

Campylobacter and risk factors associated with dog ownership: a retrospective study in household and shelter dogs

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Campylobacter,
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Summary

Campylobacteriosis has been the most frequently reported zoonotic disease in humans in Europe. The scientific literature has reported that the role of dogs may be relevant. The objectives of this work are to improve the knowledge about *Campylobacter* spp. carriage, infection and antimicrobial resistance in household and shelter dogs in Italy, and to assess risk factors at the dog/human interface. During the 2015-2016 period, rectal swabs were collected from 431 household vet-visiting dogs and 173 dogs housed in shelters. A total of 3 veterinary clinics, located in three Italian regions (Abruzzo, Molise and Tuscany) and 10 shelters, five in Abruzzo and five in Molise, were included in the study. Relevant risk factors for the transmission of *Campylobacter* spp. from dogs to humans were assessed by means of a questionnaire administered to owners of household dogs. For *Campylobacter* spp. isolation, selective cultivation methods were used, followed by confirmation and species identification with the PCR method. Phenotypic antibiotic resistance profiles assayed using antimicrobial susceptibility testing were combined. *Campylobacter* spp. were isolated from 9 household dogs (2.1% CI 1.1% - 3.9%) and from 13 shelter dogs (7.5 % CI 4.5% - 12.4%). In household dogs *C. jejuni* was the most represented species (0.9%). In shelter dogs, the most common species was *C. jejuni* (5.2%). *Campylobacter* spp. isolates were resistant to ciprofloxacin (22.73%), nalidixic acid (22.73%), tetracyclines (27.27%), streptomycin (9.09%) and erythromycin (4.55%). The main *C. jejuni* Clonal Complex identified in dogs were CC21, CC45, CC206, CC403, CC42 and CC658. The risk of contracting Campylobacteriosis from dogs remains a concrete reality. This risk is increased in the presence of common habits, as shown by the data from the questionnaire. Prevalence control of *Campylobacter* spp. in household and shelter dogs would be important in order to reduce the transmission to humans.

Introduction

In recent years, campylobacteriosis has been the most frequently reported zoonotic disease in humans in Europe (EFSA 2017, Tam *et al.* 2003).

The most common sources of human campylobacteriosis are the handling or consumption of contaminated/undercooked meat (especially poultry), the handling or consumption of contaminated or unpasteurized milk and dairy products, the consumption of contaminated water, person to person contact, direct contact with carrier

farm animals, direct contact with pets and insect as flies (Adak *et al.* 2005, Mazick *et al.* 2005, Strother *et al.* 2005). The species most commonly associated with human infections are *Campylobacter jejuni*, followed by *C. coli*, *C. lari*, and *C. upsaliensis* (Kaakoush *et al.* 2005, Ibrahim *et al.* 2019, Gahamanyi *et al.* 2020).

In many symptomatic cases, campylobacteriosis occurs as mild and self-limiting gastroenteritis, but long-term effects such as reactive arthritis (ReA), post infectious irritable bowel syndrome (IBS), Guillain Barré syndrome (GBS), inflammatory bowel disease (IBD) and Reiter's syndrome (RS) may be

associated with infection (Keithlin *et al.* 2014, Esan *et al.* 2017, Brooks *et al.* 2017).

The role of dogs as a source of infection could be relevant (Gras *et al.* 2013, Koene *et al.* 2004). Owning a pet, especially a puppy, has been identified as a risk factor for *Campylobacter* sp. infection (Doorduyn *et al.* 2010).

In many cases, dogs are asymptomatic carriers of *Campylobacter* spp. Some studies found no significant relationship between diarrhoea and *Campylobacter* sp. infection status (Acke *et al.* 2009), suggesting that the organism is commensal. Conversely, other studies reported an association between infection and clinical signs particularly in relatively young dogs (Guest 2007, Chaban 2010).

Animals may be more susceptible to clinical disease when stressed by concurrent disease, hospitalization, shipment, pregnancy or surgery. Acute campylobacteriosis that develops in puppies and some adult dogs is characterized by mucus-laden, watery or bile-streaked diarrhoea (with or without blood and leukocytes) of five to 15 days duration, partial anorexia, and occasional vomiting. Elevated temperature and leukocytosis may also be present (Fox 1990, Brown *et al.* 1999).

The close relationship between humans and dogs especially family pets, can play an important role in the transmission of zoonotic agents (Stull *et al.* 2010).

The present study aimed to analyse three important aspects of campylobacteriosis:

- prevalence and diversity of *Campylobacter* species in owned and shelter dogs;
- their antimicrobial resistance;
- identification of possible risk factors for zoonotic transmission at the dog/human interface.

