PREVALENCE, ANTIMICROBIAL RESISTANCE AND RISK FACTORS FOR CAMPYLOBACTER COLONISING DOGS AND CATS IN GREECE

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Summary


The study was conducted to determine the prevalence, antimicrobial resistance and risk factors for Campylobacter colonising dogs and cats in Greece. Faecal specimens were collected from 181 dogs and 132 cats. Culture methods were applied to detect Campylobacter spp. and a multiplex PCR assay to identify the isolates. The prevalence of Campylobacter spp. was 3.8% in dogs and 12.1% in cats. The most frequently identified Campylobacter species in dogs was C. jejuni (57.1%) followed by C. coli (42.9%). All feline isolates were identified as C. jejuni apart from one isolate that was characterised as Campylobacter-like organism. Gender, age, breed, life style, diarrhoea and type of diet of dogs and cats did not significantly correlate (P>0.05) with Campylobacter isolation. Possible predictors regarding Campylobacter presence in dogs and cats were assessed by binary logistic regression. A tendency towards higher risk for Campylobacter contamination was observed in dogs consuming a homemade diet and in outdoor cats. Disk diffusion method revealed that all Campylobacter isolates exhibited susceptibility to erythromycin, gentamicin and streptomycin. Contrariwise, 66.7% of canine isolates were resistant concurrently to tetracycline and quinolones and 59.0%, 13.6% and 4.5% of feline isolates were resistant to quinolones, quinolones along with tetracycline and tetracycline alone, respectively.

Key words: antibiotic resistance, Campylobacter, C. jejuni, C. coli, cats, dogs

INTRODUCTION

Campylobacteriosis is the most frequently reported zoonosis of bacterial origin in the EU (EFSA & ECDC, 2015a). Campylobacter species are widely distributed in the gastrointestinal tract of most warm-blooded animals such as farm animals and pets, including cats and dogs, which are usually asymptomatic carriers (WHO, 2011; EFSA & ECDC, 2015a). The vast majority of human campylobacteriosis is
due to foodborne contamination with thermophilic *Campylobacter jejuni* (*C. jejuni*) and *C. coli*, but direct contact with carrier pet animals (faecal-oral route) has been also recognised as a risk factor (Rossi et al., 2008; Kittl et al., 2013; Mughini Gras et al., 2013; EFSA & ECDC, 2015a). In particular, epidemiological data indicate that up to 9.0% of human campylobacteriosis incidents are attributed to *Campylobacter* strains acquired from pet animals (Rossi et al., 2008, Kittl et al., 2013).

Healthy dogs and cats can be potential carriers of *C. jejuni*, *C. coli*, *C. helveticus*, *C. hyointestinalis*, *C. upsaliensis*, *C. lari*, *C. fetus*, *C. gracilis*, *C. curvus*, *C. mucosalis*, *C. rectus*, *C. showae* and *C. sputorum* (Sandberg et al., 2002; Hald et al., 2004; Wieland et al., 2005; Chaban et al., 2010). Isolation of *Campylobacter* spp. from healthy dogs and cats varies considerably among studies from different countries (dogs 5–76%, cats 5–41.9%) and relates to the characteristics of the sample population (e.g. age, life style) and to the applied diagnostic methods (Sandberg et al., 2002; Wieland et al., 2005; Gargiulo et al., 2008; Rossi et al., 2008; Carbonero et al., 2012). *Campylobacter* contamination and the presence of diarrhoea in dogs and cats do not always co-exist since similar patterns of shedding between healthy and diarrhoeic animals have been observed (Sandberg et al., 2002; Rossi et al., 2008; Chaban et al., 2010). It has been reported that animals less than one year old carry campylobacters more often than older animals (Acke et al., 2010; Parsons et al., 2010; Salihu et al., 2010; Carbonero et al., 2012; Amar et al., 2014) and that *Campylobacter* is transmitted between animals of the same or different species when they come in contact (Wieland et al., 2005).

