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Prevalence and risk factors for colonization of *Campylobacter* spp. in household dogs in Metro Manila, Philippines

Ma. Socorro Edden P. Subejano[2,1*] and Gil M. Penuliar[1,2]
[1]Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City 1101 Philippines
[2]Natural Sciences Research Institute, University of the Philippines, Diliman, Quezon City 1101 Philippines
[*]Corresponding author e-mail: epsubejano@up.edu.ph
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Abstract. *Campylobacter* is one of the four leading causes of diarrheal diseases worldwide, with the number of cases surpassing those of salmonellosis and shigellosis. Contact with companion animals such as cats and dogs has been implicated in human infections. This study aimed to determine the prevalence and risk factors for *Campylobacter* spp. colonization among household dogs in Metro Manila, Philippines. Faecal samples were collected from 195 dogs and processed using selective enrichment. *Campylobacter* spp. were detected and identified through PCR amplification of genus- and species-specific genes. The overall prevalence of *Campylobacter* spp. was 9.74% (19/195), with *C. upsaliensis* as the predominant species with a prevalence of 7.2% (14/195), followed by *C. jejuni* at 2.05% (4/195). Both *C. upsaliensis* and *C. jejuni* were observed in 15.8% (3/19) of samples positive for *Campylobacter* spp. Furthermore, *Campylobacter* colonization in dogs was associated with the gender of the dog owner and presence of other pets in the household. These results reinforce the need for good hygiene practices when handling dogs in order to reduce the possibility of acquiring campylobacteriosis resulting from pet interactions.

INTRODUCTION

The World Health Organization (WHO, 2017) lists *Campylobacter* as one of the four key global causes of diarrheal diseases. It is considered one of the most important enteropathogens in developing and industrialized countries, with the number of cases surpassing those of salmonellosis and shigellosis (Allos, 2001; Uzunović-Kamberović, 2005). Due to campylobacteriosis, substantial economic losses have been documented due to clinical treatment costs and lost working hours (Gibreel & Taylor, 2006).

Campylobacter infections are considered mild but can be fatal to young, elderly, and immunocompromised individuals (Acke *et al.*, 2009). Clinical signs include abdominal discomfort, vomiting, fever, and sometimes, bloody diarrhoea (Leonard *et al.*, 2011; Carbonero *et al.*, 2012). Campylobacteriosis is generally self-limiting and may last for 3-11 days (Abdollahpour *et al.*, 2015). However, severe or prolonged cases may lead to complications such as Guillain-Barré syndrome (Peterson, 1994; Butzler, 2004; Yan *et al.*, 2005), Miller Fisher syndrome (Salloway *et al.*, 1996; Yuki, 1997), Reiter's syndrome (Peterson, 1994), and haemolytic uremic syndrome (Chamovitz *et al.*, 1983).

Broiler chickens are commonly colonized with *Campylobacter* spp., and consumption of poultry and poultry products has been identified as the principal risk factor for contracting *Campylobacter* infections. Other risk factors include consumption of raw or unpasteurized milk, drinking untreated water, handling raw meat, and contact with food-producing

animals and domestic pets (Acke *et al.*, 2006; Carbonero *et al.*, 2012; Abdollahpour *et al.*, 2015; Lim *et al.*, 2017). The infective dose is small; as low as 500 cells is enough to cause an infection (Allos, 2001).

Approximately 6% of human *Campylobacter* infections are due to contact with pets (Carbonero *et al.*, 2012; Iannino *et al.*, 2017). Companion animals, such as cats and dogs, are reservoirs and may be asymptomatic carriers (Moser, 2001; Hald *et al.*, 2004; Giacomelli *et al.*, 2015). The role of dogs as a source of human infections has been documented (Wolfs *et al.*, 2001; Labarca *et al.*, 2002; Man, 2011). While *Campylobacter jejuni* and *C. coli* are more commonly associated with human infections, less common species such as *C. upsaliensis* and *C. lari* have also been implicated in a small number of cases (Goossens *et al.*, 1995; Jimenez *et al.*, 1999; Man, 2011; Iannino *et al.*, 2017). *Campylobacter upsaliensis* and *C. jejuni* are considered the primary species of veterinary importance in companion animals (Hald & Madsen, 1997; Baker *et al.*, 1999; Koene *et al.*, 2004; Leonard *et al.*, 2011). Reported prevalence rates of *Campylobacter* spp. in pets vary extremely (Giacomelli *et al.*, 2015, Bojaniæ *et al.*, 2016), and in dogs, carriage rates range from 2.7–97% (Tsai *et al.*, 2007; Chaban *et al.*, 2010).