Materials and methods

The study has been divided into three phases:

- drawing up and administration of questionnaires to dog owners visiting veterinary clinics;
- collection of samples and laboratory examinations;
- data analysis.

Questionnaire design and administration

A total of 431 questionnaires were administered to different dog owners and compiled in case of the owner approval.

The number of completed questionnaires was 412.

Each questionnaire collected data on the risk factors for zoonotic transmission at the dog/human interface, such as habits and behaviors of the hosting families of the dog and/or other pets or domestic animals.

The questionnaires included 28 questions grouped in different sections (Table I). The introductory questions referred to the owner's data and the identification and description of the veterinarian and the dog. The first group of questions referred to the veterinary visit and the general conditions of the dog (purpose of the veterinary-visit, health conditions, and nutritional status). The second group of questions referred to the dog's risk factors for carriage and infection (nutrition, travel abroad, origin, life habits, places frequented). The third group of questions referred to the human risk factors for infection (contact with humans, dog's life habits, presence of other animals, composition of the family unit).

Collection of samples and laboratory examinations

During the 2015-2016 period, rectal swabs were collected from 431 household dogs in veterinary clinics and 173 shelter dogs. A total of 3 veterinary clinics, located in the Abruzzo, Molise and Tuscany regions and 10 shelters, five in the Abruzzo and five in the Molise region, were included in the study.

From each dog, 2 rectal swabs were collected. The rectal swabs were gathered from the rectum of the animals using culture swab transport system (Transystem™ Amies with charcoal, Copan, Brescia, Italy). All samples were transported at 4 °C in refrigerated boxes and processed immediately upon arrival to the laboratory and not later than 72 hours after sampling (at 4 °C).

The isolation of *Campylobacter* spp. was performed according to the World Organisation of Animal Health (WOAH) (OIE 2008) in modified charcoal cefoperazone deoxycholate agar (mCCDA) (Thermo Scientific Oxoid, Milan, Italy) and Karmali agar (Italian Biolife, Milan, Italy). Both methods involved directly plating swabs and enriching in Preston broth (Italian Biolife, Milan, Italy) for 24 hours in a microaerophilic atmosphere.

After enrichment, 100 microliters of the Preston broth were plated in duplicate on mCCDA and Karmali plates and all plates (directly and after enrichment) were incubated under a microaerobic atmosphere at 41.5 °C and 37 °C for 48 hours.

After incubation, the plates were examined to detect the presence of suspected of *Campylobacter* sp. colonies. The suspect *Campylobacter* colonies were identified by a multiplex PCR method, as described by Wang and colleagues (Wang *et al.* 2002) for thermotolerant *Campylobacter* and by sequencing of the 16S region

Table 1. Questionnaire completed by dog owners visiting veterinary clinics. —cont'd

