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Detection of *Campylobacter* Species in Feces of Persian Sheepdogs, Pigeons and Squirrels

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Abstract: *Campylobacter* species, in particular *Campylobacter jejuni* and *Campylobacter coli*, are considered to be the most frequent bacterial cause of human enteritis whereas their role as enteric pathogens in poultry, dogs and other pets is much less evident. The present study was conducted to determine the prevalence of *Campylobacter* spp. in Persian sheepdogs, pigeons and squirrels fecal samples in Iran. From February 2011 to August 2011, a total of 64 samples of fresh feces from Persian sheepdogs (n = 25), pigeon (n= 24) and squirrel (n = 15) were collected at Shahrekord and Isfahan provinces, Iran. In this study, 11 of 64 fecal samples (17.2%) were positive for *Campylobacter*. *Campylobacter* spp. were isolated from 6 Persian sheepdogs (24.0%), 4 pigeon (16.7%) and one squirrels (6.7%). The most prevalent *Campylobacter* species isolated from Persian sheepdogs and pigeons samples was *C. upsaliensis* (66.7%) and *C. jejuni* (100%), respectively. To establish the zoonotic significance of canine, squirrels and pigeon *Campylobacter*, isolates need to be future characterized and compared with those of human.

Key words: Sheepdogs · *Campylobacter* · Pigeon · Squirrel

INTRODUCTION

Campylobacter is the commonest bacterial cause of infective gastroenteritis in the developed world and frequently causes foodborne illness. Most cases occur sporadically, but common-source outbreaks have also been occasionally reported [1, 2].

Poultry is generally considered to be the most important single reservoir for campylobacters. However, there is some evidence based on the temporal occurrence of serotypes and genotypes shared by humans and poultry and on weekly data for poultry and human isolates that suggests that there is a common source of campylobacters instead of direct poultry-human transmission [3-5]. In addition, genotyping data on campylobacters of human and animal origin have raised the question of whether the role of poultry as a source of *Campylobacter* infections has been overestimated [6, 7] or not. Also, there is an evidence of increased risk of *Campylobacter* infection in humans associated with dog or pet ownership [8, 9].

C. jejuni is carried by most of these animal reservoirs and is the predominant species isolated from chickens and cattle [4-10, 11]. However, some *Campylobacter* species tend to be associated with particular animal hosts. *C. coli, C. hyointestinalis* and *C. mucosalis* are usually isolated from the intestines of pigs [9-12]. *C. upsaliensis* and *C. helveticus* are predominantly associated with dogs and cats [13-15]. Wild birds are a large reservoir of *Campylobacter* spp. including urease positive thermophilic campylobacters, *C. jejuni* and *C. lari* [15-17].

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 pathogen can be interspecies specific rather than

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just limited to warm blooded hosts as was once thought [17].

There is limited information regarding the prevalence *Campylobacter* in pet animals in Iran. The present study was conducted to determine the prevalence of *Campylobacter* spp. in sheepdogs, pigeons and squirrels fecal samples at Shahrekord and Isfahan, Iran.

MATERIALS AND METHODS

Sample Collection: From February 2011 to August 2011, a total of 64 samples of fresh feces from sheepdogs (n=25), pigeon (n=24) and squirrel (n=15) was collected at Shahrekord 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.

Microbiological Analysis: The fecal 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 42ºC for 24 h in a microaerophilic condition (85% N2, 10% CO2 and 5% O2), 0.1 mL of the enrichment broth 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 42ºC for 48 h under the same condition. One presumptive *Campylobacter* colony from each selective agar plate was subcultured and identification of a presumptive *Campylobacter* species was performed using standard microbiological and biochemical procedures [18, 19].

