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Original Scientific Article

PREVALENCE OF *CAMPYLOBACTER* SPECIES IN FECAL SAMPLES FROM CATS AND DOGS IN IRAN

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Summary: *Campylobacter* spp. are one of the most frequent pathogens of acute bacterial gastroenteritis in human beings. The present study was conducted to determine the prevalence of *Campylobacter* spp. from dog and cat fecal samples in Iran. From August 2010 to August 2011, a total of 173 samples of fresh feces from pet dogs (n = 126) and cats (n = 47) were collected by the owners in Fars and Isfahan provinces, Iran. 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%). Twenty-five C. upsaliensis, 18 *C. jejuni*, 5 *C. coli* isolates from dogs and 1 *C. upsaliensis*, 8 *C. helveticus*, 4 *C. jejuni*, isolates from cats were identified using both the cultural method and the PCR assay. The prevalence of *Campylobacter* in adult dogs (29.6%) was lower than in young dogs (49.1%). However, there were not significant differences in the prevalence of *Campylobacter* between adult (29.4%) and juveniles cats (26.7%). Also, no statistically significant correlation was found between the isolation of *Campylobacter*, and the presence of gastroenteric disorders, in either dogs or cats. To the authors' knowledge, the present study is the first report on the prevalence of *Campylobacter* in dogs and cats in Iran.

Key words: dogs; *Campylobacter*; cats; zoonosis

Introduction

The family Campylobacteriaceae comprises small, spiral form, Gram-negative bacteria with 25 species and 11 sub-species (1, 2). They are essentially microaerophilic, growing best in an atmosphere containing approximately 10% CO_2 and approximately 5% O_2. *Campylobacter* species, in particular *C. jejuni* and *C. coli*, are considered to be the most frequent bacterial cause of human enteritis but in a small proportion of cases *C. upsaliensis* has been reported (3, 4). *Campylobacter* species are widely distributed in nature and have been associated with poultry, pigs, cattle, sheep, shellfish, dogs and cats (5, 6). Consumption of undercooked meat, unpasteurized milk, and contaminated drinking water is considered an important risk factor for campylobacteriosis (7, 8). Cross-contamination of ready to eat foods during food preparations with *Campylobacter* spp. as well as direct contact with pet animals have been reported (4, 8). There is evidence of increased risk of *Campylobacter* infection in humans associated with dog or pet ownership (6, 9) with studies indicating an association between *C. jejuni* (10), and *C. upsaliensis* (11) infection in humans and dogs in the same household. Dogs are regarded as important reservoir for *C. upsaliensis,* and cats were shown to be carriers of *C. helveticus* (5, 12) a thermophilic *Campylobacter* species which is difficult to differentiate from *C. upsaliensis* by biochemical tests (5, 12). The development of more sensitive detection methods has allowed for more accurate detection, isolation, and classification of *Campylobacter* spp. These advances in surveillance technology have provided improved information on the prevalence of *Campylobacter* spp. worldwide and now demonstrate that this

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pathogen can be interspecies specific rather than just limited to warm blooded hosts as was once thought (5).

Such information is important for epidemiological purposes and could help in assessing the role of *Campylobacter* as a pathogen in these animals. Campylobacter has been reported in dogs and cats in some countries of the world (2, 4-12) and campylobacters in cat and dog populations are of concern for the animals themselves and for members of the public on account of the possible risks of zoonotic infection. Currently, there is limited information regarding the prevalence of *Campylobacter* in pet animals in Iran. The present study was conducted to determine the prevalence of *Campylobacter* spp. in dog and cat fecal samples in Fars and Isfahan provinces, Iran.

Materials and methods

Sample collection

From August 2010 to August 2011, a total of 173 samples of fresh feces from pet dogs (n = 126) and cats (n = 47) were collected by the owners in Fars and Isfahan provinces, Iran. All samples were placed in separate sterile plastic bags to prevent spilling and cross contamination and were immediately transported to the laboratory in a cooler with ice packs. Age distribution of animals was as follows: 71 dogs were adult (>12 months), 55 dogs were younger than 1 year. Cat samples were obtained from 17 adult and 30 juveniles were provided. Diarrhea was reported in 38 dogs and 11 cats. The remaining animals had no clinical signs reported by their owner.

Microbiological analysis

The samples were processed immediately upon arrival and at latest six hours after sampling, 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 Preston 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 including Gram staining, production of catalase, oxidase, hippurate hydrolysis, urease activity, indoxyl acetate hydrolysis, growth in the presence of 1% (w/v) glycine and 0.04% (w/v) 2,3,5-triphenyltetrazolium chloride (TTC), H2S production in triple sugar iron (TSI) agar and susceptibility to cephalotin (13, 14).

DNA extraction and identification of Campylobacter species

Only *Campylobacter* spp. isolates identified by bacteriological methods were tested by PCR. Briefly, 1 mL of pure culture of *Campylobacter* was centrifuged at 13000 g for 5 min at room temperature. The DNA was then extracted using a genomic DNA purification kit (Fermentas, GmbH, Germany, K0512) according to the manufacturer's protocol. The isolates underwent genus specific PCRs for *Campylobacter* (15). The isolates were identified at the species level by *C. jejuni,* and *C. coli* specific multiplex PCR (16), *C. upsaliensis,* and *C. helveticus* specific duplex PCR (17).