Owner		
Name and surname		
Home address and telephone number		
Owner occupation		
Veterinarian		
Name and surname		
Name of veterinary clinic and address		
Animal		
Transponder (microchip)	Name	
Breed	Size	
Sex	Date of birth	
Coat description		
Reason for clinical examination		
<input type="checkbox"/> 1. Routine examination/vaccination	<input type="checkbox"/> 2. Fever	<input type="checkbox"/> 3. Diarrhoea
<input type="checkbox"/> 4. Vomiting	<input type="checkbox"/> 5. Trauma	<input type="checkbox"/> 6. Dermatitis
<input type="checkbox"/> 7. Other		
Antibiotic administration		
Have antibiotics been administered? <input type="checkbox"/> yes <input type="checkbox"/> no if you answered 'Yes' provide details:		
<input type="checkbox"/> In the last month	<input type="checkbox"/> In the last 3 months	<input type="checkbox"/> In the last year Trade name of the drug
Feeding and body condition score		
Feeding regime		
Wet food (canned food) 70% to 80% of moisture content		
<input type="checkbox"/> Regularly (principal component of food)	<input type="checkbox"/> Weekly (one or more times per week)	<input type="checkbox"/> Monthly (occasionally) <input type="checkbox"/> Never
Semi-moist food (snacks) 15% to 40% of moisture content		
<input type="checkbox"/> Regularly (principal component of food)	<input type="checkbox"/> Weekly (one or more times per week)	<input type="checkbox"/> Monthly (occasionally) <input type="checkbox"/> Never
Dry food (pellets) less than 10% of moisture content		
<input type="checkbox"/> Regularly (principal component of food)	<input type="checkbox"/> Weekly (one or more times per week)	<input type="checkbox"/> Monthly (occasionally) <input type="checkbox"/> Never
Cooked food or food for human consumption		
<input type="checkbox"/> Regularly (principal component of food)	<input type="checkbox"/> Weekly (one or more times per week)	<input type="checkbox"/> Monthly (occasionally) <input type="checkbox"/> Never
Raw meat		
<input type="checkbox"/> Regularly (principal component of food)	<input type="checkbox"/> Weekly (one or more times per week)	<input type="checkbox"/> Monthly (occasionally) <input type="checkbox"/> Never
Body condition score		
<input type="checkbox"/> 1 Ribs, lumbar vertebrae, pelvic bones and all bony prominences evident from a distance. No discernible body fat. Obvious loss of muscle mass.		
<input type="checkbox"/> 1.5 Ribs, lumbar vertebrae and pelvic bones easily visible. No palpable fat. Some evidence of other bony prominence. Minimal loss of muscle mass.		
<input type="checkbox"/> 2 Ribs easily palpated and may be visible with no palpable fat. Tops of lumbar vertebrae visible. Pelvic bones becoming prominent. Obvious waist.		
<input type="checkbox"/> 2.5 Ribs easily palpable, with minimal fat covering. Waist easily observed when viewed from above. Abdominal tuck evident.		
<input type="checkbox"/> 3 Ribs palpable without excess fat covering. Waist observed behind ribs when viewed from above. Abdomen tucked when viewed from the side.		
<input type="checkbox"/> 3.5 Ribs palpable with slight excess fat covering. Waist is discernible when viewed from above but is not prominent. Abdominal tuck apparent.		
<input type="checkbox"/> 4 Ribs palpable with difficulty; heavy fat cover. Noticeable fat deposits over lumbar area and base of tail. Waist absent or barely visible. Abdominal tuck may be present.		
<input type="checkbox"/> 4.5 Ribs not palpable under very heavy fat cover, or palpable only with significant pressure. Heavy fat deposits over lumbar area and base of tail. Waist absent. No abdominal tuck. Obvious abdominal distension may be present.		
<input type="checkbox"/> 5 Massive fat deposits over thorax, spine and base of tail. Waist and abdominal tuck absent. Fat deposits on neck and limbs. Obvious abdominal distention		
Origin of the animal		
Breeding Stray dog Other family Shelter Other		
Travel abroad, life habits, places frequented Travel abroad in the last 3 months <input type="checkbox"/> yes <input type="checkbox"/> no		
How long has the dog been housed with the current family?		Years Months
Where does the dog live?	<input type="checkbox"/> Only inside the household	<input type="checkbox"/> Only outside the household <input type="checkbox"/> Inside and outside the household

continued

Table I. Questionnaire completed by dog owners visiting veterinary clinics. —cont'd

Do you take your dog to public area? <input type="checkbox"/> yes <input type="checkbox"/> no	
Does the dog lick family members' hands and face? <input type="checkbox"/> yes <input type="checkbox"/> no	
What do you use to collect the dog's feces?	
<input type="checkbox"/> Paper towels <input type="checkbox"/> Plastic bags	<input type="checkbox"/> Shovel <input type="checkbox"/> I do not collect the feces
Other	
Do you clean your hands after any food manipulation and administration? <input type="checkbox"/> yes <input type="checkbox"/> no	
Do you wash your hands after any contact with the dog? <input type="checkbox"/> yes <input type="checkbox"/> no	
Do you touch your dog while you consume food? <input type="checkbox"/> yes <input type="checkbox"/> no	Do you allow the dog to get on the sofa/bed? <input type="checkbox"/> yes <input type="checkbox"/> no
Living environment of the family <input type="checkbox"/> urban <input type="checkbox"/> rural	Does the family have other animals? <input type="checkbox"/> yes <input type="checkbox"/> no
Family members ID member	Sex <input type="checkbox"/> F <input type="checkbox"/> M Kinship
Profession	Date of birth

(AbiPrism 3500, Applied Biosystem) for other *Campylobacter* spp. Genomic DNA was extracted using an Ultraclean microbial DNA kit (Mo Bio Laboratories, Solana Beach, CA, USA) according to the manufacturer's instructions and quantified using a Nanodrop Spectrophotometer (NanoDrop Technologies, Celbio Srl., Milan, Italy). *Campylobacter* strain susceptibility to antibiotics was evaluated with the microbroth dilution method using Sensititre® custom susceptibility plates, EUCAMP 2 (Trek Diagnostic Systems, Biomedical Service, Venice, Italy).

The colonies were harvested in Columbia agar for 24 hours then inoculated in Mueller Hinton Broth supplemented with blood and dispensed into Eucamp microtiter plates (TREK Diagnostic Systems, Biomedical Service, Italy), containing known scalar concentrations of the following antibiotics: gentamicin (Gm) (0.12-16 µg/ml), streptomycin (S) (0.25-16 µg/ml), ciprofloxacin (Cip) (0.12-16 µg/ml), tetracyclines (Te) (0.5-64 µg/ml), erythromycin (E) (1-128 µg/ml) and nalidixic acid (NA) (1-64 µg/ml). After inoculation, the plates were incubated at 42 °C under a microaerophilic atmosphere for 24 hours and then screened. *C. jejuni* strain NCTC 11351 was used as a quality control.

