylobacter* carriage rates in diarrheic and healthy pet animals. *Zentralbl Vet. B.,* 1992; **39**: 175–180.
5. Clinical and Laboratory Standards Institute, Performance Standards for Antimicrobial Disk Susceptibility Tests, Approved standard-Ninth Edition (M2-A9). Clinical and Laboratory Standards Institute, Wayne, PA 2006.
6. Cokal Y, Caner V, Sen A, Cetin C, Karagenc N., *Campylobacter* spp. and their antimicrobial resistance patterns in poultry: An epidemiological survey study in Turkey. *Zoonosis Public Health*, 2009; **56**(3):105-110.
7. Damborg P, Olsen KE, Moller Nielsen E, Guardabassi L., Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. *J. Clin. Microbiol*., 2004; **42**: 1363–1364.
8. Denis M, Soumet C, Rivoal K, Ermel G, Blivet D, Salvat G, Colin P., Development of a m-PCR assay for simultaneous identification of *Campylobacter jejuni* and *C. coli*. *Lett. Appl. Microbiol*., 1999; **29**: 406–410.
9. Guardabassi L, Schwarz S, Lloyd DH., Pet animals as reservoirs of antimicrobial-resistant bacteria. *J. Antimicrob. Chemother.,* 2004; **54**: 321–332.
10. Hald B, Madsen M., Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. *J. Clin. Microbiol.,* 1997; **35**: 3351–3352.
11. Hald B, Pedersen K, Waino M, Jorgensen JC, Madsen M., Longitudinal study of the excretion patterns of thermophilic *Campylobacter* spp. in young pet dogs in Denmark. *J. Clin. Microbiol*., 2004; **42**: 2003–2012.
12. Hussain I, Mahmood MS, Akhtar M, Khan A., Prevalence of *Campylobacter* species in meat, milk and other food commodities in Pakistan. *Food Microbiol*., 2007; **24**: 219-222.
13. Lawson AJ, Linton D, Stanley J, Owen RJ., Polymerase chain reaction detection and speciation of *Campylobacter upsaliensis* and *C. helveticus* in human faeces and comparison with culture techniques. *J. Appl. Microbiol.,* 1997; **83**: 375-380.

14. Lentzsch P, Rieksneuwohner B, Wieler LH, Hotzel H, Moser I., Highresolution genotyping of *Campylobacter upsaliensis* strains originating from three continents. *J. Clin. Microbiol*., 2004; **42**: 3441-3448.
15. Linton D, Owen RJ, Stanley J., Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res. Microbiol*., 1996; **147**: 707–718.
16. Misawa N, Shinohara S, Satoh H, Itoh H, Shinohara K, Shimomura K, Kondo F, Itoh K., Isolation of *Campylobacter* species from zoo animals and polymerase chain reaction-based random amplified polymorphism DNA analysis. *Vet. Microbiol*., 2000; **71**: 59-68.
17. Modolo JR, Giuffrida R, Lopes CA, De M., Antimicrobial susceptibility of 51 *Campylobacter* strains isolated from diarrheic and diarrhea-free dogs. *Arquivos Instituto Biologico.,* 2003; **70**: 283-286.
18. Monfort JD, Donahue JP, Stills FH, Beech-Nielsen SH., Efficacies of erytromycin and chloramphenicol in extinguishing fecal shedding of *Campylobacter jejuni* in dogs. *J. Am. Vet. Med. Assoc*., 1990; **1**: 1069-1072.
19. Rossi M, Ha¨nninen ML, Revez J, Hannula M, Zanoni RG., Occurrence and species level diagnostics of *Campylobacter* spp., enteric *Helicobacter* spp. and *Anaerobiospirillumn* spp. in healthy and diarrheic dogs and cats. *Vet Microbiol.,* 2008; **129**: 304-314.
20. Sandberg M, Bergsjo B, Hofshagen M, Skjerve E, Kruse H., Risk factors for *Campylobacter* infection in Norwegian cats and dogs. *Prevent. Vet. Med.,* 2002; **55**: 241–253.
21. Tenkate TD, Stafford RJ., Risk factors for *Campylobacter* infection in infants and young children: a matched case-control study. Epidemiol. Infect., 2001; **127**: 399-404.
22. Westgarth C, Pinchbeck GL, Bradshaw JW, Dawson S, Gaskell RM, Christley RM, Dog-human and dog–dog interactions of 260 dog-owning households in a community in Cheshire. *Vet. Rec*., 2008; **162**: 436–442.
23. Workman SN, Mathison GE, Lavoie MC., Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *J. Clin. Microbio.,* 2005; **43**: 2642–2650.