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Occurrence of *Campylobacter* in dogs and cats in Selangor Malaysia and the associated risk factors

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ABSTRACT

Aims: *Campylobacter* is the most widely reported zoonotic bacterial agent that causes enteric disease in humans worldwide with millions of cases recorded far exceeding salmonellosis in Europe and United States. The objective of this study was to determine the occurrence of *Campylobacter* in dogs and cats and their associated risk factors.
Methodology and results: A total of 101 rectal swabs were collected from both pets (n=40) and stray dogs (n=61) for the study. Similarly, a total of 86 rectal swabs were taken from stray cats (n=46) and pet cats (n=40) from client pets at a university veterinary hospital and from stray dogs and cats from animal shelters. *Campylobacter* were isolated by culture, identified by biochemical tests and confirmed and speciated, using mPCR assay. The result showed occurrence of *Campylobacter* in stray dogs and stray cats were 16.3% and 32.6% respectively, while in pet dogs and cats were 12.5 % each. Based on the mPCR assay, three species of *Campylobacter* were identified in dogs namely *Campylobacter upsaliensis* (66.6%), *C. jejuni* (6.7%) and *C. heliviticus* (20%), while *C. upsaliensis* (55%), *C. helviticus* (20%) and *C. jejuni* (6.7%) were identified in cats. The risk factors for the presence of *Campylobacter* in the animals were analysed but none was significantly associated, however the occurrence in cats was found higher in adults, females, those kept outdoors and residing in town areas, multipets household, cats with no history of being given antibiotics in past infections and being fed on raw meat and fish while the occurrence of *Campylobacter* was high in dogs of local breeds, females, of young age, being kept outdoors and fed raw meat and fish.
Conclusion, significance and impact of study: These findings showed that *Campylobacter* were quite prevalent in both stray and pet dogs and cats which may contaminate other animals and spread in the environment as *Campylobacters*. It is of public health concern as humans can contract the disease if they do not practice proper hygiene after coming into contact with an infected animal or contaminated environment.

Keywords: Campylobacter, cat, dog, risk factors

INTRODUCTION

Campylobacter is one of most important causes of enteritis in humans, with *Campylobacter jejuni* and *C. coli* mostly responsible for the infections in developing and developed countries (Man, 2011). Most of the studies reported *Campylobacter* enteritis are foodborne with consumption of undercooked poultry meat and poultry products, seafoods and raw or unpasteurised milk and dairy products as well as contaminated vegetables and water as sources of infections. There were also reports that humans can contract the infection upon handling or in contact with infected animals. Studies have showed that animals such as livestocks, dogs and cats to be both symptomatic and asymptomatic carriers of *Campylobacter* spp. (Gras *et al.*, 2013; Kittl *et al.*, 2013; Lazou *et al.*, 2014). *Campylobacter* is prevalent in livestocks, in particular chickens and pigs. The occurrence of *Campylobacter* in pet dogs ranged from 4.8-19.4%, while in pet cats from 9.9-11.7% (Andrzejewska *et al.*, 2013; Holmberg *et al.*, 2015; Callejon *et al.*, 2015). In stray dogs

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it ranged from 23.8 to 51.3% (Tsai *et al.*, 2007) and 16.8% in stray cats (Gargiulo *et al.*, 2008).

The incidence of campylobacteriosis and intestinal carriage of *Campylobacter* in pets and stray dogs is of important public health importance both in developed and developing countries (Kaakoush *et al.*, 2015) . The commonly isolated *Campylobacter* species in dogs and cats are *C. upsaliensis*, *C. helviticus*, *C. coli* and *C. jejuni* and they are regarded as important reservoirs of the organisms. These species are present with or without the animals showing any symptoms, Animals may experience diarrhoea (sometimes bloody), decreased appetite, vomiting and possibly fever with the symptoms usually clear up on their own in 3 to 7 days; the disease is generally more severe in young animals (Andrzejewska *et al.*, 2013; Callejon *et al.*, 2015).