For the evaluation of the minimum inhibitory concentration (MIC), the Swin v3.3 software (TREK Diagnostic Systems, Biomedical Service, Italy) was used in accordance with the epidemiological cutoff values (ECOFFs) defined by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org) to interpret susceptibility.

Multilocus sequence typing (MLST) was performed using standard protocols as previously described (Dingle et al. 2001).

The DNA of the samples subjected to MLST was extracted from the strains using a Maxwell® 16 System automatic extractor (Promega, IT) according to the manufacturer's indications. Sequence types (OIE 2008), and clonal complexes

(CCs) were assigned by submitting the DNA sequence to the *Campylobacter* MLST database website (<http://pubmlst.org/campylobacter>).

Data analysis

For the comparison of the prevalence rates between the two groups of data (household and shelter dogs), according to the literature (Berger 1985), it was decided to use a Bayesian approach, using a Beta distribution with the relative 95% confidence intervals (CIs).

Results

Questionnaire administration

Table II shows the results of the questionnaires administered to the dog owners. Most owners prefer dry food, even though a not negligible percentage of owners (4.8%) more or less regularly feed their dog with raw meat. Most dogs live at home (37.4%), while 32.0% live outdoor, and 30.6% in both environments. In most cases, owners have contact with their dogs during meals (51.7%), and they have been licked by from their pets on their hands and face (72.8%). Most of the dogs participants in the study live in an urban environment (254 out of 412, 61.7%) and in 69.7% of cases the majority of owners do not have other pets or domestic animals.

Sample and data analysis

Campylobacter spp. were isolated from 9 out of 431 household dogs (2.1%, CI 1.1%-3.9%) and from 13 out of 173 shelter dogs tested (7.5%; CI 4.5%-12.4%) (Table III). The 95% CIs of the prevalence rates calculated by the Beta distribution do not overlap, thus showing a significant difference between the two percentages.

Four out of the 9 isolates in household dogs were identified as *C. jejuni*, 2 as *C. upsaliensis*, 1 was identified as *C. coli*, 1 as *C. lari* and 1 as *C. vulpis*.

In shelter dogs, nine isolates were identified as *C. jejuni*, 3 as *C. lari* and 1 as *C. coli* (Table III).

Our finding showed that the principal *C. jejuni* Clonal Complex identified in dogs were CC21, CC45, CC206, CC403, CC42 and CC658 (Table IV).

Campylobacter sp. isolates have demonstrated resistance mainly to tetracyclines, ciprofloxacin, nalidixic acid, and streptomycin. The resistance to

Table II. Results of the questionnaires compiled by 412 owners of dogs visiting a veterinary clinic. — cont'd

Description of the sample						
Age	average age: 5.60 (+/-3.602 Standard Deviation)					
Sex	Breed					
	Sex	No. of sampled dogs	%	Breed	No. of sampled dogs	%
	Female	137	33.3%	Mongrel	105	25.5%
	Male	275	66.7%	Purebred dogs	307	74.5%
	Total	412	100.0%	Total	412	100.0%
Dog Size	Body condition Score					
	Size	Number	%	Score	Number	%
	Large sized dog (adult weight more than 25 kg)	133	32.3%	1		0%
	Medium sized dog (adult weight between 10 kg and 25 kg)	137	33.3%	2		0%
	Small-sized dog (adult weight between 1 kg and 10 kg)	142	34.5%	3	5	1.5%
	Total	412	100.0%	4	22	6.4%
				5	62	18.1%
				6	108	31.6%
				7	105	30.7%
				8	37	10.8%
				9	3	0.9%
				Total	342	100.0%
Presence of diarrhoea in the previsit period and antibiotic administration						
Diarrhoea	Antibiotic administration					
	Onset time	Number	%	Administration	Number	%
	In the last three months	80	64.0%	In the last month	56	77.8%
	In the last 6 months	21	16.8%	In the last 3 months	8	11.1%
	In the last year	24	19.2%	In the last year	8	11.1%
	Total	125	100.0%	Total	72	100.0%
Antibiotic active						
	Active ingredient	Frequency of use for each antibiotic		%		
	Amoxicillin	28		38.9%		
	Cephalosporin	16		22.2%		
	Metronidazole	6		8.3%		
	Amoxicillin, Cephalosporin	4		5.6%		
	Cephalosporin, Metronidazole	4		5.6%		
	Amoxicillin, Metronidazole	2		2.8%		
	Enrofloxacin	2		2.8%		
	Marbofloxacin	2		2.8%		
	Metronidazole, Spiramycin	2		2.8%		
	Tylosin, Metronidazole	2		2.8%		
	Amoxicillin, Cephalosporin, Metronidazole	1		1.4%		
	Amoxicillin, Itraconazole	1		1.4%		
	Amoxicillin, Metronidazole, Tylosin	1		1.4%		
	Enrofloxacin, Cephalosporin	1		1.4%		
		72		100%		

continued

Table II. Results of the questionnaires compiled by 412 owners of dogs visiting a veterinary clinic. — cont' d

