

The Prevalence of *Campylobacter* spp. and Occurrence of Virulence Genes Isolated from Dogs

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Abstract

This study was conducted to determine the prevalence of *Campylobacter* spp. isolated from dogs' faecal samples. From June 2012 to June 2013, a total of 210 faecal samples from pet dogs living in different kennels (n = 210) were collected by the owners in Greater Poland Voivodeship, Poznań District, Poland. The study revealed that 105 out of 210 faecal samples (50%) contained *Campylobacter*. The highest prevalence of *Campylobacter* spp. occurred in spring (81%), followed by winter (64%). The *cadF* gene was found in 100% of the isolates tested. The occurrence of the other genes was variable. The isolates from young dogs were characterized by higher occurrence of virulence genes.

Key words: *Campylobacter* spp., dogs, virulence genes

Salmonella spp., *Escherichia coli* and *Campylobacter* spp. are the main causes of diarrhoea both in animals and humans all over the world (Silva *et al.*, 2011). According to the EFSA (EFSA, 2014), campylobacteriosis is the most frequently reported zoonotic disease. The most common sources of infection are products of animal origin: meat (primarily poultry), milk and water from natural reservoirs (Acke *et al.*, 2011). The most frequently isolated species from dogs in Denmark are: *Campylobacter upsaliensis* 75%, *Campylobacter jejuni* 19.4% and *Campylobacter coli* 0.7% (Hald *et al.*, 2004). These *Campylobacter* species are more often isolated from dogs aged between 3 and 12 months than from older dogs (Hald *et al.*, 2004). Moreover, some researchers also reported seasonal differences in prevalence (Rahimi and Saljooghian Esfahani, 2010; Andrzejewska *et al.*, 2013). The factors which are associated with the pathogenicity of *Campylobacter* include: motility, chemotaxis as well as adhesion, and invasiveness (Bang *et al.*, 2001; Krutkiewicz, 2008) and toxicity. These traits are associated with certain virulence genes identified from these bacteria, for example: *flaA*, *cadF*, *iam* and *cdtB* (Biswas, 2011; Selwet and Galbas, 2012a). The investigations which have been carried out so far have revealed varying distribution of these virulence genes among strains. The aim of this study was to determine the frequency of occurrence of *C. upsaliensis*, *C. jejuni*

and *C. coli* isolated from two different age groups of healthy dogs and from dogs with the symptoms of diarrhoea in Greater Poland Voivodeship, Poznań District. Apart from that, the frequency of occurrence of selected virulence genes was determined in the collected isolates. From June 2012 to June 2013, a total of 210 samples of faeces from pet dogs living in 5 kennels were collected by their owners in Greater Poland Voivodeship Poznań District, Poland. All the samples were obtained by veterinarian by means of swab kits with a transport substrate (Euro Tubo Collection Swab Rubi, Spain). The animals were aged as follows: 105 dogs were adult (> 12 months), 105 dogs were younger than 1 year. Diarrhoea was reported in 25 dogs. The owners of the other dogs did not report clinical symptoms of diarrhoea. The faecal samples were cultured at 42 ± 1°C in Campy Selective Agar Base (Preston) (Neogen Europe, Scotland UK) for 48 h under microaerophilic conditions. DNA was extracted by means of CHELEX-100 chelating resin (Bio-Rad, CA, USA). Bacterial colonies were suspended in 100 µl Tris buffer and 45 µl 20% CHELEX and boiled for 10 min. The samples were immediately placed on ice for 1 min. and centrifuged at 13.000 g for 10 min. at room temperature. A PCR was used for the detection of *C. upsaliensis*, *C. jejuni* and *C. coli*. (Andrzejewska *et al.*, 2011). The following positive controls were included in the PCR: *C. upsaliensis*

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Table I
The prevalence of *Campylobacter* spp. isolated from diarrhoeic and healthy dogs