In dogs and cats, several risk factors have been associated with *Campylobacter* infection. These factors include age, kennel cough, history of vomiting, common shelter with dog carrying *Campylobacter* spp., and antibiotic treatment (Friedman *et al.*, 2004; Acke *et al.*, 2009). Younger dogs appear to harbour the organism more than older dogs and it is more prevalent in kennelled dogs with the carriage rate in dogs and cats for *C. upsaliensis* ranging between 5.0 to 66.2% (Stanley *et al.*, 1992; Moreno *et al.*, 1993; Madsen, 1997; Baker *et al.*, 1999; Hald *et al.*, 2004).

In Malaysia, a number of studies were carried out in livestocks, in particular poultry such as chickens and ducks, as well as on poultry meat and wild birds and flies were also found to carry *Campylobacter* (Saleha, 2004). However there are no available data regarding the occurrence of *Campylobacter* spp. in dogs and cats in Malaysia. Thus, the aim of this study was to determine the occurrence of *Campylobacter* in dogs and cats, to identify the species using mPCR assay and to determine the risk factors associated with campylobacteriosis in the animals.

MATERIALS AND METHODS

Collection of samples

Samples were collected after due approval from the Animal Care and Use Committee (ACUC) of Universiti Putra Malaysia. The samples collected were rectal swabs from apparently healthy clients owned dogs and cats at a university veterinary hospital after seeking the consent of the owners, stray cats from an animal shelter, and stray dogs from an animal pound. One hundred and one rectal swabs were collected from both pets (n = 40) and stray dogs (n = 61) and 86 rectal swabs from stray cats (n = 46) and pet cats (n = 40). Each of the rectal swabs was then placed into a universal bottle containing Cary-Blair medium (Oxoid) as transport medium and labelled accordingly. All samples were then transported to the laboratory in a cool box containing ice and cultured within 2 to 4 h of collection.

Isolation of *Campylobacter*

Each rectal swab was directly streaked onto a plate of Modified *Campylobacter* Blood Free Selective Agar (mCCDA) (Oxoid) supplemented with cefoperazone, amphotericin and teicoplanin (CAT) (Oxoid) selective supplement. The plates were then incubated for 48 h at 42 ºC in gas jars under microaerophilic conditions generated by gas packs (BD CampyPak^{TM}; Becton, Dickinson and Company). Presumptive *Campylobacter* isolates were then subjected to phenotypic identification based on cellular morphology by Gram staining and motility characteristic by hanging drop method under phase contrast microscopy. Suspected *Campylobacter* colonies from the mCCDA plates were subcultured onto Columbia blood agar (CBA) (Oxoid) with 5% horse blood added to obtain pure colonies.

Presumptive identification of *Campylobacter* isolate

Bacterial colonies with the organisms showing curved or spiral motile rods with darting corkscrew movement as viewed under phase-contrast microscopy and gave Gram negative reaction were presumptively identified as *Campylobacter.* All suspected *Campylobacter* isolates were further identified by oxidase, catalase production, hippurate hydrolysis and indoxyl acetate hydrolysis tests that phenotypically could differentiate the species of the isolates (Table 1).

Table 1: Biochemical tests of differentiate four common *Campylobacter* species in dog and cat.

Species	*Catalase*	*Oxidase*	*Hippurate hydrolysis*	*Indoxyl acetate hydrolysis*	*Growth on potato starch*
C. upsaliensis	-/w*	+	_	+	+
C. helviticus	_	+	_	+	-
C. jejuni	+	+	+	+	n
C. coli	+	+	_	+	n

*, Bourke *et al.* (1998); Steinhauserova *et al.* (2001); -/w, weak reaction, -,negative; +, positive; n, not known

Confirmation and speciation of isolates by multiplex polymerase chain reaction (mPCR) assay

Bacterial DNA was extracted according to the manufacturer's instruction using Wizard Genomic DNA extraction kit (Promega, Madison, WI, USA). The mPCR assay conducted in this study was as described by Yamazaki-Matsune *et al.* (2007). The primers used are as shown in Table 2. The mastermix for the final multiplex PCR comprised: 5 µL of DNA template; 0.1 µL of primers

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Table 2: Primers used for the amplification of different *Campylobacter* species genes.