Feeding regime						
	Administered monthly (occasionally)		Administered regularly (main component of food)		Administered weekly (one or more times per week)	
	Number	%	Number	%	Number	%
Wet food	7	1.7%	71	17.2%	14	3.4%
Semi-moist food	19	4.6%	7	1.7%	56	13.6%
Dry food	10	2.4%	316	76.7%	7	1.7%
Homemade food	46	11.2%	69	16.7%	52	12.6%
Raw meat	12	2.9%	5	1.2%	3	0.7%

Origin and living place						
Origin			Where does the dog live?			
	Number	%	Total		%	
Breeding	189	45.9%	Only inside the household		154 37.4%	
Other family	140	34.0%	Only outside the household		132 32.0%	
Shelter	40	9.7%	Inside and outside		126 30.6%	
Stray dog	36	8.7%	Total		412 100.0%	
Born at home	3	0.7%				
Pet Shop	1	0.2%				
Other	3	0.7%				
Total	412	100.0%				

Habits				
	Yes		No	
	Number	%	Number	%
Does the dog lick your hands and face?	300	72.8%	112	27.2%
Do you clean the hands after any food manipulation and administration?	340	82.5%	72	17.5%
Do you wash your hands after any contact with the dog?	186	45.1%	226	54.9%
Do you touch your dog while consuming your food?	213	51.7%	199	48.3%
Do you allow the dog to get on the sofa/bed?	184	44.7%	228	55.3%

What do you use to pick up dog's feces?		
	Number	%
Plastic bags	168	48.8%
Paper towels	37	10.8%
Shovel	106	30.8%
I don't take the feces	28	8.1%
Other	5	1.5%
Total	344	100%

Table III. *Campylobacter* species in household and shelter dogs.

Type of dog lifestyle	<i>Campylobacter</i> species isolated	No. of positive samples	%
Household dogs	<i>Campylobacter jejuni</i>	4	0.9%
	<i>Campylobacter upsaliensis</i>	2	0.5%
	<i>Campylobacter vulpis</i>	1	0.2%
	<i>Campylobacter lari</i>	1	0.2%
	<i>Campylobacter coli</i>	1	0.2%
	Total	9	2.1%
Shelter dogs	<i>Campylobacter jejuni</i>	9	5.2%
	<i>Campylobacter lari</i>	3	1.7%
	<i>Campylobacter coli</i>	1	0.6%
	Total	13	7.5%

Table IV. *C. jejuni* Clonal Complex (CC).

Clonal Complex (CC)	Sequence type (ST)	N° strains
21	50	2
45	538	1
	2,854	1
206	3,335	1
	122	1
403	403	2
	177	1
42	6,532	1
658	1,044	3

Table V. Antimicrobial resistance of the isolated *Campylobacter* spp.

	No. of resistant isolates	% on total isolates
Tetracyclines	6	27.27%
Ciprofloxacin	5	22.73%
Nalidixic acid	5	22.73%
Streptomycin	2	9.09%
Erythromycin	1	4.55%

tetracyclines was the highest, while the resistance to erythromycin was the lowest (Table V). There was a high number of strains with intermediate sensitivity (10 isolates, 76.9%).

Discussion

This study obtained information on:

- the assessment of behaviors in household dogs, which can be considered risk factors for the transmission of *Campylobacter* and other zoonotic agents
- the prevalence of *Campylobacter* spp. in household and shelter dogs and its characterization, including the *C. jejuni* Clonal Complex
- the antibiotic resistance of the *Campylobacter* spp.

With regard to the first point, there are few data in the literature on the living habits of households' dogs, especially based on market studies (Boya et al. 2012) or sociological studies (Charles 2016). The present study evaluated some behaviors and habits as possible risk factors for the transmission of zoonotic agents. Notably, among these, the "Habits" section of Table II shows very high percentages associated with habits that could be considered risky in the presence of infected dogs. Moreover, the habit of feeding dogs with raw meat, as reported in the "Feeding regime" section of the Table II, is carried out in a non-negligible percentage of cases (4.8%), therefore representing an additional risk factor (Hellgren et al. 2019).

