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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Identification of *Campylobacter* spp. with multiplex PCR assay in healthy and diarrheic dogs

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Abstract

Campylobacter is an important and common cause of human gastroenteritis. Dogs are considered as reservoir of *Campylobacter* spp. and can infect people and other animals by shedding these organisms in stool. In this study, prevalence of *Campylobacter* and its species in diarrheic and healthy dogs were determined. Faecal samples from 75 healthy and 75 diarrheic dogs were acquired and assessed for the presence of *campylobacter* species by Multiplex PCR. *Campylobacter* spp. prevalence was significantly more in diarrheic group (45.3% and 64.0% in healthy and diarrheic groups respectively). Out of 82 positive samples, 52.4% contained only *C. jejuni*, and 37.8% *C. upsaliensis*, whereas 9.8% harboured both species. The age had a significant effect on *Campylobacter* infection and the highest shedding prevalence was observed in young diarrheic dogs (less than 6 months). According to the results of this study, diarrheic and young pets should be considered as important source of zoonotic pathogen for humans.

Keywords: *Campylobacter* spp.; diarrhoea; dogs; multiplex PCR

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Introduction

Campylobacteriosis is a universal and common enteric disease of humans, which is caused by bacteria of the genus *Campylobacter*, mainly *C. jejuni* and *C. coli*. However, other species of this genus such as *C. upsaliensis* have also been recognized as causative pathogens (Tsai et al., 2006; Yamazaki-Matsune et al., 2007; Chaban et al., 2010; Parsons et al., 2010).

In developed countries, the most important routes of *Campylobacter* transmission are consumption of undercooked poultry meat, unpasteurized milk, or contaminated water (Hald and Madsen, 1997; Tsai et al., 2006). Birds, cows, sheep, pigs, dogs and cats have been documented as *Campylobacter* reservoirs (Chaban et al., 2010). Pet ownership, especially dogs and cats, is considered a significant risk factor for transmission of this zoonotic disease (Hald and Madsen, 1997; Steinhauserova et al., 2000; Koene et al., 2009; Chaban et al., 2010; Parsons et al., 2010); with studies that demonstrated correlation between *C. jejuni* (Damborg et al., 2004) and *C. upsaliensis* (Lentzsch et al., 2004) infection in dogs and their owners. By shedding bacteria in their stools, reservoir dogs can infect their owners and other animals (Parsons et al., 2010; Fox, 2012).

Most of the infected dogs are asymptomatic, and the clinical disease usually occurs in puppies under 6 months. Campylobacteriosis usually manifests with diarrhoea (from loose feces to bloody mucoid), anorexia, vomiting, and fever (Fox, 2012).

Various methods include direct microscopy, culture, serology, PCR and etc. were developed to detect *Campylobacter* infection (Fox, 2012). Conventional culture methods for *Campylobacter* identification are limited by fastidious and slowly growth nature of this organism (Wang et al., 2002; Chaban et al., 2009; Wangroongsarb et al., 2011).

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Similarity between biochemical profiles of several species of *Campylobacter* and other bacteria such as *Arcobacter* and *Helicobacter* spp. also confuse definitive identification based on biochemical assays (Hill et al., 2006; Yamazaki-Matsune et al., 2007; Chaban et al., 2009). So, various DNA-based methods have been established to resolve these limitations (Persson and Olsen, 2005; Hill et al., 2006; Chaban et al., 2009). Among these methods, multiplex PCR has some advantages such as rapid and reliable detection of *Campylobacter* strains and clinical isolates from species by high specificity (Wang et al., 2002; Persson and Olsen, 2005; Wangroongsarb et al., 2011).

Role and pathogenicity of *Campylobacter* strains of dogs remains inconclusive. Despite significant importance, no study has yet been undertaken regarding *Campylobacter* infections in dogs and cats, especially in carrier state, in Iran. So, the goal of this study was determine the prevalence of *Campylobacter* and its species as zoonotic pathogens in diarrheic and healthy dogs.

Materials and Methods

Sample collection

Dogs referred to small animal hospital of Faculty of Veterinary Medicine, University of Tehran were considered as target population. Faecal samples were collected by rectal swabs from 75 healthy and 75 diarrheic animals. Healthy group selected from dogs that were visited for routine examination or vaccination and they were considered healthy by their owners. Diarrheic group was confirmed by the presence of diarrhoea regardless of the etiology. Only one pet from each household was included in this study. Furthermore, age of animals was recorded to assess the effect of age on *campylobacter* prevalence.