Species	Size (bp)	Target gene	Primer	Sequences (5' to 3')	References
All *Campylobacter*	854	16S rRNA	CCCJ609F CCCJ1442R	5'- AATCTAATGGCTTAACCATTA-3' 5'-GTA ACT AGT TTA GTA TTC CGG-3'	Linton *et al.* *(1996)*
C. upsaliensis	86	lpxA	CU61F CU146R	5'-CGATGATGTGCAAATTGAAGC-3' 5'-TTCTAGCCCCTTGCTTGATG-3'	Yamazaki-Matsune *et al.* (2007)
C. jejuni	161	cj0414	C-1 C-3	5'-CAAATAAAGTTAGAGGTAGAATGT-3' 5'-CCATAAGCACTAGCTAGCTGAT-3'	Wang *et al.* (2002)
C. coli	502	cueE	CC18F CC519R	5'-GGTATGATTTCTACAAAGCGAG-3' 5'-ATAAAAGACTATCGTCGCGTG-3'	Linton *et al.* (1997)
C. helviticus	1225	16S rRNA	CHCU 146F CH 1371R	5'-GGGACAACACTTAGAAATGAG-3' 5'-CCGTGACATGGGCTGATTCAC-3'	Moyaert *et al.* (2008)

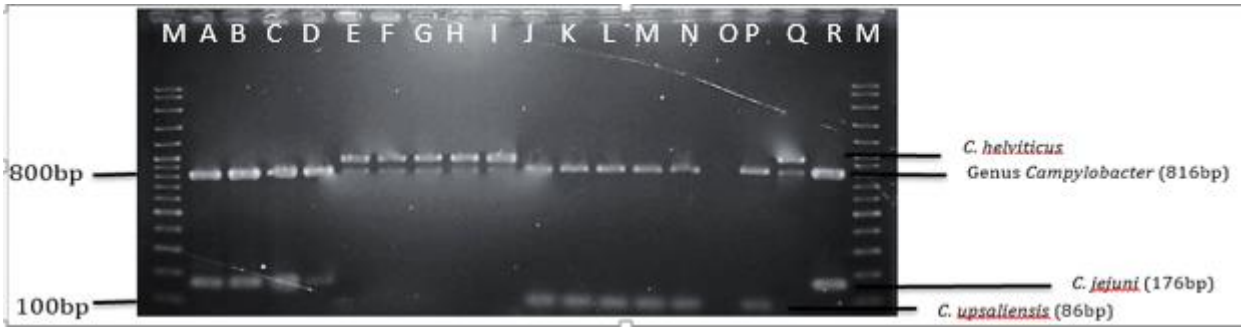

Figure 1: PCR amplification of *Campylobacter* spp isolated from dogs and cats: Lane M, marker; Lanes A, B, C and D, *C. jejuni*; Lanes E, F, G, H and I, *C. helviticus*; Lanes J, K, L, M and N, *C. upsaliensis*; Lane O, negative control; Lane P, *C. upsaliensis* (CCUG17801); Lane Q, *C. helviticus* (ATCC 51209); Lane R, *C. jejuni* (CCUG 17812).