Available data suggest that isolation of *C. upsaliensis* and *C. helveticus* from dogs and cats can exceed that of *C. jejuni* (Hald et al., 2004; Sandberg et al., 2002; Rossi et al., 2008; Chaban et al., 2010; Carbonero et al., 2012) and that there is an epidemiological correlation between these animals and human campylobacteriosis caused by *C. upsaliensis* and *C. jejuni*, especially regarding children (Damborg et al., 2004; Ramonaite et al., 2014). Moreover, Damborg et al. (2004) have reported direct transmission of quinolone-resistant *Campylobacter* from dogs and cats to humans, highlighting the importance of these pet animals to the inter-species spreading of antibiotic-resistant campylobacters.

In Greece, there are no available data regarding the prevalence of *Campylobacter* spp. in dogs and cats. Thus, the objective of the present study was twofold: first, to investigate the role of dogs and cats as reservoirs of campylobacters along with the significance of possible predictors associated with their contamination in the region of Thessaloniki, Greece, and second – to determine the potential antimicrobial resistance of the identified *Campylobacter* isolates.

**MATERIALS AND METHODS**

**Study population and sample collection**

A total of 181 dogs and 132 cats presented at the Companion Animal Clinic of the Veterinary School, Aristotle University of Thessaloniki, with various clinical signs, were randomly selected for the study. One faecal specimen using a sterile cotton-tipped swab was taken directly from the rectum of each individual animal. Owners were asked to complete a questionnaire with information relevant to the history of the animal (gender, age, breed,
life style, diarrhoea and type of diet) and to give their consent for sampling their animals. Regarding life style, dogs and cats that lived indoors but were allowed to spend time outdoors, even occasionally, were regarded as outdoor animals. Complete history data regarding diarrhoea were not available for stray animals (5 dogs and 69 cats) that were about to be sheltered in animal welfare unions and visited the clinic for veterinary care immediately after collection, whereas, consent for sampling these animals was provided by the animal shelter representative. Missing data for these animals were addressed as 'unknown'.

Microbiological analysis

Samples were transported in a temporary culture media (Transwab, Medical Wire & Equipment Co. Ltd., Corsham, England) under refrigeration (<4 °C) to the Laboratory of Hygiene of Food of Animal Origin, School of Veterinary Medicine, Aristotle University of Thessaloniki, where they were analysed within four hours from the time of collection. The rectal swabs were directly inoculated onto a modified Charcoal Cefoperazone Deoxycholate agar (Campylobacter blood-free selective agar, mCCDA, Merck, Germany) and a Karmali agar (Oxoid Ltd., Basingstoke, Hampshire, UK) plate that were subsequently incubated at 41.5 °C for 44±4 h under microaerophilic conditions. Pure cultures were examined for morphology, motility, catalase and oxidase activity and aerobic growth at 25 °C. Isolates were stored at −80 °C in Nutrient Broth No. 2 (Oxoid) supplemented with 5% lyzed horse blood (Oxoid) and 20% glycerol (BDH Laboratory Suppliers, Poole, England) for forthcoming analysis.

Identification of Campylobacter species

DNA was extracted using an in-house developed protocol as previously described (Lazou et al., 2014). The multiplex PCR (m-PCR) assay developed by Wang et al. (2002) was used to identify Campylobacter at species level. The assay simultaneously detects the hipO gene for C. jejuni; the glyA gene for C. coli, C. lari, and C. upsaliensis; and the sapB2 gene for C. fetus subsp. fetus. Moreover, the assay includes a pair of primers as an internal control for Campylobacter 23S rRNA and the corresponding amplicon is detected in Campylobacter, Arcobacter, and Helicobacter isolates. Specific identification was achieved by applying Restriction Fragment Length Polymorphism (RFLP) digestion using the restriction endonucleases BsrD1, AluI, Apol, DdeI, BclI and HhaI, resulting in specific restriction fragments for each Campylobacter species (Wang et al., 2002). The strains C. jejuni ATCC 33291, C. coli ATCC 43478, C. lari ATCC 35221, C. upsaliensis ATCC 43954, and C. fetus subsp. fetus ATCC 25936 were included as positive controls. The strain E. coli ATCC 11303 and a blank were included as negative controls.