Finally, rectal swabs were stored at -20°C until DNA extraction.

DNA extraction

Extraction of bacterial DNA was performed using commercial stool DNA extraction kit (AccuPrep, Bioneer, Korea) according to the manufacturer's instructions with only slight modification. The DNA was eluted into 100 µl volume for higher concentration and stored at -20°C before PCR analysis.

Multiplex PCR

To detect presence of *Campylobacter* and identify the most common species, Multiplex PCR technique was done as previously described (Yamazaki-Matsune et al., 2007). Primers were used for PCR are listed in Table 1.

Multiplex PCR was carried out in a final volume of 25 µl containing 2.5 µl of 10X PCR buffer (500 mM KCl, 200 mM Tris-HCl, SinaClon, Iran), 0.5 µl of dNTP mixes (10 mM, SinaClon, Iran), 2mM $MgCl_2$ (50 mM, SinaClon, Iran), 2 µl template DNA, 0.2 µM of primers C412F, C1228R, C-1, C-3, CC18F, CC519R, CU61F, CU146R, MG3F, CF359R, CLF, CLR, HYO1F and HYOFET23SR; and 1U of CinnaGen Smar *Taq* DNA polymerase (SinaClon, Iran). Amplification of DNA was done in a Techne TC-512 Thermal Cycler (Techne TC- 512, England). The PCR conditions were 95°C for 15 min followed by 25 cycles of 95°C for 0.5 min, 58°C for 1.5 min and 72°C for 1 min, and finally 72°C for 7 min.

The PCR products were analyzed by electrophoresis in 2% (w/v) agarose gel in TBE buffer (0.5X), then stained with ethidium bromide, and visualized under UV light.

Sequencing analysis was performed on one of each *C. jejuni* and *C. upsaliensis* isolates to compare with the National Center for Biotechnology Information GenBank database for best matches and used as positive control.

Table 1: Characteristics of multiplex PCR primers and products sizes for detection of *Campylobacter* species

Species	Size (bp)	Target gene	Primer	Sequence (5' to 3')
Genus *Campylobacter*	816	16S rRNA	C412F	5'-GGATGACACTTTTCGGAGC-3'
			C1228R	5'-CATTGTAGCACGTGTGTC-3'
C. hyointestinalis subsp. *hyointestinalis*	611	23S rRNA	HYO1F	5'-ATAATCTAGGTGAGAATCCTAG-3'
			HYOFET23SR	5'-GCTTCGCATAGCTAACAT-3'
C. coli	502	ask	CC18F	5'-GGTATGATTTCTACAAAGCGAG-3'
			CC519R	5'-ATAAAAGACTATCGTCGCGTG-3'
C. fetus	359	cstA	MG3F	5'-GGTAGCCGCAGCTGCTAAGAT-3'
			CF359R	5'-AGCCAGTAACGCATATTATAGTAG-3'
C. lari	251	glyA	CLF	5'-TAGAGAGATAGCAAAAGAGA-3'
			CLR	5'-TACACATAATAATCCCACCC-3'
C. jejuni	161	cj0414	C-1	5'-CAAATAAAGTTAGAGGTAGAATGT-3'
			C-3	5'-CCATAAGCACTAGCTAGCTGAT-3'
C. upsaliensis	86	lpxA	CU61F	5'-CGATGATGTGCAAATTGAAGC-3'
			CU146R	5'-TTCTAGCCCCTTGCTTGATG-3'

Yamazaki-Matsune et al. (2007)

Statistical analysis

Finally, statistical analysis of data was carried out with SPSS software (version 21; SPSS Inc.) and P<0.5 was considered significant.

Results

Stool samples from 75 healthy and 75 diarrheic dogs were acquired and assessed for the presence of *campylobacter* species in this study. So, DNA extraction from rectal swabs was done and specimens analyzed by multiplex PCR for identification of 6 *campylobacter* species (Fig. 1).

Two different samples subjected for sequencing analysis best matched with *C. jejuni* and *C. upsaliensis* from Genbank database and confirmed precision of multiplex PCR assay.