Data belonging to the section "Origin and living place" especially with regard to attendance in outdoor spaces, cannot be directly related to *Campylobacter* infection, even if they can be considered as particularly significant risk factors.

Again, in the section "habits", data show that the percentage of people who declare that they do not collect feces (8.1%) is still very high, thus contributing to increase the risk of pathogen's transmission due to environmental contamination. Collection with plastic bags, which is the most valid method for a thorough removal of feces, accounts for 48%. This indicates that much still needs to be done to

encourage dog owners to carry out an appropriate collection of feces.

With regard to the second point, the prevalence of *Campylobacter* spp. recorded in shelter dogs (13 out of 173, 7.5%, CI 4.5%-12.4%) has been significantly higher than the prevalence recorded in household dogs (9 positive out of 431, 2.1%, CI 1.1%-3.9%).

Among dogs resulted positive for *Campylobacter* spp., no one had diarrhoea at the time of sampling, one had diarrhoea in the previous three months, one in the previous six months and one in the last year, confirming what is reported in the literature, i.e. that infected dogs usually do not show clear symptoms.

In the literature reports, *Campylobacter* sp. prevalence in dogs varies greatly between authors (Leonard et al. 2011, Acke et al. 2009, Giacomelli et al. 2005, Holmberg et al. 2015) depending on the sampled population, the study design, and the analysis method. However, a higher prevalence is generally reported in shelter dogs, probably due to different hygiene and life conditions, increased stress factors, cohabitation with other dogs and contact with other animals, such as mice and rats. The majority of investigated household dogs have home habits, which means a reduced risk of contracting the pathogens investigated in the present study. Life conditions of shelter dogs, on the contrary, are characterized by the housing in boxes which may include a different number of dogs and the presence of open common areas, delimited by fences, allowing more frequent contacts between animal and zoonotic agent's carriers. In both cases, however, the prevalence recorded suggests the need to adopt precise hygiene protocols in the man/dog relationship.

The prevalence of *Campylobacter* spp. was lower than that generally found in the literature. The presence of 13.6% of household dogs treated with antibiotics in the month preceding the veterinary visit could have influenced the results of the diagnostic tests. It was not possible to obtain information on the use of antibiotics in the shelters, but it would be appropriate to investigate this circumstance with further studies.

This record of *Campylobacter* spp. in Italian dogs, and of *Campylobacter jejuni* in particular, further highlights the risk related to the zoonotic potential of the pathogen. Its diffusion may be favored by the lifestyle in man/animal relationship and by the close contact that many companion animals have with their owners.

The third aspect of this study concerns the antibiotic resistance of *Campylobacter* spp.

In veterinary practice, in the daily clinics, the antibiotics administered to pets may represent a source of molecular pressure on the microorganisms,

which, if put in favorable conditions, may acquire resistance to these molecules and transmit it to their offspring. However, restriction of the use of antimicrobials due to introduction of electronic prescription (Ministero della Salute 2019) should further improve this condition.

The prevalence of antimicrobial resistance in ciprofloxacin (22.73%), nalidixic acid (22.73%), tetracyclines (27.27%), streptomycin (9.09%) and erythromycin (4.55%) found in this study was confirmed by other studies (Andrzejewska *et al.* 2013). This issue is worrying as these antibiotics are also commonly used in humans. Antimicrobial resistance genes can be transferred to the intestinal microbial flora, and resistant commensal bacteria can constitute a reserve of resistant genes for potential pathogens (Amar *et al.* 2014).

The principal *C. jejuni* CCs in dogs were CC21, CC45, CC206, CC403, CC42 and CC 658. *C. jejuni* CCs CC21 and CC45 are regularly isolated from multiple animal species, hindering a human source attribution.

Conclusions

Data regarding the prevalence of *Campylobacter* infection in household and shelter dogs confirms a real risk of transmission to humans by dogs, even though the prevalence was not very high compared to other studies. This risk is higher in the presence

of particular common habits, as shown by the data from the questionnaire. The very high percentages of people who do not wash their hands after contact with their dogs, allow their dogs to lick their face and hands and allow their dogs to sleep on the bed and sofa or eat raw meat increase the risk of zoonotic disease transmission.

The higher prevalence of infection recorded among shelter dogs suggests that particular commitment should be devoted to staff training from people managing these premises, especially for people who is used to direct manage dogs and for this reason are more exposed to the risk of being in contact with *Campylobacter* spp.

The risk of contracting Campylobacteriosis from dogs thus remains a concrete reality. Prevalence control of *Campylobacter* spp. in household and shelter dogs would be considered important in order to reduce the transmission to humans.