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

Table 1 shows the prevalence of *Campylobacter* spp. isolated from 173 samples of fresh feces from pet dogs and cats in Fars and Isfahan provinces, Iran. Overall, 61 of 173 fecal samples (35.3%) were positive for *Campylobacter* spp. using both the cultural method and the PCR assay. *Campylobacter* spp. were isolated from 48 dogs

Table 1: Prevalence of Campylobacter spp. from dogs and cats using both the cultural method and the PCR assay

Samples	No. of samples	*Campylobacter* spp. positive*	*C. upsaliensis*	*C. helveticus*	*C. jejuni*	*C. coli*
Dogs	126	48 (38.1) [a]	25 (52.1) [a]	0 (0.0) [a]	18 (37.5) [a]	5 (10.6) [a]
Cats	47	13 (27.7) [b]	1 (7.7) [b]	8 (61.5) [b]	4 (30.8) [a]	0 (0.0) [b]
Total	173	61 (35.3)	26 (42.6)	8 (13.1)	22 (36.1)	5 (8.2)

* Results expressed as the number of Campylobacter-positive samples / number of samples analyzed (%).
[a, b] Values in the same column with different superscripts are significantly different (P < 0.05).

Table 2: Prevalence of Campylobacter spp. isolated from healthy and diarrheic dogs and cats using both the cultural method and the PCR assay

Samples		No. of samples	*Campylobacter* spp. positive*	*C. upsaliensis*	*C. helveticus*	*C. jejuni*	*C. coli*
Dogs	Healthy	88	19 (52.8)*[a]	0 (0.0) [a]	13 (36.1) [a]	13 (36.1) [a]	4 (11.1) [a]
	Diarrhoeic	38	6 (50.0) [a]	0 (0.0) [a]	5 (41.7) [a]	5 (41.7) [a]	1 (8.3) [a]
Cats	Healthy	36	0 (0.0) [a]	7 (70.0) [a]	3 (30.0) [a]	3 (30.0) [a]	0 (0.0) [a]
	Diarrhoeic	11	1 (33.3)[b]	1 (33.3) [b]	1 (33.3) [a]	1 (33.3) [a]	1 (33.3) [b]

* Results expressed as the number of Campylobacter-positive samples / number of samples analyzed (%)
a, b In each column values with no common superscripts are significantly different (P < 0.05)

Table 3: Prevalence of Campylobacter spp. isolated from young and adult dogs and cats using both the cultural method and the PCR assay

Samples		No. of samples	*Campylobacter* spp. positive	*C. upsaliensis*	*C. helveticus*	*C. jejuni*	*C. coli*
Dogs	Adult*	71	21 (29.6)**[a]	9 (42.9) [a]	0 (0.0) [a]	8 (38.1) [a]	4 (19.0) [a]
	Young	55	27 (49.1) [b]	16 (59.3) [a]	0 (0.0) [a]	10 (37.0) [a]	1 (37.0) [b]
Cats	Adult	17	5 (29.4) [a]	1 (20.0) [a]	2 (40.0) [a]	2 (40.0) [a]	0 (0.0) [a]
	Young	30	8 (26.7) [a]	0 (0.0) [b]	6 (75.0) [b]	2 (25.0) [a]	0 (0.0) [a]

* Adult (> 12 months), Young (< 12 months)
** Results expressed as the number of Campylobacter-positive samples / number of samples analyzed (%)
a, b In each column values with no common superscripts are significantly different (P < 0.05)

Table 4: Seasonal prevalence of Campylobacter spp. isolated from dogs and cats using both the cultural method and the PCR assay

Season	Fecal samples*		Total
	Dogs	Cats	
Summer	17/40 (42.5)	4/14 (28.6)	21/54 (38.9)
Fall	11/38 (36.8)	3/10 (30.0)	14/48 (29.2)
Winter	9/24 (37.5)	2/8 (25.0)	11/32 (34.4)
Spring	11/24 (45.8)	4/15 (26.7)	15/39 (38.5)

* Results expressed as the number of Campylobacter-positive samples / number of samples analyzed (%)

Table 4 shows the seasonal prevalence of *Campylobacter* spp. in dog and cat fecal samples. The highest prevalence of *Campylobacter* spp. occurred in summer (38.9%) followed by spring (38.5%). The prevalence rates of *Campylobacter* spp. in fall and winter were 29.2% and 34.4%, respectively. No significant differences in the prevalence rates of *Campylobacter* spp. were observed for dog and cat fecal samples taken in different seasons in Isfahan, and Fars provinces, Iran.