Thirty-four out of 75 (45.3%) healthy samples were positive for *Campylobacter* spp. while in diarrheic group, 48 (64.0%) positive cases were detected. The difference between these groups was statistically significant (P = 0.022).

In healthy dogs, *C. jejuni* identified in 17 (50%) and *C. upsaliensis* in 13 (38.2%) of *campylobacter* positive samples, whereas 4 (11.8%) samples contained both species. Twenty-six (54.2%) *C. jejuni* and 18 (37.5%) *C. upsaliensis* were detected in diseased group and 4 (8.3%) specimens were positive for both of them (Table 2). *C. hyointestinalis*, *C. coli*, *C. fetus* and *C. lari* were not identified in any samples.

Among *Campylobacter* negative animals included in this study, 30 (44.1%) were under 6 months and 38 (55.9%) were more than 6 months, while in positive samples 50 (61%) and 32 (39%) dogs were existed younger and older than 6 months respectively. Despite insignificant correlation between age and Campylobacter shedding within healthy and diarrheic groups separately, the influence of age on *Campylobacter* shedding was generally determined to be significant (P = 0.039) by statistical analysis. Prevalence of *Campylobacter* according to the age is summarized in Table 3.

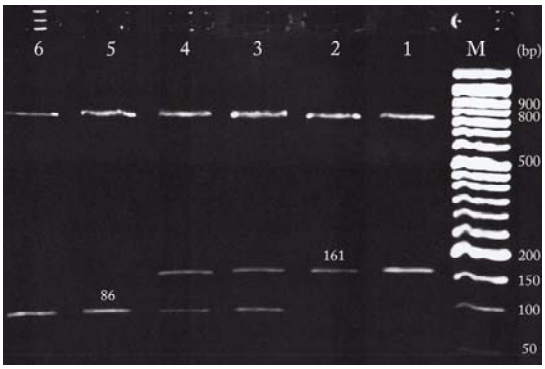

Fig. 1: Electrophoresis analysis of multiplex PCR assay for identification Campylobacter species. M: 50 bp DNA Ladder (CinaColon, Iran). Lane 1: *C. jejuni* positive sample. Lane 2: *C. jejuni* positive control. Lane 3 and 4: Samples with both *C. jejuni* and *C. upsaliensis* infection. Lane 5: *C. upsaliensis* positive control. Lane 6: *C. upsaliensis* positive sample. Lane 7: Negative control

Table 2: Frequency of *Campylobacter* species in healthy and diarrheic groups

Health Statues	*Campylobacter* Species			Total
	C. jejuni	*C. upsaliensis*	Both	
Healthy	17	13	4	34
Diarrheic	26	18	4	48
Total	43	31	8	82

Discussion

Campylobacter is one of the most important causes of bacterial gastroenteritis in humans (Tsai et al., 2006; Yamazaki-Matsune et al., 2007; Chaban et al., 2010; Parsons et al., 2010). *Campylobacter jejuni* is commonly associated with diarrhoea in dogs and cats. Other *campylobacter* species such as *C. coli* and *C. upsaliensis* have also been isolated from asymptomatic and diarrheic dogs and cats and their prevalence is raising recently (Fox, 2012). Dogs and cats owners' are at increased risk of *campylobacter* transmission (Hald and Madsen, 1997; Steinhauserova et al., 2000; Koene et al., 2009; Chaban et al., 2010; Parsons et al., 2010). This zoonotic disease has gained importance since there has been increasing interest in pet owning in Iran. So, the present study was designed to provide information on *Campylobacter* prevalence and its species in diarrheic and healthy dogs to the people of Iran.

The overall prevalence of *Campylobacter* spp. in this study was 54.7% (82 out of 150 samples). In comparison to our results, other researchers reported 38% to 77% prevalence of *Campylobacter* spp. (Koene et al., 2004; Acke et al., 2009; Parsons et al., 2010).

We found *Campylobacter* spp. in 45.3% of healthy dogs and 64.0% in diarrheic animals. Chaban et al. (2010) detected *Campylobacter* spp. shedding in 58% of healthy and 97% of diarrheic dogs by qPCR.