C412F, C-1, C-3, CC18F, CC519R, CU61F, CU146R, CH1371R, CHCU146F, CLF, and CLR 25 µL of Top taq master mix (Qiagen®). The final volume was adjusted to 50 µL. Amplification procedure using appropriate primers and cycling condition was conducted in a thermal cycler (Eppendorf). Initial denaturation was performed at 95 °C for 15 min, followed by 25 cycles each of 95 °C for 30 sec, annealing at 58 °C for 1.5 min and elongation at 72 °C for 1 min, and ending with a final extension time at 72 °C for 7 min. Reference strains were used as positive control and distilled water as negative control. The amplified PCR products were then subjected to electrophoresis in 1% agarose gel prepared in 1× TBE buffer (40 mM Tris-Borate, 2 mM EDTA, pH 7.5) at 90 V for 120 min (Figure 1).

Risk factors

Information on age, breed, sex, single or multi-pet household, recent treatment with antibiotics, housing of the dogs and cats sampled, source of drinking water and place of residence of the owner were collected using a simple questionnaire. No pilot study to evaluate the questionnaires was conducted, but the questionnaire was based on a questionnaire used in a similar project and was also discussed and evaluated by the project group (Sandberg *et al.*, 2002).

Statistical analysis

The occurrence of *Campylobacter* in the dogs and cats was determined using SPSS version 20.0 (SPSS Chicago, USA) was used. Descriptive statistic and frequency distribution were calculated and the occurrence rate was determined. Pearson Chi-square test and logistic regression statistics were used to determine the association between risk factors and occurrence of *Campylobacter* based on the questionnaire completed by pet owners. The results were considered statistically significant at p-value ≤ 0.05 at 95% confidence interval.

RESULTS

The occurrence of *Campylobacter* in pets and stray dogs and cats were shown in Table 3. Isolates were presumptively identified as *C. helviticus*, *C. upsaliensis* and *C. jejuni* by colony and cellular morphology and biochemical tests. The mPCR assay confirmed the three species of *Campylobacter* isolates (Figure 1). In stray and pet dogs, there was no significant difference ($\chi^2 = 0.290$, $P = 0.590$) in terms of occurrence; however, stray dogs had higher carriage (16.4%) when compared to pet dogs (12.5%). In cats, there was a significant difference in the occurrence between pet and stray cats ($\chi^2 = 4.847$, $P =$

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Table 3: Summary of occurrence of *Campylobacter* in dogs and cats from various sources.

Animals	Category	No. of samples	No. of samples	Percentage (%) of positive sample	Chi square	P-value	Odds Ratio	Confidence intervals	
								Lower	Upper
Dogs	Stray	61	10	16.3	0.2896	0.590	1.368	8.956	27.81
	Pet	40	5	12.5	Ref	Ref	Ref	26.58	4.993
	Total	101	15	14.85					
Cats	Stray	46	15	32.6	4.847	0.028*	3.34	47.09	47.09
	Pet	40	5	12.5	Ref	Ref	Ref	26.58	26.58
	Total	86	20	23.2					

Ref, Reference group; *, statistically significant

0.028) where the occurrence in stray and pet cats was 32.6% and12.5% respectively. Generally, there was no significant difference in terms of occurrence of different species of *Campylobacter* in dogs and cats (χ^2 = 0.826, P = 0.843). Among *Campylobacter* species, *C. upsaliensis* showed the highest occurrence at 60%, followed by *C. helviticus* at 20% and *C. jejuni* at 11.4%. *Campylobacter upsaliensis* was isolated more from dogs at 66.7% compared to cats at 55% as shown in Table 4. As shown in Table 5, there was no significant association between the breed in terms of occurrence of *Campylobacter* in dogs (*p* = 0.397) and cats (*p* = 0.457); however the percentage carriage rate was higher in local breeds of cats and dogs at 16.7% and 17.6% respectively compared to pedigree. Similarly, Campylobacteriosis was more common in young dogs. Puppies showed higher carriage of *Campylobacter* (18.2%). There was no difference in carriage rate in dogs that were kept outdoors by the owner compared to those kept indoors. Dogs with history of administration of antibiotics within the previous month showed no significant difference in occurrence of *Campylobacter* infection (*p* = 0.72). However pets with recent history of antibiotic administration showed lower carriage of *Campylobacter* than those without any record of antibiotic usage. Pets from a multi-pet household had no association with regards to *Campylobacter* carriage (*p* = 0.141). Consumption of raw meat and fish, contact with other animals and source of drinking water all showed no significant difference in terms of occurrence of *Campylobacter* in dogs.