Antimicrobial susceptibility testing

The disk diffusion method according to Bauer et al. (1966) was applied in order to screen the antibiotic susceptibility profile
of the Campylobacter isolates towards six critically important antimicrobials as recommended by the World’s Health Organization (WHO, 2009), including ciprofloxacin, erythromycin, gentamicin, nalidixic acid, streptomycin, and tetracycline (BBL-DIFCO Microbiology, Becton, Dickinson and Company, USA). The observed inhibition zones were interpreted according to a) the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (EUCAST, 2013) for Campylobacter as regards ciprofloxacin, erythromycin and tetracycline, b) the Clinical and Laboratory Standards Institute (CLSI) guidelines for Enterobacteriaceae (CLSI, 2010) as regards gentamicin and streptomycin (Maćkiw et al., 2012), and c) the British Society for Antimicrobial Chemotherapy (BSAC) guidelines for Campylobacter (BSAC, 2012) regarding nalidixic acid. The strains C. jejuni ATCC 33560 and C. coli ATCC 33559 were included as quality controls.

Statistical Analysis

Chi-square analysis was used to compare categorical traits between positive and Campylobacter-free dogs and cats. Moreover, two binary logistic regression models were built in order to assess the effects of possible predictors regarding the infection with Campylobacter spp. in dogs and cats. Variables used for the models were selected after a stepwise regression analysis as being the most significant ones. Gender (2 levels, male and female), age (2 levels, <1 year and >1 year) and life style (2 levels, outdoors and indoors) were forced as possible predictors in both models. Especially in dogs, diet (2 levels, homemade and commercial diet) was also used as a predictor variable. All statistical analyses were performed using the statistical package IBM SPSS Statistics 21.

RESULTS

In total, 23 out of 313 dogs and cats (7.3%) were Campylobacter-positive. The identification of species by m-PCR and RFLP is presented in Table 1 and indicative PCR amplification products are illustrated on Fig. 1.

The most frequently identified Campylobacter species in dogs was C. jejuni (57.1%) followed by C. coli (42.9%) whereas co-infection with both C. jejuni and C. coli was not detected in any dog. In cats, the vast majority of isolates were identified as C. jejuni (94.7%). The single isolate from a stray cat, although it yielded the 23S rRNA amplicon, could not be identified at species level by m-PCR despite repeated attempts and was characterised as Campylobacter-like organism (CLO) (Fig. 1).

Chi-square test revealed no statistically significant association (P>0.05) between the presence/absence of Campylo-

<p>| Table 1. Isolation rates of Campylobacter species in dogs (n=181) and cats (n=132) |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Species</strong></th>
<th><strong>Absence of diarrhoea</strong></th>
<th><strong>Diarrhoeic</strong></th>
<th><strong>No data regarding diarrhoea (stray animals)</strong></th>
<th><strong>Total</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dogs</strong></td>
<td>n=154</td>
<td>n=22</td>
<td>n=5</td>
<td>n=181</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>2 (1.3%)</td>
<td>2 (9.1%)</td>
<td></td>
<td>4 (2.2%)</td>
</tr>
<tr>
<td>C. coli</td>
<td>3 (1.9%)</td>
<td>–</td>
<td></td>
<td>3 (1.6%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>7 (3.8%)</td>
<td></td>
</tr>
<tr>
<td><strong>Cats</strong></td>
<td>n=57</td>
<td>n=6</td>
<td>n=69</td>
<td>n=132</td>
</tr>
<tr>
<td>C. jejuni</td>
<td>4 (7.0%)</td>
<td>1 (1.7%)</td>
<td>11 (15.9%)</td>
<td>16 (12.1%)</td>
</tr>
</tbody>
</table>
Prevalence, antimicrobial resistance and risk factors for Campylobacter colonising dogs and cats

When the full regression models were tested against constant only models, the result was not statistically significant for both of the models (chi square = 6.789, P=0.147 with df=4 and chi square = 6.966, P=0.073 with df=3, for dog and cats, respectively), indicating their low predictive power. Moreover, the two models failed to produce a strong relationship between the predictors used and the infection with Campylobacter (Nagelkerke $R^2$ = 11.4% and 9.7% for dogs' and cats' model, respectively). The effects of the predictors for the two models are summarised in Table 2. However, among the predictors, diet tended to have a significant effect on Campylobacter infection in dogs; dogs eating a homemade diet were about 6.2 times more likely to be infected with Campylobacter when compared to dogs consuming strictly a commercial diet (P=0.09, CI 95% 0.75 to 52.06). None of the other predictors was found to have a significant effect on the presence of Campylobacter. In cats, life style displayed a tendency to affect infection with Campylobacter since indoor cats were 3.4 times less likely to be infected (P=0.07, CI 95% 0.92 to 12.91) compared to outdoor cats. The effects of the rest predictors, in both models, were also not statistically significant.