Sources		No. of samples	<i>Campylobacter</i> spp. positive	<i>Campylobacter upsaliensis</i>	<i>Campylobacter jejuni</i>	<i>Campylobacter coli</i>
Adult dogs	Healthy	95	37 (38.9%)	18 (48.6%)	15 (40.5%)	4 (10.8%)
	Diarrhoeic	10	5 (50%)	1 (20%)	2 (40%)	2 (40%)
Young dogs	Healthy	90	54 (60%)	29 (53.7%)	18 (33.3%)	7 (13.0%)
	Diarrhoeic	15	9 (60%)	2 (22.2%)	5 (55.5%)	2 (22.2%)

ATCC 43954, *C. jejuni* ATCC 33560 and *C. coli* ATCC 33559. The presence of the *flaA*, *cadF*, *cdtB* and *iam* genes was determined with the primers according to the authors (Nachamkin *et al.*, 1993; Konkel *et al.*, 1999; Bang *et al.*, 2001; Carvalho *et al.*, 2001). The statistical analysis was performed according to the GLM procedure of the SAS program (SAS, 1999) and the significance of differences was verified with Tukey's test. The prevalence of *Campylobacter* spp. was determined in the 210 faecal samples. PCR revealed the presence of *Campylobacter* spp. in 105 samples (50%). *Campylobacter* spp. was isolated from 42 samples (40%) from adult individuals and from 63 samples (60%) from young dogs. The numbers of isolates differed significantly between the age groups ($P < 0.05$). The most frequent species in the group of adult dogs were: 45.2% *C. upsaliensis*, 40.5% *C. jejuni* and 14.3% *C. coli*. In the group of young dogs the isolation percentage was similar, *i.e.* 49.2% *C. upsaliensis*, 36.5% *C. jejuni* and 14.3% *C. coli*. There were significant differences observed in the prevalence of *C. upsaliensis* between the age groups of the dogs under study ($P < 0.05$). Out of the 210 samples examined 25 samples derived from individuals with diarrhoea: 10 samples from adult dogs and 15 samples from young individuals (Table I). As far as the adult dogs are concerned, the predominant species were: 45.2% *C. upsaliensis*, 40.5% *C. jejuni* and 14.3% *C. coli* whereas in the young dogs these were: 49.2% *C. upsaliensis*, 36.5% *C. jejuni* and 14.3% *C. coli*. In summer

Table II
The seasonal prevalence of *Campylobacter* spp. isolated from dogs

Seasons	Sources		Total
	Adult dogs	Young dogs	
Spring	14/22 (63.6%)	20/20 (100%)	34/42 (80.9%)
Summer	12/45 (26.7%)	20/40 (50%)	32/85 (37.6%)
Autumn	10/28 (35.7%)	13/30 (43.3%)	23/58 (39.6%)
Winter	6/10 (60%)	10/15 (66.7%)	16/25 (64%)
Total	42/105	63/105	105/210

and autumn the frequency was similar with: 37.6% and 39.6%, respectively (Table II). The analyses of the percentages of the selected virulence genes (Table III) revealed that the *cadF* gene was found in 100% of all the species. Also, the *flaA* gene was determined in 100% of *C. jejuni* and *C. coli*. As far as the *cdtB* and *iam* genes are concerned, they were detected more frequently in the younger individuals. Although poultry is considered to be the main source of infection with *Campylobacter* spp. in humans, according to Biswas *et al.* (2011), the role of other animals as vectors spreading this bacterium should also be recognised. Parsons *et al.* (2010) in UK and Andrzejewska *et al.* (2013) in Poland reported that the frequency of *Campylobacter* spp. isolation in dogs ranges from 17% in Brazil and Argentina to 76.2% in Denmark. According to the study by Workman *et al.* (2005), the age of animals may also exert significant influence on the degree of occurrence

Table III
The number and percentages of virulence genes in *Campylobacter* spp. isolated from dogs