As shown in Table 6, there was no significant association between the age category of cats sampled in terms of occurrence of *Campylobacter* (P = 0.457); however, the percentage of occurrence was higher in adult cats (33%) compared to juvenile (13%) and kitten (7.1%). In terms of occurrence of *Campylobacter* among the male and female sampled, there was no significant difference (*p* = 0.481) however females appeared to have high carriage (20%) than males (5%). Similarly, Campylobacteriosis was more common in local breeds of cats although there was no significant difference (*p* = 0.386) in the occurrence when compared with pedigree. There was high-level of carriage for cats that were kept outdoors by the owner than those staying indoors but was not statistically significant (*p* = 0.471). Also, cats with history of administration of antibiotics within the previous month showed no significant difference in occurrence of *Campylobacter* infection (*p* = 0.633). Similarly, pets with recent antibiotic administered showed lesser carriage of *Campylobacter* than those without any record of antibiotic usage. Cats that lived together with other animals had no association with regards to *Campylobacter* carriage (*p* = 0.53). Water source, contact with other animals, predatory habit and consumption of raw meat and fish were all shown to be not significant in the occurrence of *Campylobacter* in cats.

Table 4: Different species of *Campylobacter* isolated from dogs and cats.

Species	Stray dogs n=10	Pet dogs n=5	Stray cats n=15	Pet cats n=5	Total in dogs (%)	Total in cats (%)
C.upsaliensis	6*	4	8	3	66.6**	55
C. helviticus	2	1	3	1	20	20
C. jejuni	1	-	2	1	6.7	15
C. coli	1	-	2	-	6.7	10
Total no. of isolates	10	5	15	5		

*, No. of isolates; **, No. of each *Campylobacter* spp. in dog or cat / Total no. of isolates in dog or cat

DISCUSSION

The presence of *Campylobacter* has previously been reported in Malaysia mainly in chicken and meat products and wild birds and *C. jejuni* and *C. coli* were frequently isolated (Saleha, 2004). The overall prevalence of *Campylobacter* in dogs and cats were 14.85% and 23.25% respectively. *C. upsaliensis* was the predominant species in both dogs and cats followed by *C. helviticus*. These results were similar to the findings of studies conducted by various researchers from various countries across the globe. Baker *et al.* (1999) reported the

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Table 5: Univariate analyses of risk factors and occurrence of Campylobacter in dogs.

Variable	Category	Prevalence (%)	P-value	Odds ratio	95% Confidence interval	
					Lower	Upper
	Puppy	18.2	0.863	1.250	0.100	15.647
Age	Juvenile	11.1	0.542	2.222	0.171	28.856
	Adult	9.1	NA	Ref	Ref	Ref
Sex	Female	21.1	0.120	0.625	0.062	6.301
	Male	4.8	NA	Ref	Ref	Ref
Breed category	Local	17.6	0.397	0.444	0.066	3.010
	Pedigree	8.7	NA	Ref	Ref	Ref
Owner's residence	Urban	13.8	0.688	0.625	0.062	6.301
	Town	9.1	NA	Ref	Ref	Ref
Housing	Outdoor	12.5	1.000	1.000	0.148	6.772
	Indoor	12.5	Ref	Ref	Ref	Ref
Household type	Multi-pet	17.2	0.141	0.686	0.548	0.858
	Single	0	NA	Ref	Ref	Ref
Antibiotic history	No	14.3	0.720	1.417	0.210	9.548
	Yes	10.5	NA	Ref	Ref	Ref
Antibiotic duration	<1 month	14.3	0.720	1.417	0.210	9.548
	>1 month	10.5	NA	Ref	Ref	Ref
Predatory habit	Yes	14.7	0.315	0.829	0.713	0.963
	No	0	NA	Ref	Ref	Ref
Water source	Unfiltered	13.8	0.688	1.600	0.159	16.131
	Filtered	9.1	NA	Ref	Ref	Ref
Raw meat or fish consumption	Yes	14.3	0.875	1.208	0.114	12.811
	No	12.1	NA	Ref	Ref	Ref
Contact with other animals	Yes	12.8	0.702	0.971	0.918	1.028
	No	0	NA	Ref	Ref	Ref

