Prevalence of Campylobacter jejuni and Campylobacter coli species in cats and dogs from Bydgoszcz (Poland) region

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Abstract

The aim of this study was to investigate the role of cats and dogs as a potential reservoir of Campylobacter spp. Rectal swabs from 83 dogs and 71 cats were examined. Samples were obtained from the animals aged between 2 weeks and 24 months living in shelters, private households, farms and from veterinary clinics located in Bydgoszcz region during routine check-up.

Campylobacter spp. were isolated from 4.81% dogs and 9.86% cats, respectively. C. jejuni was predominant in this study. All strains were isolated in autumn and winter from the animals living in farms and private houses. All the animals positive for Campylobacter prevalence had access to small water basins, accidental source of food and had contact with wild birds, poultry or their feaces.

Isolates characterization revealed high prevalence of Campylobacter virulence genes-flaA, cadF and cdtB. 91% of isolated strains were susceptible to erythromycin. 81% among isolated strains were susceptible to azithromycin, 64% to tetracycline and 36% to ciprofloxacin. For 2 C. jejuni strains isolated from cats Random Amplified Polymorphic DNA (RAPD) profiling indicated 80% homology between them.

Key words: Campylobacter jejuni, Campylobacter coli, cats, dogs, antimicrobial susceptibility, genetic similarity, virulence genes

Introduction

Campylobacter is a leading bacterial cause of food-borne diarrheal illness worldwide (Chaban et al. 2010, Zhao et al. 2010). In the EU campylobacteriosis is the most commonly reported zoonosis followed by salmonellosis and yersiniosis (EFSA 2010).

The handling or consumption of undercooked/contaminated meat (especially poultry) is considered to be significant sources of human Campylobacter spp. infection. Other risk factors for infection include ingestion of contaminated dairy products, drinking contaminated water, foreign travel, and swimming in natural sources of water (Workman et al. 2005, Acke et al. 2011).

Campylobacter spp. are wide spread across the world. Natural reservoirs of the bacteria is the gastrointestinal tract of farm and wild animals. Direct contact with carriers animals was found to be a possible source of infection (Salihu et al. 2010). Living...
with dogs and cats has been documented to be a specific risk factor for Campylobacter infection (Lopez et al. 2002, Hald et al. 2004). Many questions of the epidemiology of Campylobacter spp. infection from domestic animals remain unanswered.

Species of veterinary importance in animals include C. upsaliensis and C. jejuni. Asymptomatic carriers are common, but the organism has also been associated with gastrointestinal disease, especially in younger animals (Acke et al. 2010). The rates of isolation of Campylobacter species from dogs and cats vary, depending on their age, species, and the season of the year (Moser et al. 2001, Sandberg et al. 2002).

Epidemiological studies on the prevalence of Campylobacter in dogs and cats are not available in the study area. The aim of this research was to define Campylobacter spp. Rectal swabs were collected from 83 dogs and 51 cats. Samples were obtained from the animals aged between 2 weeks and 24 months living in shelters, private households, farms and from veterinary clinics located in Bydgoszcz region during routine check-up. The animals had no signs of gastrointestinal disease.

Isolation of Campylobacter spp. Rectal swabs stored in Amies (Copan) transported medium were transmitted on Preston broth (Oxoid) and incubated in temperature 42°C for 48 h under micro-aerobic condition (Generbox microaer-BioMerieux). Next, bacterial suspension from Preston broth were spread on surface of CCDA plates (Oxoid). The plates were incubated in temperature 42°C for 48 h under micro-aerobic condition. Colonies suspected as being Campylobacter spp. were examined for cell morphology by Gramm method staining, motility, catalase, oxydase, and hippurate hydrolysis reactions.

Species identification. Bacterial chromosomal DNA was isolated from 24-h culture on Columbia agar with 5% sheep blood (Oxoid) by conventional boiling method. For species identification a multiplex PCR for the simultaneous detection of the C. jejuni and C. coli was performed (On et al. 2003). The following positive strains: C. jejuni ATCC 33291, C. coli ATCC 33559 were also included.

Materials and Methods

Sample collection. A total of 154 animals were analyzed for the presence of Campylobacter spp. Rectal swabs were collected from 83 dogs and 51 cats. Samples were obtained from the animals aged between 2 weeks and 24 months living in shelters, private households, farms and from veterinary clinics located in Bydgoszcz region during routine check-up. The animals had no signs of gastrointestinal disease.