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References

- Acke E., McGill K., Golden O., Jones B.R., Fanning S. & Whyte P. 2009. Prevalence of thermophilic *Campylobacter* species in household cats and dogs in Ireland. *Vet Rec*, **164**, 44-47.
- Adak G.K., Meakins S.M., Yip H., Lopman B.A. & O'Brien S.J. 2005. Disease risks from foods, England and Wales, 1996-2000. *Emerg Infect Dis*, **11**, 365-372.
- Amar C., Kittl S., Spreng D., Thomann A., Korczak B.M., Burnens A.P. & Kuhnert P. 2014. Genotypes and antibiotic resistance of canine *Campylobacter jejuni* isolates. *Vet Microbiol*, **168**, 124-130.
- Andrzejewska M., Szczepańska B., Klawe J.J., Spica D. & Chudzińska M. 2013. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* species in cats and dogs from Bydgoszcz (Poland) region. *Pol J Vet Sci*, **16**, 115-120.
- Berger J.O. 1985. Statistical decision theory and Bayesian analysis. New York, Springer-Verlag.
- Boya U.O., Dotson M.J. & Hyatt E.M. 2012. Dimensions of the dog-human relationship: a segmentation approach. *J Target Meas Anal Mark*, **20**, 133-143.
- Brooks P.T., Brakel K.A., Bell J.A., Bejcek C.E., Gilpin T., Brudvig J.M. & Mansfield L.S. 2017. Transplanted human fecal microbiota enhanced Guillain Barré syndrome autoantibody responses after *Campylobacter jejuni* infection in C57BL/6 mice. *Microbiome*, **5** (1), 1-22.
- Brown C., Martin V. & Chitwood S. 1999. An outbreak of enterocolitis due to *Campylobacter* spp. in a beagle colony. *J Vet Diagn Invest*, **11**, 374-376.
- Chaban B., Ngeleka M. & 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 Microbiol*, **10**, 73.
- Charles N. 2016. Post-human families? Dog-human relations in the domestic sphere. *Sociol Res Online*, **21**, 1-12.
- Dingle K.E., Colles F.M., Wareing D.R., Ure R., Fox A.J., Bolton F.E., Bootsma H.J., Willems R.J., Urwin R. & Maiden M.C. 2001. Multilocus sequence typing system for *Campylobacter jejuni*. *J Clin Microbiol*, **39**, 14-23.
- Domingues A.R., Pires S.M., Halasa T. & Hald T. 2012. Source attribution of human campylobacteriosis using a meta-analysis of case-control studies of sporadic infections. *Epidemiol Infect*, **140**, 970-981.
- Doorduyn Y., Van Den Brandhof W.E., Van Duynhoven Y.T.H.P., Breukink B.J., Wagenaar J.A. & Van Pelt W. 2010. Risk factors for indigenous *Campylobacter jejuni* and *Campylobacter coli* infections in The Netherlands: a case-control study. *Epidemiol Infect*, **138** (10), 1391-1404.
- Esan O.B., Pearce M., van Hecke O., Roberts N., Collins D., Violato M., McCarthy N., Perera R. & Fanshawe T.R. 2017. Factors associated with sequelae of *Campylobacter* and non-typhoidal *Salmonella* infections: a systematic review. *EBioMedicine*, **15**, 100-111.
- European Food Safety Authority and European Centre for Disease Prevention and Control (EFSA and ECDC). 2018. The European union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2017. *EFSA J*, **16**, 5500.
- Fox J.G. 1990. Campylobacteriosis. In *Infectious diseases of dog and cat*. (Greene C.E. ed) 2nd ed. Philadelphia, PA, WB Saunders, 226-248.
- Gahamanyi N., Mboera L.E., Matee M.I., Mutangana D. & Komba E.V. 2020. Prevalence, risk factors, and antimicrobial resistance profiles of thermophilic *Campylobacter* species in humans and animals in Sub-Saharan Africa: a systematic review. *Int J Microbiol*, 2092478.
- Giacomelli M., Follador N., Coppola L.M., Martini M. & Piccirillo A. 2015. Survey of *Campylobacter* spp. in owned and unowned dogs and cats in Northern Italy. *Vet J*, **204**, 333-337.
- Gras L.M., Smid J.H., Wagenaar J.A., Koene M.G.J., Havelaar A.H., Friesema I.H.M. & Busani L. 2013. Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. *Epidemiol Infect*, **141** (12), 2526-2535.
- Guardabassi L., Schwarz S. & Lloyd D. 2004. Pet animals as reservoirs of antimicrobial-resistant bacteria. *J Antimicrob Chemother*, **54**, 321-332.
- Guest C.M., Stephen J.M. & Price C.J. 2007. Prevalence of *Campylobacter* and four endoparasites in dog populations associated with hearing dogs. *J Small Anim Pract*, **48**, 632-637.
- Hellgren J., Hästö L.S., Wikström C., Fernström L.L. & Hansson I. 2019. Occurrence of *Salmonella*, *Campylobacter*, *Clostridium* and *Enterobacteriaceae* in raw meat-based diets for dogs. *Vet Rec*, **184**, 442.
- Holmberg M., Rosendal T., Engvall E.O., Ohlson A. & Lindberg A. 2015. Prevalence of thermophilic *Campylobacter* species in Swedish dogs and characterization of *C. jejuni* isolates. *Acta Vet Scand*, **57**, 19.
- Ibrahim J.N., Eghnatio E., El Ro A., Fardoun T. & Ghseini G. 2019. Prevalence, antimicrobial resistance and risk factors for campylobacteriosis in Lebanon. *J Infect Dev Ctries*, **13** (01), 11-20.
- Kaakoush N.O., Castaño-Rodríguez N., Mitchell H.M. & Man S.M. 2015. Global epidemiology of *Campylobacter* infection. *Clin Microbiol Rev*, **28** (3), 687-720.
- Kärenlampi R., Rautelin H., Schönberg-Norio D., Paulin L. & Hänninen M.L. 2007. Longitudinal study of Finnish *Campylobacter jejuni* and *C. coli* isolates from humans, using multilocus sequence typing, including comparison with epidemiological data and isolates from poultry and cattle. *Appl Environ Microbiol*, **73**, 148-155.
- Keithlin J., Sargeant J., Thomas M.K. & Fazil A. 2014. Systematic review and meta-analysis of the proportion of *Campylobacter* cases that develop chronic sequelae. *BMC Public Health*, **14**, 1203.
- Koene M.G., Houwers D.J., Dijkstra J.R., Duim B. & Wagenaar J.A. 2004. Simultaneous presence of multiple *Campylobacter* species in dogs. *J Clin Microbiol*, **42**, 819-821.