Isolates originating from the same animal displayed a common antimicrobial profile and the exhibited antimicrobial resistance profile of the examined Campylobacter isolates is presented in Table 3. Both canine and feline isolates exhibited susceptibility to erythromycin, gentamicin and streptomycin. On the other hand, 66.7% of Campylobacter isolates from dogs were resistant concurrently to tetracycline and quinolones (ciprofloxacin and
In this study, Campylobacter was isolated from approximately 4% of dogs and 12% of cats. These proportions, although similar to previous studies (Lee et al., 2004; Gariglio et al., 2008; Ramonaitė et al., 2014), could be considered rather low compared to other reported results (Wieland et al., 2005; Parsons et al., 2010; Carbonero et al., 2012). It should be noted that each animal was sampled only once according to the study design, whereas, higher frequencies have been recorded in longitudinal studies where

Table 2. The effects of the predictors used in the two binary logistic regression models for Campylobacter infection in dogs (n=181) and cats (n=132)

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Predictor</th>
<th>B</th>
<th>S.E.</th>
<th>P</th>
<th>Odds ratio</th>
<th>95% CI for Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>Gender</td>
<td>-1.14</td>
<td>0.83</td>
<td>0.17</td>
<td>0.32</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>Male (n=84)</td>
<td>Ref.*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Female (n=97)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>Age</td>
<td>0.18</td>
<td>0.86</td>
<td>0.84</td>
<td>1.19</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>&lt; 1 year (n=44)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>&gt; 1 year (n=137)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>Diet</td>
<td>1.83</td>
<td>1.08</td>
<td>0.09</td>
<td>6.23</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Homemade (n=114)</td>
<td>Ref.*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dogs</td>
<td>Life style</td>
<td>-0.69</td>
<td>0.75</td>
<td>0.36</td>
<td>0.50</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>Outdoors (n=84)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dogs</td>
<td>Constant</td>
<td>-3.70</td>
<td>1.04</td>
<td>0.00</td>
<td>0.03</td>
<td>-</td>
</tr>
<tr>
<td>Cats</td>
<td>Gender</td>
<td>0.59</td>
<td>0.53</td>
<td>0.26</td>
<td>1.81</td>
<td>0.64</td>
</tr>
<tr>
<td></td>
<td>Male (n=48)</td>
<td>Ref.*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cats</td>
<td>Age</td>
<td>-1.41</td>
<td>1.07</td>
<td>0.19</td>
<td>0.24</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>&lt; 1 year (n=20)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td>Life style</td>
<td>1.24</td>
<td>0.68</td>
<td>0.07</td>
<td>3.44</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td>Outdoors (n=91)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cats</td>
<td>Constant</td>
<td>-2.80</td>
<td>0.68</td>
<td>0.00</td>
<td>0.06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Indoons (n=41)</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Table 3. Antimicrobial resistance profile of the investigated Campylobacter isolates from dogs and cats.

<table>
<thead>
<tr>
<th>Antimicrobial resistance profile</th>
<th>Campylobacter spp. (n = 34)</th>
<th>Dog isolates</th>
<th>Cat isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. jejuni (n=8)</td>
<td>C. coli (n=4)</td>
<td>C. jejuni (n=22)</td>
</tr>
<tr>
<td>TE</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (4.5%)</td>
</tr>
<tr>
<td>CIP + NA</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>13 (59.0%)</td>
</tr>
<tr>
<td>CIP + NA + TE</td>
<td>4 (33.3%)</td>
<td>4 (33.3%)</td>
<td>3 (13.6%)</td>
</tr>
<tr>
<td>Total</td>
<td>33.3%</td>
<td>33.3%</td>
<td>17 (77.3%)</td>
</tr>
</tbody>
</table>

* Key to antimicrobial agents: TE: tetracycline; NA: nalidixic acid; CIP: ciprofloxacin.

nalidixic acid). In cats, approximately 60.0% of Campylobacter isolates were resistant to quinolones, 14.0% to quinolones along with tetracycline, and 5.0% only to tetracycline.