Isolates/Animals	Genes			
	<i>cadF</i>	<i>flaA</i>	<i>cdtB</i>	<i>iam</i>
<i>C. upsaliensis</i>				
Adult dogs (n = 19)	19 (100%)	18 (94.7%)	17 (89.5%)	18 (94.7%)
Young dogs (n = 31)	31 (100%)	30 (96.8%)	30 (96.8%)	30 (96.8%)
<i>C. jejuni</i>				
Adult dogs (n = 17)	17 (100%)	17 (100%)	16 (94.1%)	15 (88.2%)
Young dogs (n = 23)	23 (100%)	23 (100%)	23 (100%)	21 (91.3%)
<i>C. coli</i>				
Adult dogs (n = 6)	6 (100%)	6 (100%)	5 (83.3%)	5 (83.3%)
Young dogs (n = 9)	9 (100%)	9 (100%)	8 (88.9%)	8 (88.9%)

of *Campylobacter* spp. Andrzejewska *et al.* (2013) reported the highest prevalence of *Campylobacter* spp. in dogs less than one year of age. Westgarth *et al.* (2008) observed that younger dogs were more likely to harbour *C. upsaliensis* and *C. jejuni* than adult animals. It may be related with younger dogs' lower immunity. So far the relationships between the occurrence of *C. upsaliensis* and gastritis as well as diarrhoea in dogs and humans have not been sufficiently explained. However, younger individuals are believed to be the main source of occurrence of gastritis and diarrhoea, as Rahimi *et al.* (2012) noted. Salihu *et al.* (2010), claim that age is not an indicator of the risk of contamination with *C. jejuni*. In our study *C. upsaliensis* was found significantly more often in younger dogs than adult animals whereas there was no such difference observed with *C. jejuni* and *C. coli*. Infections caused by *Campylobacter* spp. are usually correlated with the seasons of the year, although there are reports indicating that the isolations of *Campylobacter* spp. from dogs were more frequent in spring (Sandberg *et al.*, 2002). Rahimi *et al.* (2012) recorded an increase of *Campylobacter* spp. isolations from young dogs aged under 1 year as well as from individuals which were over 1 year of age in warm months: in spring – 38.5% and in summer – 38.9%. In our study, the frequency of isolation of *Campylobacter* spp. was higher in spring and in winter. The study by Rizal *et al.* (2010) showed that the following factors are involved in pathogenicity of *Campylobacter* spp.: motility and chemotaxis as well as adhesion and invasiveness. Nowadays it is believed that the following genes are responsible for the potential pathogenicity of *Campylobacter* spp.: the *flaA* gene influencing motility, *cadF* – affecting adhesion, *cdtB* – responsible for toxin production (cytolethal distending toxin) and *iam* – determining invasiveness (Krutkiewicz, 2008). In our investigations the *cadF* gene was determined in 100% in *C. upsaliensis*, *C. jejuni* and *C. coli*. Biswas *et al.* (2011) confirmed the occurrence of this gene in 100% of the examined *C. jejuni* strains derived from human clinical studies and cow faeces. Selwet and Galbas (2012a; 2012b) observed that the *cadF* and *flaA* genes were found in 100% of *C. coli* and *C. jejuni* isolated from broilers, porkers, calves and piglets. All the strains of *C. jejuni*, *C. coli* *C. upsaliensis* under analysis carried the *cdtB* gene encoding the protein exhibiting toxic properties, *i.e.* cytolethal distending toxin. This exotoxin causes the inhibition of the cell cycle and DNA degradation in the host (Lara-Tejero, 2001) and it may cause the death of sensitive eukaryotic cells (Heywood *et al.*, 2005). CDT is composed of three subunits: CdtA, CdtB and CdtC, which are encoded by three genes: *cdtA*, *cdtB* and *cdtC*. All the three subunits are required for full activity (Rozynek *et al.*, 2005). In our study the *iam* gene, which is responsible for invasiveness, was

found in all the strains of *Campylobacter* spp. Carvalho *et al.* (2001) observed in their studies that the *iam* gene was detected most frequently in the strains of *C. jejuni* rather than in *C. coli*. The PCR analysis of stool isolates collected from dogs on farms in Wielkopolska (Greater Poland) region revealed the presence of *Campylobacter* spp. with the predominance of *C. upsaliensis*, which occur more frequently in young animals. The presence of *C. jejuni* and *C. coli* was frequently identified in adult dogs with the symptoms of diarrhoea, whereas *C. jejuni* was identified in young dogs. The correlation between diarrhoea and the presence of these bacteria should be taken into consideration in veterinary practice.

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