- Kurnar R., Verma A.K., Kurnar A., Srivastava M. & Lal H.P. 2012. Prevalence and antibiogram of *Campylobacter* infections in dogs of Mathura, India. *Asian J Anim Vet Adv*, **7**, 434-740.
- Leonard E.K., Pearl D.L., Janecko N., Weese J.S., Reid-Smith R.J., Peregrine A.S. & Finley R.L. 2011. Factors related to *Campylobacter* spp. carriage in client-owned dogs visiting veterinary clinics in a region of Ontario, Canada. *Epidemiol Infect*, **139**, 1531-1541.
- World Organisation for Animal Health (OIE). 2008. Manual of diagnostic tests and vaccines for terrestrial animals. Chapter 2.9.3. *Campylobacter jejuni* and *Campylobacter coli*. OIE, Paris.
- Mazick A., Ethelberg S., Møller Nielsen E., Mølbak K. & Lisby M. 2006. An outbreak of *Campylobacter jejuni* associated with consumption of chicken, Copenhagen, 2005. *Euro Surveill*, **11**, 137-139.
- Mohan V. 2015. Faeco-prevalence of *Campylobacter jejuni* in urban wild birds and pets in New Zealand. *BMC Res Notes*, **8**, 1.
- Siemer B.L., Harrington C.S., Nielsen E.M., Borck B., Nielsen N.L., Engberg J. & On S.L. 2004. Genetic relatedness among *Campylobacter jejuni* serotyped isolates of diverse origin as determined by numerical analysis of amplified fragment length polymorphism (AFLP) profiles. *J Appl Microbiol*, **96**, 795-802.
- Strother K.O., Steelman C.D. & Gbur E.E. 2005. Reservoir competence of lesser mealworm (Coleoptera: Tenebrionidae) for *Campylobacter jejuni* (Campylobacterales: Campylobacteraceae). *J Med Entomol*, **42** (1), 42-47.
- Stull J.W., Peregrine A.S., Sargeant J.M. & Weese J.S. 2013. Pet husbandry and infection control practices related to zoonotic disease risks in Ontario, Canada. *BMC Public Health*, **13** (1), 520.
- Tam C.C., O'Brien S.J., Adak G.K., Meakins S.M. & Frost J.A. 2003. *Campylobacter coli* - an important foodborne pathogen. *J Infect*, **47**, 28-32.
- Tenkate T.D. & Stafford R.J. 2001. Risk factors for *Campylobacter* infection in infants and young children: a matched case-control study. *Epidemiol Infect*, **127**, 399-404.
- Wang G., Clark C.G., Taylor T.M., Pucknell C., Barton C., Price L., Woodward D.L. & 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*. *J Clin Microbiol*, **40**, 4744-4747.