DISCUSSION

In this study, Campylobacter was isolated from approximately 4% of dogs and 12% of cats. These proportions, although similar to previous studies (Lee et al., 2004; Gariglio et al., 2008; Ramonaitė et al., 2014), could be considered rather low compared to other reported results (Wieland et al., 2005; Parsons et al., 2010; Carbonero et al., 2012). It should be noted that each animal was sampled only once according to the study design, whereas, higher frequencies have been recorded in longitudinal studies where
serial sampling was performed from each animal (Hald et al., 2004; Sandberg et al., 2002). Nevertheless, the proportion of Campylobacter-positive cats was higher than that of dogs and this difference proved to be statistically significant (P=0.012). Both higher and lower rates of Campylobacter infection in cats than in dogs or even similar infection rates between these two animal species have been previously reported (Sandberg et al., 2002; Wieland et al., 2005; Mohan, 2015). The most likely explanation of the significant divergence between the Campylobacter contamination rates of cats and dogs observed in this study could be the fact that approximately 52.3% of the cats whereas only 2.8% of the dogs examined were stray animals. Indeed, stray animals have been found more commonly infected by campylobacters than household animals that are kept mostly indoors (Tsai et al., 2007). Stray animals that are not sheltered may easily come in direct or indirect contact with wild birds that are regarded as reservoirs for Campylobacter (Mohan, 2015). Moreover, crowded animal housing with recurrent turnover in shelters and financial limitations for pathogen surveillance in shelters for stray animals have been indicated as predisposing factors for pathogen transmission between them (Tsai et al., 2007).

In dogs, C. jejuni was isolated more frequently (57.1%) followed by C. coli (42.9%) and the vast majority of isolates from cats were identified as C. jejuni (94.7%). Comparable results regarding the predominance of C. jejuni in samples from dogs and cats have been reported in previous studies (Gargiulo et al., 2008; Badlik et al., 2014; Giacomelli et al., 2015). None of the Campylobacter isolates in the present study has been identified as C. upsaliensis, though the predominance of this species over C. jejuni in faecal samples from both diarrhoeic and healthy dogs and cats is a common finding (Hald et al., 2004; Wieland et al., 2005; Chaban et al., 2010; Parsons et al., 2010; Salih et al., 2010; Carbonero et al., 2012). Available data suggest that the recovery of C. upsaliensis presupposes a series of crucial parameters such as either a filtration method or the use of up to four agar plates containing cefoperazone, teicoplanin, and amphotericin B (CAT medium) combined with extension of the incubation period for at least four days or even combination of different culture methods (Goossens et al., 1991; Moreno et al., 1993; Hald et al., 2004; Acke et al., 2009). Moreover, the higher cefoperazone content of mCCDA compared to CAT has been reported to impose an inhibitory effect to the growth of C. upsaliensis (Hald et al., 2004). Therefore, the applied methodology in the present study (direct inoculation of mCCDA and Karmali agar plates, incubation for 48 hours) could not be regarded as ideal for the isolation of C. upsaliensis.

With only 7 and 16 Campylobacter-positive dogs and cats, respectively, the power to detect significant risk factors by the applied statistical models was limited. Nevertheless, an overall tendency of higher odds for Campylobacter contamination in dogs consuming a homemade instead of a commercial diet was observed though not significant in the statistical model. This tendency could be enlightened by the fact that the hygiene status of homemade diets may vary considerably and include leftovers of raw meat, such as poultry, that serve as vehicles for Campylobacter transmission to dogs and cats. The indication of a higher risk of Campylobacter contamination for outdoor cats was also weak but may reflect the contact...
with other animals, consumption of raw meat and exposure to environments harbouring campylobacters. Conversely, indoor cats use their litter pan and have limited or even no contact with other animals. Similar findings have been reported previously and were related to the stray behaviour of cats (e.g. ground digging) (Sandberg et al., 2002; Wieland et al., 2005).