As of 2012, the Philippines has been reported to have the biggest proportion of dog owners in Asia, with one dog for every eight Filipinos (Bradley & King, 2012). This presents a significant risk factor in Filipinos, especially if their pets are infected with *Campylobacter*. To date, there are no published data on the prevalence of *Campylobacter* spp. in dogs in the Philippines. This work aimed to determine the prevalence of *Campylobacter* spp. among household dogs in the Philippines' National Capital Region of Metropolitan Manila, to identify which species of *Campylobacter* is predominantly present, and to recognize risk factors associated with *Campylobacter* spp. colonization. Selective enrichment and identification by PCR amplification of genus- and species-specific genes were employed in this study.

MATERIALS AND METHODS

Sample Collection

Faecal samples were collected from 195 dogs from the cities of Caloocan, Las Piñas, Makati, Malabon, Mandaluyong, Manila, Marikina, Muntinlupa, Navotas, Parañaque, Pasay, Pasig, Quezon, San Juan, Taguig, Valenzuela, and the municipality of Pateros. Prior to sample collection, dog owners were briefed regarding the study and given an information sheet, consent form, questionnaire, and instructions on how to collect the faecal samples using the collection kit provided, which contained a pair of disposable gloves, face mask, sterile spatula, and sterile specimen container. Households that participated in the study were sampled randomly and only those that have apparently healthy dogs were recruited. A maximum of 12 and a minimum of 10 dogs were sampled per city. Pet owners were asked to collect a faecal sample 12 hours before the scheduled pickup time using the kit provided and to store the sample in a cool, dry place. In households with several dogs, pet owners were asked to collect a faecal sample from only one dog. Samples were transported to the Medical Microbiology Laboratory of the Institute of Biology, University of the Philippines Diliman, and stored at 4°C for 24 hours before processing.

Demographic Information

A questionnaire was administered to collect demographic information about the owners and their dogs. Table 4 shows the owner and pet-related variables examined in the study.

Selective Enrichment

The enrichment protocol for *Campylobacter* using Bolton broth recommended by ISO 10272:2006-1 was followed (ISO, 2006). Briefly, a sterile cotton swab moistened with the enrichment broth was passed through the faecal sample until it was sufficiently covered with faecal material. The swab was inoculated into 5 mL of Bolton Selective Enrichment Broth (HiMedia™, Mumbai, India) supplemented with 5% (v/v) lysed horse blood and Bolton Selective Supplement

(HiMedia™, Mumbai, India) containing cefoperazone (20 mg/L), vancomycin (20 mg/L), trimethoprim (20 mg/L), and amphotericin B (10 mg/L). Samples were incubated at 37°C for 4 hours under microaerobic conditions using a candle jar, followed by incubation at 42°C for 48 hours, also under microaerobic conditions.

DNA Extraction

Genomic DNA extraction was performed using the boiling lysis method following the protocol of Queipo-Ortuño *et al.*, 2008, with modifications. Briefly, 1 mL of the 48-hour enrichment culture was transferred to a sterile 1.5-mL microcentrifuge tube. Bacterial cells were harvested by centrifugation at 6,000 rpm (LMS Mini Centrifuge MCF-1350) for 5 minutes. The supernatant was discarded, and the pellet was re-suspended in 500 µL sterile distilled water for washing and again pelleted at 6,000 rpm for 5 minutes. The washing step was performed twice more. The pellet was re-suspended in 200 µL of sterile distilled water and incubated at 100°C for 15 minutes, followed by centrifugation at 10,000 rpm for 5 minutes. The supernatant was collected and transferred to a new sterile 1.5 mL tube. DNA concentration and purity were determined using a NanoDrop™ 2000/2000c spectrophotometer.

Molecular Detection

DNA quality was further verified by PCR amplification of the 16S rDNA gene. Samples were identified as *Campylobacter* spp., *C. upsaliensis*, *C. jejuni*, and *C. coli* using the genus- and species-specific primers listed on Table 1. Uniplex PCR assays were performed in 20-µL reaction mixtures composed of 10 µL of GoTaq® Master Mix (Promega, Wisconsin, USA) (with 50 units/mL of *Taq* DNA polymerase in a proprietary reaction buffer (pH 8.5), 400 µM each of dATP, dGTP, dCTP, dTTP, and 3 mM of $MgCl_2$), 1.2 µL of each primer (0.6 µM final concentration), 6.6 µL of nuclease-free water, and 1 µL of template DNA. PCR amplification assays were performed in a MyCycler™ Thermal Cycler System (Bio-Rad, California, USA) following the cycling conditions listed in Table 2.

