PCR detection of *Campylobacter* species in feces from dogs

*PCR-detectie van Campylobacter species in feces van honden*

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**ABSTRACT**

The presence of *Campylobacter* DNA was studied by means of PCR in fecal samples from 37 dogs suffering from acute or chronic diarrhea and 50 dogs without clinical signs. In total, 47% of the fecal samples were positive for *Campylobacter* DNA, with *C. upsaliensis* being the predominant species, followed by *C. coli*, *C. jejuni* and *C. mucosalis*. *C. helveticus*, *C. lari*, *C. hyointestinalis*, *C. sputorum*, *C. fetus* and *C. lanienae* DNA was not detected in any of the samples. No significant difference was noted between the healthy dogs and the diarrheic dogs. Dogs younger than 12 months old were significantly more often infected with *Campylobacter* species than older dogs. Although a pathogenic role cannot be excluded, the detection of these organisms in fecal samples is not diagnostic for *Campylobacter*-associated disease in dogs. However, because of their frequent presence in dog feces, *Campylobacter* species may constitute a public health hazard.

**INTRODUCTION**

Diarrhea in dogs has several causes, including infections with enteropathogenic bacteria such as *Clostridium perfringens*, *Salmonella* spp., *Escherichia coli*, *Helicobacter* spp. and *Campylobacter* spp. Nonetheless, these bacterial species are also commonly isolated from apparently asymptomatic dogs (Marks and Kather, 2003). With respect to *Campylobacter* spp., mainly *C. upsaliensis* and to a lesser extent *C. coli* and *C. jejuni* are present in dogs (Bourke et al., 1998; Engvall et al., 2003; Koene et al., 2004). Although these thermotolerant *Campylobacter* species have been associated with diarrhea in dogs (Steinhauserova et al., 2000; Misawa et al., 2002; Sokolow et al., 2005), their real role in canine enteritis is not clear (Koene et al., 2004).

In humans, *Campylobacter* species are an important cause of gastroenteritis and may also cause bacteremia. Apparently, children are most sensitive to infection with these bacteria (Owen and Hernandez, 1990; Burnens et al., 1992; Hald and Madsen, 1997; Chattopadhyay et al., 2001; Wolsf et al., 2001). The aim of the present study was to examine the occurrence of *Campylobacter* species in feces from dogs in Belgium with and without diarrhea and to identify the detected *Campylobacters* up to the species level.

**MATERIALS AND METHODS**

**Sample origin**

Fresh fecal specimens were collected from 50 clinical-
ly healthy dogs from various breeds housed individually at home and 37 dogs suffering from acute or chronic diarrhea that were presented at the Department of Small Animal Medicine and Clinical Biology, Faculty of Veterinary Medicine from April 2006 to January 2007. The animals (46 females and 41 males) were between four weeks and 14 years of age. Twenty-one dogs were younger than 12 months, six of them being in the clinically healthy group and 15 of them in the diarrheic group. For four dogs, the age was unknown. All samples were stored at 4°C for maximally 24 hours until further analysis.

**Extraction of DNA**

DNA was extracted from approximately 200 mg of fecal material using a commercial QIAamp® DNA Stool Mini Kit (Qiagen, Venlo, The Netherlands). The DNA extracts were frozen at -20°C until further analysis.

**PCR and gel electrophoresis**

Analysis of the fecal samples was performed using *Campylobacter* genus- and species-specific PCR assays. The respective target genes and amplicon sizes of these PCR assays are listed in Table 1. Generally, the previously described reaction and amplification conditions were used (Inglis and Kalischuk, 2003), with the exception that 35 cycles instead of 25 cycles were used for the *C. lari*, *C. upsaliensis* and *C. mucosalis* PCR assays to enhance their sensitivity. To determine the detection limit of each PCR assay, the respective *Campylobacter* strains were grown on Mueller Hinton II agar (Becton, Dickinson and Company, Cockeysville, USA) supplemented with 5% horse blood. The plates were incubated at 37°C in jars under microaerobic conditions. DNA was extracted with guanidium thiocyanate as described by Pitcher et al. (1989). Ten-fold serial dilutions of the genomic DNA were used as a template in the respective PCR assays to evaluate their sensitivity. The concentration of the extracted DNA was determined as absorbance at 260 nm wavelength (A_{260}) with a NanoDrop® ND-1000 Spectrophotometer (Isogen Life Science, St.-Pieters-Leeuw, Belgium). The DNA purity as determined by the A_{260}/A_{280} ratio was >1.8.

For each PCR assay, DNA of corresponding type or reference strains, including *C. jejuni* LMG 6444^T, *C. coli* LMG 6440^T, *C. lari* LMG 8846^T, *C. upsaliensis* LMG 19529^T, *C. hyointestinalis* LMG 13356, *C. fetus* LMG 6442^T, *C. mucosalis* LMG 8499, *C. sputorum* LMG 11765, *C. laniænae* NCTC 13004^T and *C. helveticus* LMG 12639, was used as positive control. All PCR products were subjected to electrophoresis in an agarose gel and visualized, as described before (Baele et al., 2004).

