PCR detection of *Campylobacter* species in feces from dogs

PCR-detectie van Campylobacter species in feces van honden

[1]H. Moyaert, [1]L. Ceelen, [2]J. Dewulf, [1]F. Haesebrouck, [1]F. Pasmans

[1] Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

[2] Department of Obstetrics, Reproduction and Herd Health, Veterinary Epidemiology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, B-9820 Merelbeke, Belgium

Hilde.Moyaert@UGent.be

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.

SAMENVATTING

De aanwezigheid van *Campylobacter* DNA werd door middel van PCR onderzocht in de meststalen van 37 honden die leden aan acute of chronische diarree en van 50 honden zonder klinische symptomen. In totaal was 47% van de meststalen positief voor *Campylobacter* DNA. *C. upsaliensis* was de meest voorkomende species, gevolgd door *C. coli, C. jejuni* en *C. mucosalis*. *C. helveticus, C. lari, C. hyointestinalis, C. sputorum, C. fetus* en *C. lanienae* DNA werd in geen enkel staal teruggevonden. Er werd geen significant verschil gevonden tussen de gezonde honden en de honden met diarree. De honden jonger dan 12 maanden waren significant vaker geïnfecteerd met *Campylobacter* species dan de oudere honden. Hoewel pathogeniciteit niet uitgesloten kan worden, is het aantonen van deze kiemen in meststalen niet diagnostisch voor *Campylobacter*-geassocieerde ziekten bij honden. Doordat ze frequent aanwezig zijn in hondenfeces kunnen ze echter een gevaar inhouden voor de volksgezondheid.

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; Wolfs *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. lanienae* 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 $\alpha = 0.05$ 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. laniaenae* 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.

PCR assay	Target gene	Primer	Size (bp) of the amplicon	Detection limit (ng DNA/ reaction mixture)
Campylobacter genus	16S rRNA	C412F C1228R	816	10×10^{-4}
C. coli and *C. jejuni* multiplex				
C. coli	*ceuE*	Primary: COL3Upper MDCOL2Lower Nested: CCceuEN3F CCceuEN3R	462 330	29×10^{-7}
C. jejuni	*mapA*	Primary: MDmapA1Upper MDmapA2lower Nested: CJmapAN3F CJmapAN3R	589 413	10×10^{-6}
C. fetus and *C. lanienae* multiplex				
C. fetus	23S rRNA	Primary: FETI HYOFET23SR Nested: FETNF HYOFET23SR2	784 473	73×10^{-4}
C. lanienae	16S rRNA	Primary: CLAN76F CLAN521021R Nested: CLANNF CLANNR	920 360	44×10^{-6}
C. hyointestinalis	23S rRNA	Primary: HYO1F HYOFET23SR Seminested: HYO1F HYOFET23SR2	611 468	12×10^{-6}
C. helveticus	16S rRNA	CHCU146F CH1371R	1225-1375	93×10^{-4}
C. lari	16S rRNA	CL594F CL1155R	561	72×10^{-4}
C. mucosalis	23S rRNA	MUC1 MUC1	306	41×10^{-6}
C. sputorum	23S rRNA	SPUT1 SPUT2	588	54×10^{-4}
C. upsaliensis	16S rRNA	CHCU146F CU1024R	878	44×10^{-4}

Table 2. Prevalence of dna of different *campylobacter* species in dogs with or without diarrhea.

PCR assay	Number (%) of positive samples in dogs with diarrhea (37*)	without diarrhea (50*)
Campylobacter genus	21 (57%)	20 (40%)
Campylobacter upsaliensis	13 (35%)	13 (26%)
Campylobacter coli	3 (8%)	3 (6%)
Campylobacter jejuni	4 (11%)	1 (2%)
Campylobacter mucosalis	1 (3%)	-

* = number of animals examined

lischuk, 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 10^2) 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 (Chattopadhyay *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 evolvement 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 campylobacters. 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).

ACKNOWLEDGMENTS

This work was supported by the Research Fund of Ghent University, Belgium, Code GOA 12050602. We are grateful to Jurgen De Craene, Ellen Dewaele and Sofie De Bruyckere for their technical assistance. Julie Crucke and Joyce Verschoof are gratefully acknowledged for their contribution in collecting fecal samples and performing literature research.

REFERENCES

Adak G.K., Cowden J.M., Nicholas S., Evans H.S. (1995). The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of *Campylobacter* infection. *Epidemiology and Infection 115*, 15-22.

Altekruse S.F., Stern N.J., Fields P.I., Swerdlow D.L. (1999). *Campylobacter jejuni* – an emerging foodborne pathogen. *Emerging Infectious Diseases 5*, 28-35.

Baele M., Van den Bulck K., Decostere A., Vandamme P., Hänninen M-L., Ducatelle R., Haesebrouck F. (2004). Multiplex PCR assay for differentiation of *Helicobacter felis, H. bizzozeronnii*, and *H. salomonis*. *Journal of Clinical Microbiology 42*, 1115-1122.

Baker J., Barton M.D., Lanser J. (1999). *Campylobacter* species in cats and dogs in South Australia. *Australian Veterinary Journal 77*, 662-666.

Bourke B., Chan V.L., Sherman P. (1998). *Campylobacter upsaliensis*: waiting in the wings. *Clinical Microbiology Reviews 11*, 440-449.