Table 1. List of primers used in the PCR assays

Primer	Target organism	Sequence (5'-3')	PCR product (bp)	Reference
Unibac-F	bacteria	CGTGCCAGCCGCGGTAATACG	611	Amit-Romach *et al.*, 2004
Unibac-R		GGGTTGCGCTCGTTGCGGGAC TTAACCCAACAT		
MD16S1-F	*Campylobacter* spp.	ATCTAATGGCTTAACCATTAAAC	857	Inglis & Kalischuk, 2004
MD16S2-R		GGACGGTAACTAGTTTAGTATT		
UpsF	*C. upsaliensis*	TGGAATGGCTTTGACGCT	192	Fontanot *et al.*, 2014
UpsR		GGTATAACCAGCAGTTAGG		
MDmapA1-F	*C. jejuni*	CTATTTTATTTTTGAGTGCTTGTG	589	Inglis & Kalischuk, 2004
MDmapA2-R		GCTTTATTTGCCATTTGTTTTATTA		
Col3-F	*C. coli*	AATTGAAAATTGCTCCAACTATG	462	Gonzalez *et al.*, 1997
MDCol2-R		TGATTTTATTATTTGTAGCAGCG		

Table 2. PCR conditions for the primers used in this study

	Unibac-F Unibac-R	MD16S1-F MD16S2-R	UpsF UpsR	MdmapA1-F MdmapA2-R	Col3-F MDCol2-R	No. of cycles
Initial Denaturation	95°C, 2 m	95°C, 2 m	95°C, 2 m	95°C, 2 m	95°C, 2 m	1x
Denaturation	95°C, 1 m	95°C, 1 m	95°C, 1 m	95°C, 1 m	95°C, 1 m	35x
Annealing	60°C, 1 m	49°C, 1 m	50°C, 1 m	53°C, 1 m	53°C, 1 m	
Extension	72°C, 1 m	72°C, 1 m	72°C, 1 m	72°C, 1 m	72°C, 1 m	
Final Extension	72°C, 5 m	72°C, 5 m	72°C, 5 m	72°C, 5 m	72°C, 5 m	1x
Final hold	4°C, ∞	4°C, ∞	4°C, ∞	4°C, ∞	4°C, ∞	

Agarose Gel Electrophoresis

PCR products were electrophoresed in parallel with a 100-bp molecular weight marker (Vivantis Technologies, California, USA) on 1% (w/v) agarose (Invitrogen, California, USA) gel stained with 0.5 µg/mL ethidium bromide (Invitrogen, California, USA) in 1X Tris-Acetate-EDTA (TAE) buffer, and viewed using a UV transilluminator (UVP, California, USA).

Statistical Analysis

The prevalence of *Campylobacter* spp. was calculated based on the positive results. The Chi-square test for independence was used to determine whether there was significant association between the owner and dog-related variables considered and the presence of *Campylobacter*. Statistical significance was determined using SPSS version 21 for Windows (IBM, 2012), with the level of significance set at <0.05.

RESULTS

Prevalence of *Campylobacter* spp.

From the 195 dogs' faecal samples collected from Metro Manila, 19 (9.74%) tested positive for *Campylobacter* spp. *Campylobacter upsaliensis* was the predominant species with a prevalence of 7.2% (14/195), followed by *C. jejuni* at 2.05% (4/195). *Campylobacter coli* was not detected. One sample contained a *Campylobacter* that could not be identified as it was neither *C. upsaliensis* nor *C. jejuni* nor *C. coli*. Table 3 presents the prevalence rate by city and by species. Simultaneous presence of *C. upsaliensis* and *C. jejuni* in the same host was also observed in three (15.8%) of the 19 samples that were *Campylobacter*-positive.

Risk factor analysis

The Chi-square test for independence revealed that male dog owners and presence of other pets were significantly associated with *Campylobacter* spp. colonization. Table 4 shows the owner- and dog-related variables and their respective *p*-values.