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 64 samples of fresh feces from sheepdog, pigeon and squirrels at Shahrekord and Isfahan provinces, Iran. In this study, 11 of 64 fecal samples (17.2%) were positive for *Campylobacter* isolation. *Campylobacter* spp. was isolated from 6 sheepdogs (24.0%), 4 pigeons (16.7%) and one squirrel (6.7%). There was no significant difference ($P > 0.05$) in the level of *Campylobacter* between sheepdogs and pigeons fecal samples. The most prevalent *Campylobacter* species isolated from sheepdogs` samples were *C. upsaliensis* (66.7%) and *C. jejuni* (33.3%). The only *Campylobacter* species isolated from pigeons` and squirrels` fecal samples was *C. jejuni* (100%).

Table 1: Prevalence of *Campylobacter* spp. isolated from Persian sheepdog, pigeon and squirrels fecal Shahrekord and Isfahan provinces, Iran

Samples	No. of samples	*Campylobacter* spp. positive	*C. upsaliensis*	*C. jejuni*
Sheepdogs	25	6 (24.0%)	4 (66.7%)	2 (33.3%)
Pigeons	24	4 (16.7%)	0 (0.0%)	4 (100%)
Squirrels	15	1 (6.7%)	0 (0.0%)	1 (100%)
Total	64	11 (17.2%)	4 (36.4%)	7 (63.6%)

DISCUSSON

Generally, *Campylobacter* colonize in high concentrations in the cecum and colon of poultry. Since thermophilic *Campylobacter* grow optimally at temperatures near 42°C [17-20], the higher metabolic temperatures (42°C) found in poultry species may predispose poultry to be a prominent reservoir for thermotolerant *Campylobacter*. The increased temperature may allow the thermophilic species to regulate gene expression that benefits motility and energy regulation based on specific growth requirements within a particular environmental temperature [21].

In the present study, the prevalence rate of *Campylobacter* spp. in pigeons` fecal samples was 16.7%. The occurrence of *Campylobacter* spp. in pigeon feces has been studied in several countries worldwide. In 1981, Luechtefeld *et al.* [22] were able to isolate strains from 17% of 153 pigeons trapped at the Denver Zoo. Fenlon [23] obtained a higher isolation rate (41%) from urban pigeons in Scotland. In Japan, Kinjo *et al.* [24] isolated *C. jejuni* from 54 of 196 urban pigeons (27.6%) caught. In France, Megraud [25] was able to isolate strains from 106 of 200 pigeons trapped by direct plating. In Croatia, Vlahoviæ *et al.* [26] isolated *Campylobacter* spp. from 2 of 107 free-living bird species examined (1.9%). In Barcelona, Casanovas *et al.* [27] found *Campylobacter*

spp. in 26.2% of fecal pigeon samples. All of *Campylobacter* species isolated from pigeon fecal samples was *Campylobacter jejuni* (100%). *C. jejuni* has been reported to be the most frequent species recovered from poultry and poultry carcasses [11-29].

Domesticated pets are known to harbor *Campylobacter* spp. in their digestive tracts, with incidences ranging from 11% to as much as 92% of stool samples when evaluated and characterized by either culture, polymerase chain reaction (PCR), or pulsed-field gel electrophoresis (PFGE) [30-32]. The prevalence rate of *Campylobacter* spp. in Persian sheepdog fecal samples was 24.0% which is comparable with those reported by Wieland *et al.* [30], Rossi *et al.* [31] and Salihu OR Sandberg (Revise) *et al.* [32]; however, higher isolation rates have been reported by others [14-33]. *C. upsaliensis* was the most frequently isolated species in dogs. Frequently, *C. upsaliensis* has been found to be the most common species isolated from dogs [31- 34].

Campylobacter was detected from 1/15 (6.7%) squirrels examined. The strain was identified as *C. jejuni*. In a recent study in Southern Italy, 6 of 60 samples (10 %) were positive for *C. jejuni* using a PCR assay [35]. The role of this rodent in the epidemiology of campylobacteriosis is not clear and cannot be evaluated solely on the basis of this study.

Campylobacter jejuni is a foodborne pathogen capable of causing disease in humans. These pathogens are proficient in colonizing gut environments of warm-blooded hosts as evidenced by high prevalence in domestic, feral and wild animals. Also, to establish the zoonotic potential of canine *Campylobacter* isolates, both human and canine isolates have to be further characterized and compared.

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