In the present study, no statistically significant (P>0.05) association between Campylobacter spp. isolation and gender, age and presence of diarrhoea was observed in the dogs and cats. No significant difference in the prevalence of Campylobacter relevant to the gender of dogs and cats has been reported previously (Gargiulo et al., 2008; Salihu et al., 2010). It has been found that the age of dogs is not a risk factor for C. jejuni and C. coli infection (Wieland et al., 2005; Selwet et al., 2015) but dogs and cats younger than 36 months old have significantly higher odds of carrying C. upsaliensis and C. helveticus (Wieland et al., 2005). In a longitudinal study by Hald et al. (2004), three month-old dogs were 60% Campylobacter-positive, with the prevalence of contamination reaching almost 100% at 12 months of age and then decreasing to 67% when the dogs were 24 months old. Other studies have not detected any association between Campylobacter carriage and intestinal disease in dogs (Acke et al., 2006; Rossi et al., 2008; Parsons et al., 2010). In accordance to the results of this study, the prevalence of Campylobacter in dogs and cats with diarrhoea and healthy animals in Norway was not significantly different (Sandberg et al., 2002) and recent diarrhoeic episodes in dogs in Switzerland did not have a significant impact on C. jejuni isolation (Wieland et al., 2005). On the other hand, intestinal-related signs have been recognised as a significant factor for the isolation of Campylobacter spp., C. jejuni and C. upsaliensis in dogs in Spain (Carbonero et al., 2012). The contribution of Campylobacter to the aetiology of diarrhoea of the dogs and cats in the present study could not be excluded, although disorders in the gastrointestinal tract other than the presence of campylobacters constituted the definitive diagnoses.

Antimicrobial resistance of pathogenic bacteria towards critically important antimicrobials (WHO, 2009), as the ones applied in this study, is a topic of public health interest. All of the tested Campylobacter isolates from dogs and cats exhibited susceptibility to erythromycin, gentamicin and streptomycin, which are administered for campylobacteriosis in humans (Blaser & Engberg, 2008). This finding is comforting from a public health point of view. High susceptibility rates of Campylobacter isolates from dogs and cats to these antimicrobials have been reported in other studies (Sandberg et al., 2002; Lee et al., 2004; Carbonero et al., 2012). However, resistance to quinolones was exhibited by the majority of canine and feline Campylobacter isolates in this study, while cross-resistance to nalidixic acid and ciprofloxacin was always observed. These results can be attributed to the fact that enrofloxacin is widely used in veterinary practice in Greece and this drug has been associated to the increase in fluoroquinolone-resistant C. jejuni strains (van Looveren et al., 2001). Tetracycline is an alternative drug for campylobacteriosis treatment (Blaser & Engberg, 2008) and resistance of Campylobacter isolated from dogs and cats towards tetracycline varies among different studies (0–77.5%) (Sandberg et al., 2002; Lee et al., 2004; Carbonero et al., 2012). In the present study, approximately 67% of canine and
18% of feline *Campylobacter* isolates displayed resistance to this antimicrobial agent. Since tetracycline is not commercially available for veterinary use in dogs and cats in Greece in contrast to doxycycline, the observed resistance could be attributed to cross-resistance to the latter (Karmali et al., 1981) or to the primary infection of the dogs and cats with tetracycline-resistant isolates commonly found in other animal species and food (EFSA & ECDC, 2015b).

CONCLUSION

To our knowledge, this is the first study on the prevalence and antibiotic resistance of *Campylobacter* in dogs and cats in Greece. The data presented in the current study support previously reported results that not only diarrhoeic but also healthy dogs and cats can shed *Campylobacter*. *Campylobacter* infection of humans from dogs and cats via accidental exposure to this pathogen is possible. The awareness that even healthy dogs and cats can pose a zoonotic risk for humans may itself be a first step towards reducing its transmission by adopting good hygiene practices. Another finding of public health interest is the fact that erythromycin, gentamicin and streptomycin displayed the highest in vitro efficiency against the tested *Campylobacter* isolates of canine and feline origin. Further research is deemed necessary in order to clarify the actual role of dogs and cats as sources of human campylobacteriosis in Greece.

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