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Original article

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

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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 the frequency of *C. jejuni* and *C. coli* isolation from healthy cats and dogs living in Bydgoszcz region. Indirect aim of the work was to determine susceptibility to drugs, similarity among *C. jejuni* and *C. coli* strains and to estimate occurrence of pathogenic genes: *cadF*, *flaA*, *cdtB* and *iam*.

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 71 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.

Amplification of virulence genes. The presence of the *cadF*, *flaA*, *cdtB* 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 (bioMeriuex) 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 feaces. 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.

Animal	No. of samples	No. of *Campylobacter* spp. (%)	Isolated species
Dog	83	4 (4.81)	*C. jejuni* (2), *C. coli* (2)
Cat	71	7 (9.86)	*C. jejuni* (6), *C. coli* (1)
Total	154	11 (7.14)	*C. jejuni* (8), *C. coli* (3)

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	cat	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 house-holds	private house-holds	farm	farm	farm	farm	private house-holds	private house-holds
Season of a year	autumn	autumn	winter	autumn	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. coli*	*C. coli*
Antimicrobial susceptibility	EM – S AZ – R CI – R TC – S	EM – S AZ – S CI – R TC – S	EM – R AZ – R CI – R TC – S	EM – S AZ – S CI – S TC – S	EM – S AZ – S CI – S TC – R	EM – S AZ – S CI – S TC – S	EM – S AZ – S CI – R TC – R	EM – S AZ – S CI – R TC – R	EM – S AZ – S CI – R TC – S	EM – S AZ – S CI – R TC – R	EM – S AZ – S CI – S TC – S
Virulence genes	cadF (+) flaA (+) iam (–) cdtB (+)	cadF (+) flaA (+) iam (+) cdtB (+)	cadF (+) flaA (+) iam (–) cdtB (–)	cadF (+) flaA (+) iam (+) cdtB (+)	cadF (+) flaA (+) iam (–) cdtB (–)	cadF (+) flaA (+) iam (–) cdtB (+)	cadF (+) flaA (+) iam (–) cdtB (+)	cadF (+) flaA (+) iam (–) cdtB (+)	cadF (+) flaA (+) iam (+) cdtB (+)	cadF (+) flaA (+) iam (–) cdtB (–)	cadF (+) flaA (+) iam (–) cdtB (+)
RAPD-PCR	80% homology with sample 2	80% homology with sample 1	no similarity affirm	no similarity affirm	no similarity affirm	no similarity affirm	no similarity affirm	no similarity affirm	no similarity affirm	no similarity affirm	no similarity affirm

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.