**Statistical analysis**

Differences in the prevalence of *Campylobacter* species among healthy dogs and dogs with diarrhea, dogs younger than 12 months old and dogs older than 12 months, and female and male dogs were analyzed using logistic regression analysis (SPSS 12.0, Chicago, Illinois, USA). A significance level of α = 0.05 was used.

**RESULTS**

In total, 47% (41/87) of the fecal samples were positive for *Campylobacter* genus DNA: 20 samples from the clinically healthy group (40%) and 21 samples from the diarrheic group (57%) (Table 2). The observed difference in prevalence between the clinically healthy dogs and the diarrheic dogs was statistically not significant (P = 0.124).

In general, a statistically significant difference (P = 0.001) in prevalence of *Campylobacter* species was noted between dogs younger than 12 months (17 out of 21 (81%) positive) and dogs older than 12 months (21 out of 62 (34%) positive). In the clinically healthy group, five out of the six (83%) dogs younger than 12 months were positive and 15 out of the 43 (35%) samples of dogs older than 12 months were positive. In the diarrheic group, 12 out of the 15 (80%) dogs younger than 12 months had positive samples and 6 out of the 19 (32%) dogs older than 12 months had positive samples. No statistically significant difference in prevalence was observed between male and female dogs (P = 0.77).

The most frequently found species was *C. upsaliensis*, followed by *C. coli*, *C. jejuni* and *C. mucosalis* (Table 2). Two dogs were simultaneously infected with *C. upsaliensis* and *C. coli*, and in another dog both *C. jejuni* and *C. upsaliensis* DNA were present. Six samples that gave a positive result in the *Campylobacter* genus-specific PCR assay could not be identified up to the species level. Two samples in which *C. upsaliensis* DNA was detected, three *C. jejuni*-positive samples, two *C. coli*-positive samples and the *C. mucosalis*-positive sample were negative in the *Campylobacter* genus-specific PCR assay. *C. helveticus*, *C. lari*, *C. hyointestinalis*, *C. sputorum*, *C. fetus* and *C. laniænae* DNA was not detected in any of the samples.

The detection limit of each PCR assay is shown in Table 1.

**DISCUSSION**

In the present study, fecal samples were investigated for the presence of *Campylobacter* DNA by means of PCR. *Campylobacter* species are fastidious, and media used to selectively isolate the classical species like *C. jejuni* and *C. coli* contain antimicrobial agents which are known to be inhibitory to other *Campylobacter* species, including *C. lari*, *C. sputorum*, *C. upsaliensis*, *C. hyointestinalis* and *C. fetus* (Inglis and Kalischuk, 2003; Moore et al., 2005). As a result, most culture-based methods do not yield a reliable estimate of the frequency and diversity of *Campylobacter* species associated with fecal samples of different animal species. Another limitation of these methods is that at least 48 hours is needed to obtain a presumptive isolate, which then requires confirmation using phenotypic or genotypic tests (Inglis and Kalischuk, 2003). The application of PCR provides a faster and more accurate description of the prevalence of *Campylobacter* species associated with dog feces. PCR inhibitors of fecal origin like bile salts, hemoglobin degradation products and complex polysaccharides can be removed by using the commercial QIAamp® DNA Stool Mini Kit (Inglis and Ka-
Table 1. PCR assays used to identify *Campylobacter* species in dog feces.

<table>
<thead>
<tr>
<th>PCR assay</th>
<th>Target gene</th>
<th>Primer</th>
<th>Size (bp) of the amplicon</th>
<th>Detection limit (ng DNA/reaction mixture)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> genus</td>
<td>16S rRNA</td>
<td>C412F C1228R</td>
<td>816</td>
<td>10 x 10^-4</td>
</tr>
<tr>
<td><strong>C. coli and C. jejuni multiplex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. coli</td>
<td><em>ceuE</em></td>
<td>Primary: COL3Upper MDCOL2Lower Nested: CCceuEN3F CCceuEN3R</td>
<td>462</td>
<td>29 x 10^-7</td>
</tr>
<tr>
<td>C. jejuni</td>
<td><em>mapA</em></td>
<td>Primary: MDmapA1Upper MDmapA2Lower Nested: CJmapAN3F CJmapAN3R</td>
<td>589</td>
<td>10 x 10^-6</td>
</tr>
<tr>
<td><strong>C. fetus and C. lanienae multiplex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. fetus</td>
<td>23S rRNA</td>
<td>Primary: FET1 HYOFET23SR Nested: FETNF HYOFET23SR2</td>
<td>784</td>
<td>73 x 10^-4</td>
</tr>
<tr>
<td>C. lanienae</td>
<td>16S rRNA</td>
<td>Primary: CLAN76F CLAN521021R Nested: CLANNF CLANNR</td>
<td>920</td>
<td>44 x 10^-6</td>
</tr>
<tr>
<td><strong>C. hyointestinalis</strong></td>
<td>23S rRNA</td>
<td>Primary: HYO1F HYOFET23SR Seminested: HYO1F HYOFET23SR2</td>
<td>611</td>
<td>12 x 10^-6</td>
</tr>
<tr>
<td>C. helveticus</td>
<td>16S rRNA</td>
<td>CHCU146F CHI371R</td>
<td>1225-1375</td>
<td>93 x 10^-4</td>
</tr>
<tr>
<td>C. lari</td>
<td>16S rRNA</td>
<td>CL594F CL1155R</td>
<td>561</td>
<td>72 x 10^-4</td>
</tr>
<tr>
<td>C. mucosalis</td>
<td>23S rRNA</td>
<td>MUC1 MUC1</td>
<td>306</td>
<td>41 x 10^-6</td>
</tr>
<tr>
<td>C. sputorum</td>
<td>23S rRNA</td>
<td>SPUT1 SPUT2</td>
<td>588</td>
<td>54 x 10^-4</td>
</tr>
<tr>
<td>C. upsaliensis</td>
<td>16S rRNA</td>
<td>CHCU146F CU1024R</td>
<td>878</td>
<td>44 x 10^-4</td>
</tr>
</tbody>
</table>