Only 2 species of *Campylobacter*, *C. jejuni* (61.8% in normal group, 62.5% in diseased dogs) and *C. upsaliensis* (50% and 45.8% in healthy and diarrheic dogs respectively), were detected in this study. Similar to our results, in some studies *C. jejuni* was the most common species isolated (Tsai et al., 2007). The prevalence of *C. jejuni* varies from 3 to 40% in different studies (Lopez et al., 2002; Koene et al., 2004; Tsai et al., 2007). Twenty-nine percent of diarrheic and 4% of healthy dogs harboured *C. jejuni* in one study (Dillon et al., 1987). Hald and Madsen (1997) also reported *C. jejuni* (76%) as the most prevalent species followed by *C. upsaliensis* (19%) and *C. coli* (5%). In

Table 3: Prevalence of *Campylobacter* spp. according to the age in healthy and diarrheic dogs

Campylobacter statues	Healthy Group			Diarrheic Group			Total		
	< 6 months	> 6 months	Sum	< 6 months	> 6 months	Sum	< 6 months	> 6 months	Sum
Positive	18	16	34	32	16	48	50	32	82
Negative	17	24	41	13	14	27	30	38	68
Total	35	40	75	45	30	75	80	70	150

another study performed in Barbados, *C. jejuni* had the highest prevalence (cultured from 51.5% of dogs), whereas *C. upsaliensis* was isolated in 30% (Workman et al., 2005).

C. *upsaliensis* has been discovered to be the predominant species in some other studies, ranging from 17 to 59% (Sandberg et al., 2002; Engvall et al., 2003; Hald et al., 2004; Koene et al., 2004; Rossi et al., 2008). In contrast to our results, the most prevalent *Campylobacter* species isolated from dogs and cats was *C. upsaliensis*, and *C. jejuni* (Acke et al., 2009).

Interestingly, in the study of Parsons et al. (2010) *Campylobacter upsaliensis* identification rate was 98% and *Campylobacter jejuni* prevalence was only 2%. Furthermore, *C. upsaliensis* was the predominant species detected in the study of Chaban et al. (2010). Detection rate of *C. jejuni* was 7 and 46% in normal and diarrheic population of dogs respectively. It appears that differences between isolated species in various researches are related to variation between evaluated populations, geographical and environmental conditions, and identification methods. Few studies were performed regarding pathogenicity of different *Campylobacter* species in animals; while in some researches like this study common human-pathogen species (such as *C. jejuni*) were detected to be higher in samples obtained from diarrheic dogs, in other studies unusual human-pathogen species (like *C. upsaliensis*) were more prevalent. No relationship has yet been established between clinical disease and isolated *Campylobacter* species, so this warrants further investigation.

We found 4 (11.8%) cases infected with 2 species of *campylobacter* in healthy group. Four (8.3%) dogs were observed to have more than one species among diarrheic animals. Our findings are in accordance with other studies. The highest identified numbers of species per dog was 7 in healthy group and 12 in diarrheic animals (Chaban et al., 2010). Consequently, it seems that infection with more than one species of *Campylobacter* has no significant effect on disease status in dogs.

The differences mentioned between various studies in isolation rates and detected species might be due to variations in target populations and identification methods. Conventional diagnostic methods such as culture and biochemical tests are time-consuming and sometimes unable to differentiate between *Campylobacter* spp. and from other similar bacteria (Wang et al., 2002; Hill et al., 2006; Yamazaki-Matsune et al., 2007; Chaban et al., 2009; Wangroongsarb et al., 2011). Therefore, various molecular methods have been demonstrated to identify *Campylobacter* spp. based on different genes (Gonzalez et al., 1997; Lawson et al., 1998; Eyigor et al., 1999; Al Rashid et al., 2000; Bang et al., 2002; Engvall et al., 2002; Englen & Fedorka-Cray, 2002; Volokhov et al., 2003; LaGier et al., 2004, Hill et al., 2006; Chaban et al., 2009). It was demonstrated that the PCR method has more sensitivity in identifying small numbers of organisms and can be used for direct detection from stool (Wangroongsarb et al., 2011).

In contrast, some researchers reported that PCR failed to detect bacteria from samples which were positive by culture methods (Persson and Olsen, 2005; Parsons et al., 2010).

In the present study, we used Multiplex PCR to detect 6 different *Campylobacter* species, including *C. jejuni, C. upsaliensis, C. hyointestinalis, C. coli, C. fetus* and *C. lari*.