Table 6: Univariate analyses of risk factors and occurrence of *Campylobacter* in cats.

Variable	Category	Prevalence (%)	P-value	Odds ratio	95% Confidence interval	
					Lower	Upper
Age	Adult	33.3	0.581	1.950	0.183	20.827
	Juvenile	13	0.244	6.500	0.280	151.123
	Kitten	7.1	NA	Ref	Ref	Ref
Sex	Female	20	0.481	4.750	0.481	46.906
	Male	5	NA	Ref	Ref	Ref
Owner's residence	Town	15.4	0.702	1.455	0.212	9.984
	Urban	11.1	NA	Ref	Ref	Ref
Housing	Outdoor	16.7	0.471	2.000	0.296	13.511
	Indoor	9.7	NA	Ref	Ref	Ref
Household type	Multi-pet	30.0	0.53	1.67	0.23	1.197
	Single	6.7	NA	Ref	Ref	Ref
Antibiotic history	No	15	0.633	1.588	0.236	10.704
	Yes	10	NA	Ref	Ref	Ref
Antibiotic duration	>1month	15.8	0.550	1.781	0.264	12.014
	<1month	9.5	NA	Ref	Ref	Ref
Predatory habit	Yes	13.3	0.733	0.722	0.071	7.340
	No	10	NA	Ref	Ref	Ref
Water source	Unfiltered	15	0.633	0.630	0.093	4.244
	Filtered	10	NA	Ref	Ref	Ref
Raw meat or fish consumption	Yes	91.7	0.602	0.545	0.054	5.465
	No	2.3	NA	Ref	Ref	Ref
Contact with other animals	Yes	14.3	0.366	0.857	0.749	0.981
	No	0	NA	Ref	Ref	Ref

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prevalence of *C. upsaliensis* and *C. jejuni* in cats at 11% and 4% respectively, whereas 34% of dogs carried *C. upsaliensis*, 7% *C. jejuni* and 2% *C. coli*.

In Spain, Carbonero *et al.* (2012) reported that of 102 *Campylobacter* isolated from 306 dogs, 35.2% were *C. jejuni*, 58.8% *C. upsaliensis* and 2% *Campylobacter* spp. In Nigeria, Salihu *et al.* (2010) also reported the occurrence of *Campylobacter* spp. in 141 dogs and 104 cats at 27.7% and 18.3% respectively. In all the studies mentioned, *C. upsaliensis* was the predominant species isolated from both dogs and cats. Stray dogs were shown to have higher carriage rate (16.3%) compared to pet dogs (12.5%) although it was not statistically significant. This finding was similar to that obtained in a study from Taiwan which showed that 2.7% of the household dogs and 23.8% of the stray dogs were positive for *Campylobacter* (Tsai *et al.*, 2007). A higher prevalence of *Campylobacter* species was reported in dogs in Denmark at the rate of 76.2% (278/366) (Hald *et al.*, 2004). Variation in these results may be due to different dog populations sampled and areas investigated and the technique employed in the isolation process and the fastidious nature of the organism (Byrne *et al.*, 2007). Also, when the isolates are exposed to adverse condition, they may change to viable, but non-culturable (VBNC) form, which affects the isolation rate (Person and Olsen, 2005).