Burnens A.P., Angeloz-Wick B., Nicolet J. (1992). Comparison of *Campylobacter* carriage rates in diarrheic and healthy pet animals. *Zentralblatt für Veterinärmedizin Reihe B 39*, 175-180.

Chattopadhyay U.K., Rashid M., Sur S.K., Pal D. (2001). The occurrence of campylobacteriosis in domestic animals and their handlers in and around Calcutta. *Journal of Medical Microbiology 50*, 933-934.

Damborg P., Olsen K.E.P., Møller Nielsen E., Guardabassi L. (2004). Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C. jejuni*. *Journal of Clinical Microbiology 42*, 1363-1364.

Engvall E.O., Brändström B., Andersson L., Båverud V., Trowald-wigh G., Englund L. (2003). Isolation and identification of thermophilic *Campylobacter* species in faecal samples from Swedish dogs. *Scandinavian Journal of Infectious Diseases 35*, 713-718.

Goossens H., Vlaes L., Butzler J.P., Adnet A., Hanicq P., N'Jufom S., Massart D., de Schrijver G., Blomme W. (1991). *Campylobacter upsaliensis* enteritis associated with canine infections. *The Lancet 337*, 1486-1487.

Hald B., Madsen M. (1997). Healthy puppies and kittens as carriers of *Campylobacter* spp., with special reference to *Campylobacter upsaliensis*. *Journal of Clinical Microbiology 35*, 3351-3352.

Inglis G.D., Kalischuk L.D. (2003). Use of PCR for direct detection of *Campylobacter* species in bovine feces. *Applied and Environmental Microbiology 69*, 3435-3447.

Koene M.G., Houwers D.J., Dijkstra J.R., Duim B.,

Wagenaar J.A. (2004). Simultaneous presence of multiple *Campylobacter* species in dogs. *Journal of Clinical Microbiology 42*, 819-821.

Kulkarni S.P., Lever S., Logan J.M.J., Lawson A.J., Stanley J., Shafi M.S. (2002). Detection of *Campylobacter* species: a comparison of culture and polymerase chain reaction based methods. *Journal of Clinical Pathology 55*, 749-753.

Lawson G.H.K, Rowland A.C. (1974). Intestinal adenomatosis in the pig: a bacteriological study. *Research in Veterinary Science 17*, 331-336.

López C.M., Giacoboni G., Agostini A., Cornero F.J., Tellechea D.M., Trinidad J.J. (2002). Thermotolerant *Campylobacters* in domestic animals in a defined population in Buenos Aires, Argentina. *Preventive Veterinary Medicine 55*, 193-200.

Marks S.L., Kather E.J. (2003). Bacterial-associated diarrhea in the dog: a critical appraisal. *The Veterinary Clinics of North America: Small Animal Practice 33*, 1029-1060.

Misawa N., Kawashima K., Kondo F., Kushima E., Kushima K., Vandamme P. (2002). Isolation and characterization of *Campylobacter*, *Helicobacter*, and *Anaerobiospirillum* strains from a puppy with bloody diarrhea. *Veterinary Microbiology 87*, 353-364.

Modolo J.R., Giuffrida R. (2004). *Campylobacter upsaliensis* isolated from young dogs with and without diarrhea. *Revista da Sociedade Brasileira de Medicina Tropical 37*, 72-73.

Moore J.E., Corcoran D., Dooley J.S.G., Fanning S., Lucey B., Matsuda M., McDowell D.A., Mégraud F., Millar B.C., O'Mahony R., O'Riordan L., O'Rourke M., Rao J.R., Rooney P.J., Sails A., Whyte P. (2005). *Campylobacter*. *Veterinary Research 36*, 351-382.

Owen R.J., Hernandez J. (1990). Occurrence of plasmids in "*Campylobacter upsaliensis*" (catalase negative or weak group) from geographically divers patients with gastroenteritis or bacteraemia. *European Journal of Epidemiology 6*, 111-117.

Pitcher D.G., Saunders N.A., Owen R.J. (1989). Rapid extraction of bacterial genomic DNA with guanidium thiocyanate. *Letters of Applied Microbiology 8*, 151-156.

Saeed A.M., Harris N.V., DiGiacomo R.F. (1993). The role of exposure to animals in the etiology of *Campylobacter jejuni/coli* enteritis. *American Journal of Epidemiology 137*, 108-114.

Sandberg M., Bergsjo B., Hofshagen M., Skjerve E., Kruse H. (2002). Risk factors for *Campylobacter* infection in Norwegian cats and dogs. *Preventive Veterinary Medicine 55*, 241-253.

Sokolow S.H., Rand C., Marks S.L., Drazenovich N.L., Kather E.J., Foley J.E. (2005). Epidemiologic evaluation of diarrhea in dogs in an animal shelter. American *Journal of Veterinary Research 66*, 1018-1024.

Steinhauserova I., Fojtikova K., Klimes J. (2000). The incidence and PCR detection of *Campylobacter upsaliensis* in dogs and cats. *Letters of Applied Microbiology 31*, 209-212.

Wolfs T.F., Duim B., Geelen S.P., Rigter A., Thomson-Carter F., Fleer A. (2001). Neonatal sepsis by *Campylobacter jejuni*: genetically proven transmission from a household puppy. *Clinical Infectious Diseases 32*, E97-99.