Table 2. Prevalence of DNA of different *Campylobacter* species in dogs with or without diarrhea.

<table>
<thead>
<tr>
<th>PCR assay</th>
<th>Number (%) of positive samples in dogs with diarrhea (37*)</th>
<th>Number (%) of positive samples in dogs without diarrhea (50*)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Campylobacter</em> genus</td>
<td>21 (57%)</td>
<td>20 (40%)</td>
</tr>
<tr>
<td><em>Campylobacter</em> upsaliensis</td>
<td>13 (35%)</td>
<td>13 (26%)</td>
</tr>
<tr>
<td><em>Campylobacter</em> coli</td>
<td>3 (8%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td><em>Campylobacter</em> jejuni</td>
<td>4 (11%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><em>Campylobacter</em> mucosalis</td>
<td>1 (3%)</td>
<td>-</td>
</tr>
</tbody>
</table>

* = number of animals examined
lishchuk, 2003), as was done in the present survey. On the other hand, a disadvantage of PCR-based methods is the lack of isolates and hence the inability to perform antimicrobial sensitivity testing. Furthermore, performing PCR assays is rather expensive and does not make it possible to distinguish between the simple DNA detection of dead micro-organisms and the presence of viable Campylobacter species (Kulkarni et al., 2002).

There appeared to be some discrepancy between the results obtained with the Campylobacter genus-specific PCR assay and those from the species-specific assays. This can partly be explained by a difference in sensitivity of the tests. Indeed, as seen in Table 1, the C. jejuni, C. mucosalis and C. coli PCR assays were more sensitive (by a factor of 10²) than the genus-specific PCR assay. The finding of six samples that were positive in the genus-specific PCR but not in the species-specific PCR assays could have resulted from the presence of DNA from other Campylobacter species than those tested in this survey.

The present study shows that fecal material from 57% of the dogs with diarrhea, but also from 40% of the clinically healthy dogs, harbored Campylobacter DNA. The finding of similar percentages in both groups is in accordance with previous studies (Chat- topadhyay et al., 2001; López et al., 2002; Modolo and Giuffrida, 2004) and may call into question the presumed association of Campylobacter with gastrointestinal disease in dogs. However, the encountering of Campylobacter DNA in feces from gastrointestinal patients as well as from clinically healthy dogs does not necessarily exclude this microorganism from being pathogenic. The actual evolution into enteric disease depends on bacterial factors, host characteristics and/or the interaction between host and bacterium.

In accordance with other studies (Goossens et al., 1991; Burnens et al., 1992; Altekruse et al., 1999; Baker et al., 1999; Sandberg et al., 2002), C. upsaliensis, which was shed by 30% of the dogs investigated, was the predominant Campylobacter species found. In addition, 7% and 6% of all the dogs excreted C. coli and C. jejuni, respectively. In humans, C. jejuni and C. coli are recognized as the most common causes of bacterial gastroenteritis worldwide, although less common species, including C. upsaliensis, are increasingly being implicated in human disease (Moore et al., 2005). One diarrheic dog was positive for C. mucosalis. To the authors’ knowledge, this is the first report of the presence of C. mucosalis DNA in another animal species than pigs (Lawson and Rowland, 1974).

The higher carriage rate of Campylobacter species found in the dogs younger than 12 months (81%) compared with the dogs older than 12 months (34%) agrees with earlier findings (Sandberg et al., 2002; Engvall et al., 2003). Frequently, the highest Campylobacter prevalences have been described in puppies with diarrhea (Saeed et al., 1993; Adak et al., 1995), but in the current study, as many as 83% of the healthy young dogs shed campylobacters. These data underscore the fact that both diarrheic and non-diarrheic household puppies, which often live in close proximity to humans, frequently shed campylobac-

ters. This fact may have an impact on public health. Especially in families with children, careful hygiene with dogs should be practiced (Wolfs et al., 2001; Damborg et al., 2004).

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