In this study, the result of biochemical tests and mPCR assay clearly showed that the biochemical and molecular methods are equally reliable for detection and confirmation of *Campylobacter*. However, mPCR is often preferred for the simultaneous confirmation and species differentiation because of its high sensitivity and faster time to complete than biochemical tests (Stoyanchev, 2004). Biochemical characterization as a basis for the presumptive identification and species of the isolates are cumbersome and often leads to ambiguous results (Steinhauserova *et al.,* 2001). For this reason, mPCR assay becomes more important to confirm and to differentiate *Campylobacter* species.

Campylobacter upsaliensis is reported as the most prevalent species in dogs and cats but less common in human (Bourke *et al.*, 1998). The high carriage rate of *Campylobacter* in dogs and cats indicates the organism may be intestinal commensal in the animals (Sandsedt *et al*.,1983).

Also in this study, 11.4% of dogs and cats carried *C. jejuni*. Pet dogs and cats have also been implicated in the transmission of *C. jejuni* to humans according to Deming *et al.* (1987). In their study 30% of cases of Campylobacteriosis in human were accounted by contact with infected cats and it was observed that an association of sporadic cases of *Campylobacter* enteritis with handling kittens was observed. Some studies in clinically normal cats and healthy dogs were found to yield *Campylobacter.* Stray dogs and cats have been shown to have higher prevalence of *Campylobacter* infection than those under their owner's care due to the fact that stray dogs and cats are often in contact with contaminated environment and wild birds and wild rodents which are considered the reservoirs of *Campylobacter* (Bungay *et al.*, 2005; Moore *et al.*, 2002; Newell and Fearnley, 2003).

Similar to stray dogs, stray cats are known to be a potential source of *C. jejuni* infection for human and given that they cohabit with humans in places like parks, public gardens, and harbour areas. It would be important to minimize the risk of zoonotic spread by encouraging humans to follow good hygiene practices and to reduce contact with stray animals (Gargiulo *et al.*, 2008). *Campylobacter* spp. occurs in the environment between animal and human host where they are exposed to less optimum growth conditions such as low oxygen, temperatures ranges outside their minimum growth requirement, desiccation and other stress factors. Unlike other foodborne pathogens, *Campylobacter* spp. are fragile and apparently unable to grow in the presence of air and multiply outside the animal host and are highly susceptible to a number of environmental conditions (Park, 2002). However, they may change to VBNC form and remain infective.

Several studies conducted across the globe found that younger dogs are more likely to carry *Campylobacter* spp. than older dogs, and *C. upsaliensis* is the most common species isolated (Hald *et al*., 2004; Sandberg *et al.*, 2002; Wieland *et al.*, 2005). This is similar to the finding of this study. Older animals appear to have less occurrence of *Campylobacter* probably due to the immunity developed from previous infections as immunity to *Campylobacter* have been observed in monkeys initially with the infection (Russell *et al.*, 1989). *Campylobacter* carriage has no relation with presence of other animals living together. This is similar to the findings of a study done in Argentina by Lopez *et al.* (2002). Stray animals sampled in an animal shelter had higher carriage rate of *Campylobacter* than stray animals sampled from an animal pound. The system of management may play a role in the spread of the organism because dogs are housed in separate kennels in animal pound while cats are kept together in an animal shelter. *Campylobacter* are shed in the faeces which in turn may contaminate and spread in the environment. Generally, the high prevalence observed in this study could be associated with the incidence in stray dogs and cats due to their exposure to environmental sources of *Campylobacter* infection. However, the carriage rate may be attributed to the population sampled and the methods used in their isolation (Sandberg *et al.*, 2002).

CONCLUSION

The presence of *Campylobacter* in dogs and cats is an indication that they can be commensals in these animals due to their high carriage rates. Humans have to ensure good hand hygiene upon handling infected animals to prevent from being infected with *Campylobacter.* It is also important to ensure proper waste disposal so as to reduce environmental contamination and avoid direct contact with faeces.

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