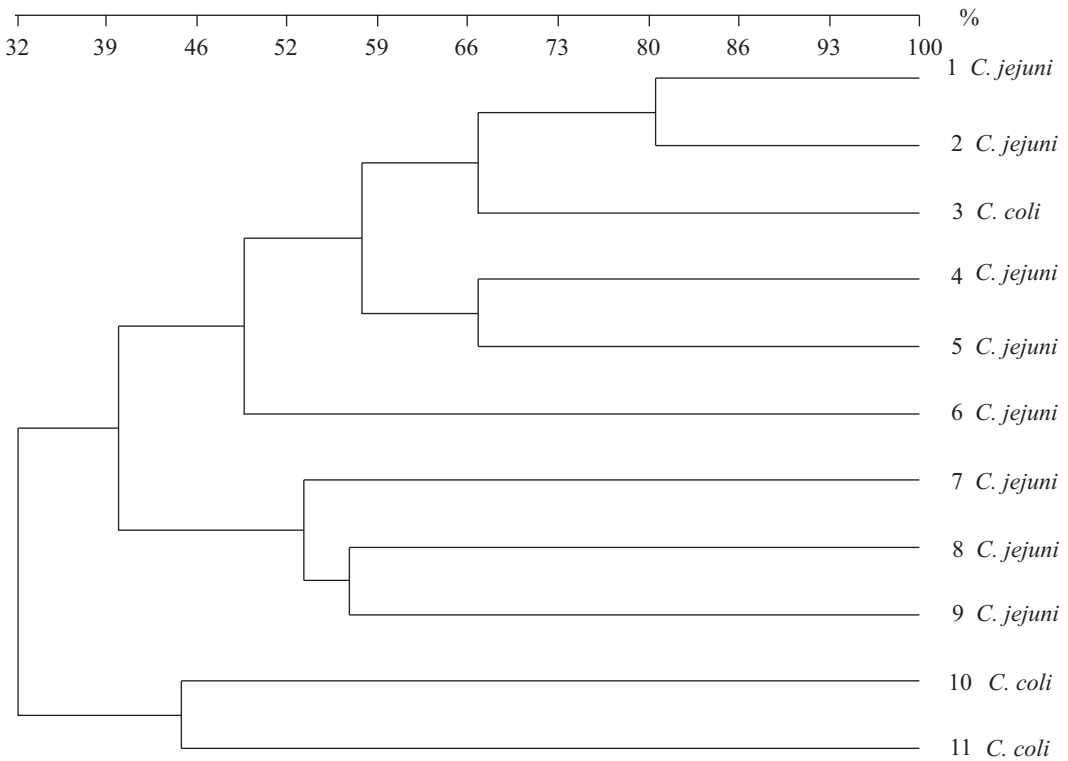

Fig. 1. Dendrogram of chromosomal DNA profiles of *C. jejuni* and *C. coli* strains isolated from cats and dogs.

Table 3. Prevalence of *Campylobacter* spp. species in cats and dogs.

Country	Dogs			Cats		
	n	positive samples for *Campylobacter* (%)	predominate species	n	positive samples for *Campylobacter* (%)	predominate species
Switzerland	634	41.2	*C. upsaliensis*	596	41.9	*C. upsaliensis*
Canada	70	58	*C. upsaliensis*	–	–	–
Brazil	76	17	*C. jejuni*	12	8	*C. jejuni*
Sweden	91	56	*C. upsaliensis*	–	–	–
Ireland	147	41.5	*C. upsaliensis*	35	42.9	*C. upsaliensis*
Great Britain	249	38	*C. upsaliensis*	–	–	–
Barbados	130	46.9	*C. jejuni*	51	37.3	*C. helveticus*
Germany	261	32.7	*C. upsaliensis*	46	47.8	*C. helveticus*
Denmark	366	76.2	*C. upsaliensis*	–	–	–
Nigeria	141	27.7	*C. upsaliensis*	104	18.3	*C. upsaliensis*
Norway	529	23	*C. upsaliensis*	301	18	*C. upsaliensis*
Argentina	293	17	*C. jejuni*	64	16	*C. jejuni*

Discussion

Although the most often isolated species from animals is *C. upsaliensis*, in humans this species causes only sporadic infections. 80% human campylobacteriosis has *C. jejuni* or less *C. coli* etiology (Sandberg et al. 2002, Wieland 2005, Acke et al. 2009). As there is increasing number of confirmed *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 *C. jejuni* and *C. coli* in dogs and cats were not conducted in Poland so far.

In this study the occurrence of *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 *Campylobacte*r 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 feaces 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

Acke E, Carroll C, O'Leary A, McGill K, Kelly L, Lawlor A, Madden RH, Moran L, Scates P, McNamara E, Moore JE, Jones BR, Fanning S, Whyte P (**2011**) Genotypic characterization and cluster analysis of *Campylobacter jejuni* isolates from domestic pets, human clinical cases and retail food. Ir Vet J 64: 6.

Acke E, McGill K, Golden O, Jones BR, Fanning S, Whyte P (**2009**) Prevalence of thermophilic *Campylobacter* species in household cats and dogs in Ireland. Vet Rec 164: 44-47.

Acke E, McGill K, Lawlor A, Jones BR, Fanning S, Whyte P (**2010**) Genetic diversity among *Campylobacter jejuni* isolates from pets in Ireland. Vet Rec 166: 102-106.

Andrzejewska M, Klawe JJ, Szczepańska B, Śpica D (**2011**) Occurence of virulence genes among *Campylobacter jejuni* and *Campylobacter coli* isolates from domestic animals and children. Pol J Vet Sci 14: 207-211.

Aquino MH, Pacheco AP, Ferreira MC, Tibana A (**2002**) Frequency of isolation and identification of thermophilic *Campylobacters* from animals in Brazil. Vet J 164: 159-161.

Bang DD, Scheutz F, Ahrens P, Pedersen K, Blom J, Madsen M (**2001**) Prevalence of cytolethal distending toxin (cdt)

genes and CDT production in *Campylobacter* spp. isolated from Danish broilers. J Med Microbiol 50: 1087-1094.