Role and pathogenic significance of *Campylobacter* strains of dogs remains inconclusive and it is still uncertain if any correlation exists between the carrier state and the development of clinical campylobacteriosis in dogs (Parsons et al., 2010). The prevalence of *Campylobacter* spp. was significantly higher in diarrheic dogs in our study. In contrast to some researchers that did not observed significant relationship between diarrhoea and *Campylobacter* infection (Sandberg et al., 2002; Engvall et al., 2003; Workman et al., 2005; Rossi et al., 2008), others found an association between infection and clinical signs (Guest et al., 2007). The prevalence of *Campylobacter* spp. was reported to be greater in dogs and cats suffered from acute or chronic gastro-enteritis (Steinhauserova et al., 2000). Also, higher prevalence was observed in dogs suffering from diarrhoea (Dillon et al., 1987; Acke et al., 2009).

In another study, *Campylobacter* spp. detection was remarkably higher in diarrheic dogs (97% compared to 58% in healthy animals); most of the *Campylobacter* species were significantly more prevalent in diarrheic group, while some had the same prevalence between these groups. In addition, 5 species were only detected in diarrheic specimens (Chaban et al., 2010). In the same study, the levels of bacteria shedding in dog stools were similar between two groups, despite the etiology of the diarrhoea. Hence, the raise in the ratio of *Campylobacter* shed in faeces compared to the total bacterial population was because

of raise in *Campylobacter* shedding during diarrhoea (Chaban et al., 2010).

Campylobacter shedding rate in animals younger than 6 months was 53.3% in this study and determined to be significantly more than older group (46.6%). *Campylobacter* species shedding in pups ranges from about 5 to 90% (Fox, 2012). Hald and Madsen (1997) also identified *Campylobacter* spp. in 29% of the puppies (11 to 17 weeks old) by culture.

In according to our findings, many researchers indicated that younger dogs were more likely to be carriers of *Campylobacter* spp. when age was assessed as a risk factor, and it might be due to lack of previous encounter and protective immunity (Sandberg et al., 2002; Wieland et al., 2005; Acke et al., 2006; Parsons et al., 2010; Fox, 2012). *C. jejuni* prevalence in clinically normal dogs was stated to be significantly higher in cases younger than 6 months old (Torre and Tello, 1993). Also, Acke et al. (2009) identified higher prevalence in dogs younger than six months old. However, in small numbers of reports, no correlation was expressed between the age and *Campylobacter* spp. shedding in dogs (Steinhauserova et al., 2000; Tsai et al., 2007).

Conclusions

According to the results of this study, it was determined that *Campylobacter* spp. shedding in diarrheic animals and dogs younger than 6 months is higher. *Campylobacter* is an important cause of enteric disease in humans, and dogs can serve as the sources of infection. The fact that a few hundred bacteria can cause clinical disease in people shows the importance of this issue. So, veterinarians should warn pet owners regarding the zoonotic potential of this organism, especially in younger and diarrheic pets.

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References

Acke, E., McGill, K., Golden, O., Jones, B.R., Fanning, S. and Whyte, P. 2009. Prevalence of thermophilic Campylobacter species in household cats and dogs in Ireland. Veterinary Record, 164: 44-47.

Acke, E., Whyte, P., Jones, B.R., McGill, K., Collins, J.D. and Fanning S. 2006. Prevalence of thermophilic Campylobacter species in cats and dogs in two animal shelters in Ireland. Veterinary Record, 158: 51-54.

Al Rashid, S.T., Dakuna, I., Louie, H., Ng, D., Vandamme, P., Johnson, W. and Chan, V.L. 2000. Identification of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, Arcobacter butzleri, and A. butzleri-like species based on the glyA gene. Journal of Clinical Microbiology, 38: 1488-1494.

Bang, D.D., Wedderkopp, A., Pedersen, K. and Madsen, M. 2002. Rapid PCR using nested primers of the 16S rRNA and the hippuricase (hip O) genes to detect Campylobacter jejuni and Campylobacter coli in environmental samples. Molecular and Cellular Probes, 16: 359-369.

Chaban, B., Musil, K.M., Himsworth, C.G. and Hill, J.E. 2009. Development of cpn60-based real-time quantitative PCR assays for the detection of 14 Campylobacter species and application to screening of canine fecal samples. Applied and Environmental Microbiology, 75: 3055-61.

Chaban, B., Ngeleka, M. and Hill, J.E. 2010. Detection and quantification of 14 Campylobacter species in pet dogs reveals an increase in species richness in feces of diarrheic animals. BMC Microbiology, 10: 73-79.