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Amplification of virulence genes. The presence of the cadF, flaA, cdB and iam genes was determined with the PCR method with primers and procedures described previously (Nachamkin et al. 1993, Konkel et al. 1999, Bacon et al. 2001, Carvalho et al. 2004). PCR primers were synthesized by Oligo (Poland). The PCR products were analyzed by electrophoresis in 1.5% agarose gel. The DNA bands were visualized by staining with Midori Green Stain (Fermentas) and photographed using the IG/L-E InGenius L documentation system (TK Biotech). The size of the PCR amplicons was compared to the 100 bp DNA marker (Fermentas).

Genotypic relatedness of C. jejuni and C. coli isolates. The chromosomal DNA was separated by Random Amplified Polymorphic DNA (RAPD) method with using random primer OPA-11 5'-CAATCGCCGT-3' (Hernandez et al. 1995). The results were interpreted using GeneTool (Syngene). Strains showing 94% genetic similarity were considered identical, showing 86-92% genetic similarity were identified as closely related (Tenover et al. 1995).

Antimicrobial susceptibility testing. Minimal Inhibitory Concentration (MIC) for erythromycin, azithromycin, ciprofloxacin, and tetracycline was determined with the E-test method (bioMerieux) on Mueller-Hinton agar plates with 5% sheep blood (bioMerieux). Strains were considered as resistant for MIC values to erythromycin ≥ 32 mg/l, azithromycin ≥ 32 mg/l, ciprofloxacin ≥ 4 mg/l and tetracycline ≥ 16 mg/l, according to recommendation of CLSI (Clinical and Laboratory Standards Institute).

Results

During the study period, total of 154 samples were tested. The frequency of Campylobacter prevalence in cats and dogs from Bydgoszcz region is shown in Table 1. The results indicated that 7.1% of samples were positive for Campylobacter spp. The frequency of Campylobacter in examined samples taken from dogs was 4.81%. Discussed microorganisms were found in 9.86% samples from cats. All strains were isolated in autumn (November, December) and winter (January). All the animals positive for Campylobacter prevalence had access to small water basins, accidental source of food and contact with wild birds, poultry or their feces. Predominant species in the study was C. jejuni, which was isolated from 8 animals. C. coli was less frequent and was confirmed in 2 dogs and 1 cat.

Further studies included phenotypic and genotypic characterization of isolated C. jejuni and C. coli. The comparison of Campylobacter spp. strains isolated from cats and dogs is summarised in Table 2.
Table 1. The frequency of Campylobacter spp. in examined samples taken from animals.

<table>
<thead>
<tr>
<th>Animal</th>
<th>No. of samples</th>
<th>No. of Campylobacter spp. (%)</th>
<th>Isolated species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dog</td>
<td>83</td>
<td>4 (4.81)</td>
<td>C. jejuni (2), C. coli (2)</td>
</tr>
<tr>
<td>Cat</td>
<td>71</td>
<td>7 (9.86)</td>
<td>C. jejuni (6), C. coli (1)</td>
</tr>
<tr>
<td>Total</td>
<td>154</td>
<td>11 (7.14)</td>
<td>C. jejuni (8), C. coli (3)</td>
</tr>
</tbody>
</table>

Table 2. Comparison of Campylobacter spp. strains isolated from cats and dogs.

No of strain | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11
---|---|---|---|---|---|---|---|---|---|----|----
Animal | cat | cat | cat | cat | cat | cat | dog | dog | dog | dog | dog
Animal age (months) | 6 | 15 | 20 | 4 | 6 | 12 | 12 | 24 | 6 | 8 | 21
Place of sampling | farm | farm | farm | private households | private households | farm | farm | farm | private households | private households
Season of a year | autumn | autumn | winter | autumn | autumn | winter | winter | winter | autumn | winter
Contact with poultry/wild birds | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes
Access to water basins | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes | yes

Campylobacter species | C. jejuni | C. jejuni | C. coli | C. jejuni | C. jejuni | C. jejuni | C. jejuni | C. jejuni | C. jejuni | C. jejuni | C. coli | C. coli
Virulence genes | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+) | cadF (+)
cflaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+) | flaA (+)
iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–) | iam (–)
cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+) | cdTB (+)
RAPD-PCR | 80% homology, 80% similarity | with sample 2 | with sample 1
EM – erythromycin, AZ – azithromycin, CI – ciprofloxacin, TC – tetracycline, S (Susceptible), R (Resistant)

All Campylobacter spp. isolates from cats and dogs had cadF gene responsible for adherence, and flaA gene involved in strains motility. CdtB gene associated with toxin production was present in 72.7% of Campylobacter strains. Iam – gene linked with invasiveness of Campylobacter spp. was found in 27.2% of isolated strains.

There was no genetic similarity > 90% between strains isolated in this study. For 2 C. jejuni strains isolated from cats RAPD profiling indicated 80% homology between them (Fig. 1).