(38.1%) and from 13 cats (27.7%). There were not significant differences (P > 0.05) in the prevalence of *Campylobacter* between different fecal samples. The most prevalent *Campylobacter* species 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%). No statistically significant correlation was found between the isolation of *Campylobacter*, and the presence of gastroenteric disorders, in either dogs or cats (Table 2). Also, no significant differences in the prevalence rates of *Campylobacter* spp. were observed between fecal samples isolated in Fars and Isfahan provinces (data not shown). In this study the prevalence of *Campylobacter* in adult dogs (29.6%) was lower than in young dogs (49.1%) (P < 0.05). However, there were not significant differences (P > 0.05) in the prevalence of *Campylobacter* between adult (29.4%) and juveniles cats (26.7%) (Table 3).

Discussion

The prevalence rate of *Campylobacter* spp. in dog and cat fecal samples was 38.1% and 27.7% respectively, which is comparable with those reported from Denmark, Norweg, Switzerland, Italy, Nigeria, The UK (3, 6, 12, 18-20); however, higher prevalence rates have been reported by others (21-24). *C. upsaliensis* was the most frequently isolated species in dogs and *C. helveticus* from cats while the isolation rates of *C. jejuni* were similar in both animals. The prevalence of dogs carrying *Campylobacter* spp. varies widely, depending on the population sampled and probably also on the detection methods used (3, 18-20, 23, 25). Frequently, *C. upsaliensis* has been found to be the most common species isolated from dogs (6, 19, 20, 25), although in other studies, *C. jejuni* predominated (18, 26, 27). In any case, cats predominantly carry *C. helveticus* rather than *C. upsaliensis* (3, 20, 22, 26).

When age was investigated as a risk indicator for *Campylobacter* spp. carriage in dogs, the majority of studies found that younger rather than older dogs were more likely to carry *C. upsaliensis* and *C. jejuni* (3, 4, 6, 19, 21, 23). Similar to other studies, we found that younger dogs were more likely to be carriers of *C. upsaliensis* than older dogs and that this is probably a consequence of age-related immunity. However, a small number of reports have suggested that age is not a risk indicator for *C. jejuni* infection (3, 12, 20, 27). There was no statistically significant association between *Campylobacter* carrier status and clinical history or signs as has been reported by others (6, 19-21, 23, 26); however, higher prevalence rates in diarrheic cat rather than and healthy cat have been reported by Queen et al. (22).

Although various outbreak and seasonal peak of *Campylobacter* have been reported in the warmer months (5), in our study no apparent pattern in the seasonality of *Campylobacter* prevalence was observed. This observation is in agreement with the findings reported by Hudson et al. (7).

The high prevalence of *Campylobacter* carriers found in dogs and cats in this and previous studies suggests the bacteria may be intestinal commensals in this species. Although the relationship between the presence of *C. upsaliensis* and gastroenteritis in both dogs and humans is still unclear, it is worth highlighting that younger dogs in particular may pose a zoonotic risk (4). However the prevalence of *C. jejuni*, the most common *Campylobacter* spp. associated with disease in humans, was the second most common *Campylobacter* species isolated from dogs and cats in our study. To establish the zoonotic potential of canine *Campylobacter* isolates, both human and canine isolates have to be further characterized and compared. To the authors' knowledge, the present study is the first report on the prevalence of *Campylobacter* in dogs and cats in Iran.

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PREVALENCA BAKTERIJ VRSTE KAMPILOBAKTER V VZORCIH BLATA PSOV IN MAČK V IRANU

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Povzetek: Okužba z bakterijami kampilobakter *(Campylobacter spp.)* je eden izmed najpogostejših povzročiteljev akutnega bakterijskega gastroenteritisa pri ljudeh. Namen naše raziskave je bil določiti razširjenost bakterij *Campylobacter spp.* v iztrebkih psov in mačk v Iranu. Od avgusta 2010 do avgusta 2011 smo s pomočjo lastnikov zbrali 173 vzorcev svežih iztrebkov hišnih psov (n = 126) in mačk (n = 47) v provincah Fars in Isfahan v Iranu. V 61 vzorcih (35,3 %) smo potrdili prisotnost bakterij kampilobakter, in sicer pri 48 vzorcih psov (38,1 %) in 13 vzorcih mačk (27,7 %). Posamezne vrste bakterij kampilobakter smo določili z mikrobiološko metodo in metodo PCR in pri psih ugotovili *C. upsaliensis* v 25 vzorcih, *C. jejuni* v 18 in *C. coli* v 5. Pri mačkah smo potrdili *C. upsaliensis* v enem vzorcu, *C. helveticus* v 8 in *C. jejuni* v 4 vzorcih. Razširjenost bakterij kampilobakter pri odraslih psih (29,6 %) je bila nižja kot pri mladih (49,1 %), pri mačkah pa ni bilo značilne razlike med odraslimi (29,4 %) in mladimi živalmi (26,7 %). Prav tako ni bilo statistično pomembne povezave med prisotnostjo bakterij kampilobakter v iztrebkih in gastrointestinalnimi motnjami tako pri psih kot pri mačkah. Ta raziskava je prvo poročilo o razširjenosti bakterij kampilobakter pri psih in mačkah v Iranu.

Ključne besede: psi; mačke; bakterije kampilobakter; zoonoza