Damborg, P., Olsen, K.E., Moller Nielsen E. and Guardabassi, L. 2004. Occurrence of Campylobacter jejuni in pets living with human patients infected with C. Jejuni. Journal of Clinical Microbiology, 42: 1363-1364.

Dillon, A.R., Boosinger, T.R. and Blevins, W.T. 1987. Campylobacter enteritis in dogs and cats. Compendium on Continuing Education for the Practising Veterinarian, 9: 1176-1183.

Englen, M.D. and Fedorka-Cray, P.J. 2002. Evaluation of a commercial diagnostic PCR for the identification of Campylobacter jejuni and Campylobacter coli. Letters in Applied Microbiology, 35: 353-356.

Engvall, E.O., Brandstrom, B., Andersson, L., Baverud, V., Trowald-Wigh, G. and Englund, L. 2003. Isolation and identification of thermophilic Campylobacter species in faecal samples from Swedish dogs. Scandinavian Journal of Infectious Diseases, 35: 713-718.

Engvall, E.O., Brandstrom, B., Gunnarsson, A., Morner, T., Wahlstrom, H. and Fermer, C. 2002. Validation of a polymerase chain reaction/restriction enzyme analysis method for species identification of thermophilic campylobacters isolated from domestic and wild animals. Journal of Applied Microbiology, 92: 47-54.

Eyigor, A., Dawson, K.A., Langlois, B.E. and Pickett, C.L. 1999. Cytolethal distending toxin genes in Campylobacter jejuni and Campylobacter coli

isolates: detection and analysis by PCR. Journal of Clinical Microbiology, 37: 1646-1650.

Fox, J.G. 2012. Campylobacter infections, in: Greene, C.E. (editor), Infectious Diseases of the Dogs and Cats. Saunders, St Louis, pp: 370-374.

Gonzalez, I., Grant, K.A., Richardson, P.T., Park, S.F. and Collins, M.D. 1997. Specific identification of the enteropathogens Campylobacter jejuni and Campylobacter coli by using a PCR test based on the ceuE gene encoding a putative virulence determinant. Journal of Clinical Microbiology, 35: 759-763.

Guest, C.M., Stephen, J.M. and Price, C.J. 2007. Prevalence of Campylobacter and four endoparasites in dog populations associated with hearing dogs. Journal of Small Animal Practice, 48: 632-637.

Hald, B. and 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.

Hald, B., Pedersen, K., Waino, M., Jorgensen, J.C. and Madsen, M. 2004. Longitudinal study of the excretion patterns of thermophilic Campylobacter spp. in young pet dogs in Denmark. Journal of Clinical Microbiology, 42: 2003-2012.

Hill, J.E., Paccagnella, A., Law, K., Melito, P.L., Woodward, D.L., Price, L., Leung, A.H., Ng, L.K., Hemmingsen, S.M. and Goh, S.H. 2006. Identification of Campylobacter spp. And discrimination from Helicobacter and Arcobacter spp. by direct sequencing of PCR-amplified cpn60 sequences and comparison to cpnDB, a chaperonin reference sequence database. Journal of Medical Microbiology, 55: 393-399.

Koene, M.G., Houwers, D.J., Dijkstra, J.R., Duim, B. and Wagenaar, J.A. 2004. Simultaneous presence of multiple Campylobacter species in dogs. Journal of Clinical Microbiology, 42: 819-821.

Koene, M.G., Houwers, D.J., Dijkstra, J.R. Duim, B. and Wagenaar, J.A. 2009. Strain variation within Campylobacter species in fecal samples from dogs and cats. Veterinary Microbiology, 133: 199-205.

LaGier, M.J., Joseph, L.A., Passaretti, T.V., Musser, K.A. and Cirino, N.M. 2004. A real-time multiplexed PCR assay for rapid detection and differentiation of Campylobacter jejuni and Campylobacter coli. Molecular and Cellular Probes, 18: 275-282.

Lawson, A.J., Shafi, M.S., Pathak, K. and Stanley, J. 1998. Detection of campylobacter in gastroenteritis: comparison of direct PCR assay of faecal samples with selective culture. Epidemiology & Infection, 121: 547-553.