DISCUSSION

Dogs are widely recognized as carriers of *Campylobacter* and may or may not manifest any of the clinical signs of campylobacteriosis (Damborg *et al.*, 2016). In this study, 9.74% of the household dogs examined harboured the bacteria. Prevalence rates in published studies vary extremely between countries. Prevalence rates in Canada, New Zealand, and some countries in Europe range from 22 to 43% (Leonard *et al.*, 2011; Carbonero *et al.*, 2012; Procter *et al.*, 2014; Bojaniæ *et al.*, 2016), whereas some countries reported lower prevalence rates. A study in Taiwan reported a prevalence rate of 2.7% (Tsai *et al.*, 2007) while a study in Poland detected *Campylobacter* in 4.8% of dogs (Andrzejweska *et al.*, 2013). A carriage rate of 11% was reported by a study in Italy (Giacomelli *et al.*, 2015).

Table 3. Prevalence of *Campylobacter* spp., *C. upsaliensis*, *C. jejuni*, and *C. coli* in household dogs in Metro Manila, Philippines

City	No. of samples	*Campylobacter* spp. Positive (%)	*C. upsaliensis* Positive (%)	*C. jejuni* Positive (%)	*C. coli* Positive (%)
Caloocan	12	1 (8.3)	1 (8.3)	0	0
Las Piñas	12	3 (25)	2 (16.7)	0	0
Makati	12	1 (8.3)	1 (8.3)	0	0
Malabon	11	0	0	0	0
Mandaluyong	11	0	0	0	0
Manila	10	0	0	0	0
Marikina	12	2 (16.7)	2 (16.7)	0	0
Muntinlupa	12	1 (8.3)	0	1 (8.3)	0
Navotas	12	3 (25)	2 (16.7)	1 (8.3)	0
Parañaque	12	1 (8.3)	0	0	0
Pasay	12	2 (16.7)	1 (8.3)	0	0
Pasig	10	0	0	0	0
Pateros	11	2 (18. 2)	2 (18.2)	2 (18.2)	0
Quezon	12	2 (16.7)	2 (16.7)	0	0
San Juan	10	1 (10)	1 (10)	0	0
Taguig	12	0	0	0	0
Valenzuela	12	0	0	0	0
TOTAL	**195**	**19 (9.74)**	**14 (7.2)**	**4 (2.05)**	**0**

This high variability may be explained by factors such as the age of the dogs, detection methods used, and geographical location (Giacomelli *et al.*, 2015; Bojaniæ *et al.*, 2016). Several works have cited high carriage rates in young dogs compared to adult dogs (Hald & Madsen, 1997; Acke *et al.*, 2009; Carbonero *et al.*, 2012; Procter *et al.*, 2014).

There is no recommended single standard method for the isolation and detection of all *Campylobacter* species because of their varying temperature requirements, microaerobic conditions, nutrients, and susceptibility to antibiotics used to increase selectivity. Culture-independent molecular methods, specifically PCR, reveal higher detection rates than cultural methods (Van Dyke *et al.*, 2010). PCR methods may be used as a primary tool or in combination with a culture-dependent method for *Campylobacter* detection (Huang *et al.*, 2015).

Campylobacter upsaliensis was the predominant species in dogs in this study, followed by *C. jejuni*. This finding concurs with the results of several other studies (Hald *et al.*, 2004; Acke *et al.*, 2009; Chaban *et al.*, 2010; Carbonero *et al.*, 2012; Procter *et al.*, 2014; Bojaniæ *et al.*, 2016). These reports support the notion that dogs are predominantly carriers of *C. upsaliensis* and, due to their close association with humans, can be an important source of human campylobacteriosis. Transmission is via the faecal-oral route and may occur directly or indirectly through fomites (Damborg *et al.*, 2016). Dogs may shed the bacteria without manifesting any clinical signs of the disease but can also be infected and exhibit the same signs of gastrointestinal distress as in humans. There are a few studies, however, that report *C. upsaliensis* as only the second most common species isolated from dogs, after *C. jejuni* (Hald & Madsen, 1997; Tsai *et al.*, 2007). While *C. coli* was not detected in this work, other studies have reported its presence in dogs, although less frequently (Hald *et al.*, 2004; Acke *et al.*, 2009; Andrzejweska *et al.*, 2013).