Antimicrobial susceptibility testing shown that 91% of strains isolated in the study were susceptible to erythromycin. For 82% of strains susceptibility to azithromycin was confirmed. 64% among Campylobacter strains were susceptible to tetracycline. The lowest antimicrobial susceptibility was observed for ciprofloxacin. Only 36% among isolated strains were susceptible to this drug.
Discussion

Although the most often isolated species from animals is \textit{C. upsaliensis}, in humans this species causes only sporadic infections. 80\% human campylobacteriosis has \textit{C. jejuni} or less \textit{C. coli} etiology (Sandberg et al. 2002, Wieland 2005, Acke et al. 2009). As there is increasing number of confirmed \textit{Campylobacter} infections getting from animals, it is important to estimate the prevalence of these pathogens in animals living in a close relationship with humans. Researches on carrying of \textit{C. jejuni} and \textit{C. coli} in dogs and cats were not conducted in Poland so far.

In this study the occurrence of \textit{Campylobacter} spp.
in cats and dogs from Bydgoszcz region was estimated on 7.1% and the frequency of isolation was higher in cats. Obtained data from samples isolated from dogs are inconsistent with reference, but it is necessary to underline that the research include only animals from one region. References data were based on researches performed in entire countries or many regions. The frequency of isolation of Campylobacter spp. in dogs varied from 17% (Brazil, Argentina) to 76.2% (Denmark), while in cats isolation values ranged from 8% in Brazil to 47.8% in Germany (Moser et al. 2001, Aquino et al. 2002, Lopez et al. 2002, Sandberg et al. 2002, Engvall et al. 2003, Hald et al. 2004, Wieland et al. 2005, Workman et al. 2005, Acke et al. 2009, Chaban et al. 2010, Parsons et al. 2010, Salihu et al. 2010). The prevalence of Campylobacter spp. species in cats and dogs obtained in those studies is shown in Table 3.

In most reference data is assumed that infections with Campylobacter spp. in animals are not related to the seasonal peaks. However, a study carried out in Norway (Sandberg et al. 2002) shows that the frequency of isolation of Campylobacter spp. positive samples from faeces of dogs is higher in spring. In turn, in Argentina these microorganisms were frequently isolated from dogs in the summer, and from cats during the autumn/winter (Lopez et al. 2002). Also in Switzerland more positive samples from cats were collected during the winter (Wieland et al. 2005). In this study similar results were obtained, all Campylobacter positive samples were collected in the autumn and winter.

The age of an animal has a significant impact on the possibility of Campylobacter occurrence. Confirmation of this are researches of Aquino et al. (2002) and Workman et al. (2005). In those studies Campylobacter spp. were frequently isolated from young animals. Young dogs and cats below one year old are also more prone to infection risk.

The prevalence of Campylobacter spp. in dogs and cats living in shelters is higher (Wieland et al. 2005, Acke et al. 2009). In contrary, the study did not reveal the occurrence of Campylobacter in animals from shelters. This varying data need more researches to be carried out to verify whether residing in larger gatherings increase the number of Campylobacter isolation.

Seven of 11 Campylobacter isolates were collected from the animals living in farms. This may confirm the fact that the presence of Campylobacter spp. in animals depends on the conditions in which an animal is kept and the access to the sources of the pathogen (contact with poultry or their faeces and the proximity of water containers).

The flaA and cadF gene were present in all Campylobacter spp. isolates derived from cats and dogs, which might indicate their virulence and the possibility of human infection. The results obtained are similar to those of previous studies (Andrzejewska et al. 2010, Krutkiewicz et al. 2010). In both studies high percentage of flaA, cadF and cdtB genes among tested strains isolated from domestic animals was revealed. This similar observation confirmed the important role of Campylobacter virulence genes in pathogenesis process.

The present study on the antimicrobial susceptibility of strains isolated from dogs and cats confirmed high level of macrolides susceptibility. The highest level of resistance was to ciprofloxacin and tetracycline. Similar research was described in the contribution of Krutkiewicz et al. (2010). Antimicrobial susceptibility testing in this study also revealed Campylobacter spp. strains isolated from domestic animals resistant to erythromycin.

Similarity test confirmed genetic relationship of 2 C. jejuni isolates from cats living in the farms. This may suggest that Campylobacter spp. strains isolated from animals living in particular environment (e.g. the countryside) have common genetic origin. It is possible that further investigation of RAPD fingerprinting would be helpful in this regard.

Further studies involving larger populations, a variety of sampling groups, testing for multiple Campylobacter spp. and specialized molecular techniques are needed to clarify the role of cats and dogs in human campylobacteriosis and to improve the understanding of the complex epidemiology of Campylobacter infections.

References