Lentzsch, P., Rieksneuwohner, B., Wieler, L.H., Hotzel, H. and Moser, I. 2004. Highresolution genotyping of Campylobacter upsaliensis strains originating from three continents. Journal of Clinical Microbiology, 42: 3441-3448.

Lopez, C.M., Giacoboni, G., Agostini, A., Cornero, F.J., Tellechea, D.M. and Trinidad, J.J. 2002. Thermotolerant campylobacters in domestic animals in a defined population in Buenos Aires, Argentina. Preventive Veterinary Medicine, 55: 193-200.

Parsons, B.N., Porter, C.J., Ryvar, R., Stavisky, J., Williams, N.J., Pinchbeck, G.L., Birtles, R.J., Christley, R.M., German, A.J., Radford, A.D., Hart, C.A., Gaskell, R.M. and Dawson, S. 2010. Prevalence of Campylobacter spp. in a cross-sectional study of dogs attending veterinary practices in the UK and risk indicators associated with shedding. The Veterinary Journal, 184: 66-70.

Persson, S. and Olsen, K.E.P. 2005. Multiplex PCR for identification of Campylobacter coli and Campylobacter jejuni from pure cultures and directly on stool samples. Journal of Medical Microbiology, 54: 1043-1047.

Rossi, M., Hänninen, M.L., Revez, J., Hannula, M. and Zanoni, R.G. 2008. Occurrence and species level diagnostics of Campylobacter spp., enteric Helicobacter spp. and Anaerobiospirillum spp. in healthy and diarrheic dogs and cats. Veterinary Microbiology, 129: 304-314.

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

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

Torre, E. and Tello, M. 1993. Factors influencing fecal shedding of Campylobacter jejuni in dogs without diarrhoea. American Journal of Veterinary Research, 54: 260-262.

Tsai, H.J., Huang, H.C., Lin, C.M., Lien, Y.Y. and Chou, C.H. 2007. Salmonella and Campylobacters in household and stray dogs in Northern Taiwan. Veterinary Research Communications, 31: 931-939.

Tsai, H.J., Huang, H.C., Tsai, H.L. and Chang, C.C. 2006. PCR-Based Restriction Fragment Length Polymorphism (RFLP) Analysis of Campylobacter jejuni isolates from Humans, Chickens and Dogs in Northern Taiwan. Journal of Veterinary Medical Science, 68: 815-819.

Volokhov, D., Chizhikov, V., Chumakov, K. & Rasooly, A. 2003. Microarray-based identification of thermophilic Campylobacter jejuni, C. coli, C. lari, and C. upsaliensis. Journal of Clinical Microbiology, 41: 4071-4080.

Wang, G., Clark, C.G., Taylor, T.M., Pucknell, C., Barton, C., Price, L., Woodward, D.L. and Rodgers, F.G. 2002. Colony Multiplex PCR Assay for Identification and Differentiation of Campylobacter jejuni, C. coli, C. lari, C. upsaliensis, and C. fetus subsp. Fetus. Journal of Clinical Microbiology, 40: 4744.

Wangroongsarb, P., Jittaprasatsin, C., Suwannasing, S., Suthivarakom, K., and Khamthalang, T. 2011. Identification of Genus Campylobacter and Four Enteropathogenic Campylobacter Species by PCR. The Journal of Tropical Medicine and Parasitology, 34: 17-29.

Wieland, B., Regula, G., Danuser, J., Wittwer, M., Burnens, A.P., Wassenaar, T.M. and Stark, K.D. 2005. Campylobacter spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. Journal of veterinary medicine. B, Infectious diseases and veterinary public health, 52: 183-189.

Workman, S.N., Mathison, G.E. and Lavoie, M.C. 2005. Pet dogs and chicken meat as reservoirs of Campylobacter spp. in Barbados. Journal of Clinical Microbiology, 43: 2642-2650.

Yamazaki-Matsune, W., Taguchi, M., Seto, K., Kawahara, R., Kawatsu, K., Kumeda, Y., Kitazato, M., Nukina, M., Misawa, N. and Tsukamoto, T. 2007. Development of a multiplex PCR assay for identification of Campylobacter coli, Campylobacter fetus, Campylobacter hyointestinalis subsp. hyointestinalis, Campylobacter jejuni, Campylobacter lari and Campylobacter upsaliensis. Journal of Medical Microbiology, 56: 1467-1473.