Simultaneous detection of two *Campylobacter* species in the same host was observed in this study. The same result was described in a report that made use of four

Table 4. Results of Chi-square test for the owner- and dog-related variables examined in this study. The level of significance was set at <0.05

Owner-related variables	Category	*Campylobacter*-positive (%)	*p*-value
Gender	Male	61.1	0.048*
	Female	38.9	
Annual income	< Php200,000	80.0	1.000
	Php200,000–Php399,000	13.3	
	≥ Php400,000	6.7	
No. of household members	1-3	11.8	0.238
	4-6	41.2	
	7 or more	47.0	
Dog-related variables			
Size	Small	50.0	0.349
	Medium	38.9	
	Large	11.1	
Age	<2 months	0	0.575
	2–6 months	16.7	
	7–12 months	11.1	
	1–2 years old	22.2	
	2–5 years old	44.4	
	>5 years old	5.6	
Gender	Male	55.6	0.897
	Female	44.4	
Reproductive status (intact or spayed/neutered)	Spayed/Neutered	0	1.000
	Intact	100	
Number of dogs in the household	1	52.9	0.632
	2	35.3	
	3	5.9	
	4+	5.9	
Living with other pets	Yes	55.6	0.010*
	No	44.4	
Presence of sick pets	Yes	5.6	0.110
	No	94.4	
Veterinarian consultation in the past year	Yes	41.2	0.595
	No	58.8	
Regular veterinary care	Yes	61.1	0.890
	No	38.9	
Antibiotics in the past month	Yes	0	0.591
	No	100	
If with signs of gastrointestinal upset in the last 30 days			
Diarrhoea	Yes	0	0.738
	No	100	
Vomiting	Yes	16.7	0.471
	No	83.3	
Other sources of drinking water	Toilet	16.7	0.706
	None	83.3	

Current diet	Dog food	27.8	
	Table food	33.3	0.149
	Mixed	38.9	
Duration of diet	More than a month	23.5	
	Not more than 6 months	5.9	0.471
	More than 6 months	70.1	
Feeding frequency	Once a day	11.8	
	Twice day	35.3	0.476
	Thrice a day	47.0	
	>3x a day	5.9	
Additional products on the diet	None	5.6	1.000
	Yes	94.4	
Dog's activity	Can run freely in other places	5.5	
	Caged	22.2	
	Always kept on a leash	16.7	0.814
	Stays indoor all the time	38.9	
	Others	16.7	
Bath frequency	Daily	0	
	2–3x a week	56.25	0.204
	Weekly	25.0	
	1–2x a month	18.75	
Grooming frequency	Weekly	0	
	Every 2 weeks	56.25	0.275
	Monthly	25.0	
	Never	18.75	
Food bowl cleaning	Daily	93.75	
	1–2x a week	6.25	1.000
	3–4x a week	0	
	Never	0	
Water bowl cleaning	Daily	87.5	
	1–2x a week	6.25	0.705
	3–4x a week	6.25	
	Never	0	

*Statistically significant.

different isolation methods (Koene *et al.*, 2004). The same study also concluded that co-colonization with multiple *Campylobacter* species is a common occurrence in dogs. Another study that employed both direct plating and selective enrichment was able to recover multiple species in the same host (Andrzejweska *et al.*, 2013). Information on coinfections may be useful in epidemiological studies that involve tracing the sources of human campylobacteriosis (Koene *et al.*, 2004).

In this study, it was determined that dogs with male owners tend to be colonized by *Campylobacter* more frequently than dogs with female owners. This has never been reported in any previous work. It is possible that the hand washing behaviour of males is less effective than females in controlling the spread of the microorganism, as reported by one study (Edwards *et al.*, 2002). However, this observed association may have been confounded by other factors, such as the owner's occupation or the individual who is the primary caretaker of the dog, and as such, must be interpreted with caution.

This work was also able to determine that living with other pets can be associated with *Campylobacter* spp. carriage in dogs. This has been previously cited as a risk

factor (Leonard *et al.*, 2011). One study reported that dogs that lived in groups and have close contact with other animals were found to have a higher carriage rate of *Campylobacter* (Acke *et al.*, 2006). Pet-to-pet contact, which may involve exposure to the faeces of other pets, is said to play an important role in the transmission of this pathogen, especially in urban areas (Damborg *et al.*, 2016). Dogs have been reported to eat faeces (coprophagy) and practice xenosmophilia (preference for foreign smells), hence they roll in substances with strong odours, such as faecal matter (Frenkel & Parker, 1996). Coprophagia leads to the ingestion of the bacterium that can be shed in the faeces and rolling in faecal matter contaminates the fur. Pet animals can shed campylobacter for an extended time, and, thus, contribute to its spread in the environment (Damborg *et al.*, 2016).

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Conflict of interest
None to declare.

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