Chaban B, Ngeleka M, Hill JE (**2010**) Detection and quantification of 14 *Campylobacter* species in pet dogs reveals an increase in species richness in feces of diarrheic animals. BMC Microbiol 10: 73.

Engvall EO, Brandstrom B, Andersson L, Baverud V, Trowald-Wigh G, Englund L (**2003**) Isolation and identification of thermophilic *Campylobacter* species in faecal samples from Swedish dogs. Scand J Infect Dis 35: 713-718.

Hald B, Pedersen K, Wainz M, Jzrgensen JC, Madsen M (2004) Longitudinal study of the excretion patterns of thermophilic *Campylobacter* spp. in young pet dogs in Denmark. J Clin Microbiol 42: 2003-2012.

Hernandez J, Fayos A, Ferrus MA, Owen RJ (**1995**) Random amplified polymorphic DNA fingerprinting of *Campylobacter jejuni* and *C. coli* isolated from human faeces, seawater and poultry products. Res Microbiol 146: 685-696.

Konkel ME, Gray SA, Kim BJ, Garvis SG, Yoon J (**1999**) Identification of enteropathogens *Campylobacter jejuni* and *Campylobacter coli* based on the *cadF* virulence gene and its product. J Clin Microbiol 37: 510-517.

Krutkiewicz A, Klimuszko D (**2010**) Genotyping and PCR detection of potential virulence genes in *Campylobacter jejuni* and *Campylobacter coli* isolates from different sources in Poland. Folia Microbiol (Praha) 55: 167-175.

Krutkiewicz A, Sałamaszyńska-Guz A, Rzewuska M, Klimuszko D, Binek M Resistance to antimicrobial agents of *Campylobacter* spp. strains isolated from animals in Poland. Pol J Vet Sci 12: 465-472.

de Lamballerie X, Zandotti C, Vignoli C, Bollet C, de Micco P (**1992**) A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res Microbiol 143: 785-790.

Lopez CM, Giacoboni G, Agostini A, Cornero FJ, Tellechea DM, Trinidad JJ (**2002**) Thermotolerant *Campylobacters* in domestic animals in a defined population in Buenos Aires, Argentina. Prev Vet Med 55: 193-200.

Moser I, Rieksneuwohner B, Lentzsch P, Schwerk P, Wieler LH (**2001**) Genomic heterogeneity and O-antigenic diversity of *Campylobacter upsaliensis* and *Campylobacter helveticus* strains isolated from dogs and cats in Germany. J Clin Microbiol 39: 2548-2557.

Nachamkin I, Bohachick K, Patton CM (**1993**) Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. J Clin Microbiol 31: 1531-1536.

On ST, Jordan PJ (**2003**) Evaluation of 11 PCR assays for species-level identification of *Campylobacter jejuni* and *Campylobacter coli*. J Clin Microbiol 41: 330-336.

Parsons BN, Porter CJ, Ryvar R, Stavisky J, Williams NJ, Pinchbeck GL, Birtles RJ, Christley RM, German AJ, Radford AD, Hart CA, Gaskell RM, 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. Vet J 184: 66-70.

Salihu MD, Magaji AA, Abdulkadir JU, Kolawale A (**2010**) Survey of thermophilic *Campylobacter* species in cats and dogs in north-western Nigeria. Vet Ital 46: 425-430.

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

Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (**1995**) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33: 2233-2239.

The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2010. (**2012**) EFSA Journal 10: 2597.

Wieland B, Regula G, Danuser J, Wittwer M, Burnens AP, Wassenaar TM, Stark KD (**2005**) *Campylobacter* spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. J Vet Med B Infect Dis Vet Public Health 52: 183-189.

Workman SN, Mathison GE, Lavoie MC (**2005**) Pet Dogs and Chicken Meat as Reservoirs of *Campylobacter* spp. in Barbados. J Clin Microbiol 43: 2642-2650.

Zhao S, Young SR, Tong E, Abbott JW, Womack N, Friedman SL, McDermott PF (**2010**) Antimicrobial resistance of *Campylobacter* isolates from retail meat in the United States between 2002 and 2007. Appl Environ Microbiol 76: 7949-7956.