



Prevalence and risk factors of *Campylobacter* species infection of puppies in the Nairobi Metropolitan Region, Kenya

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ABSTRACT

Campylobacter species are bacterial pathogens of veterinary and public health significance. Despite the fact that several studies have identified close contact with puppies as a risk factor for human campylobacteriosis, the current status of *Campylobacter* species infecting puppies in Kenya is unclear. The purpose of this study was to determine the prevalence of *Campylobacter* species infection of puppies and the associated risk factors in the Nairobi Metropolitan Region, Kenya. A cross-sectional study was carried out from January 2021 to August 2021. Rectal swabs from 260 puppies were collected aseptically, and *Campylobacter* species were isolated and identified using conventional culture techniques and biochemical tests. To collect information on potential risk factors, a questionnaire survey was given to owners at the facilities where the rectal swab samples were taken. Variables were identified as risk factors for *Campylobacter* species colonization using univariable and multivariable mixed effects logistic regression analyses. *Campylobacter* species were isolated from 150 of the 260 sampled puppies yielding a prevalence of 57.7%. This study shows that *Campylobacter* species are present in Kenyan puppies. The reasons for keeping puppies, deworming status of puppies, and puppies with a recent history of vomiting had significant association with *Campylobacter* species colonization in puppies. Being a household puppy, being more than 2 months of age, being kept for security as well as having an ideal body condition or being moderately obese were identified as protective factors. Understanding the risk factors for *Campylobacter* species carriage in puppies will aid in the development of awareness and management strategies to improve puppy health and welfare while potentially lowering the risk of this pathogen being transmitted from puppies to humans.

Key words: Prevalence, Risk Factors, *Campylobacter*, Puppies

INTRODUCTION

Campylobacteriosis, which is caused by bacteria of the genus *Campylobacter*, is a significant zoonotic gastrointestinal disease affecting humans and animals, including dogs, globally (Kaakoush et al. 2015; EFSA and ECDC 2018; Elmali and Can 2019; Igwaran and Okoh 2019). Exposure to campylobacteriosis through the consumption of raw or undercooked poultry has been linked to a significant number of human cases (Sahin et al. 2015; Tresse et al. 2017; Carron et al. 2018), contaminated water (Hyllestad et al. 2020) or raw milk (Kaakoush et al. 2015). Close contact with pets has been identified as a significant source of human *Campylobacter*

species infections (Acke 2018; Mbindyo 2019) with puppies (less than one year old) serving as potential *Campylobacter* infection reservoirs, infants and young children are at an increased risk of infection (Rahimi et al. 2012).

Campylobacter species are frequently found and shed in dog feces (Goni et al. 2017), and these pathogens may eventually infect humans and other animals through environmental contamination (LeJeune and Hancock 2001). Most dogs are subclinically infected, but puppies under 6 months old or those from stressful environments may develop mild to moderate enteritis, characterized by watery, mucoid or bloody diarrhea, and tenesmus (Acke 2018).

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Campylobacter species prevalence varies widely in dogs (Kumar et al. 2012; Verma et al. 2014; Holmberg et al. 2015; Lazou et al. 2016; Torkan et al. 2018; Thepault et al. 2020), and this variation is dependent on age, geographic region, housing, diagnostic method, diarrheic versus non-diarrheic dogs, and the presence of infection or concomitant disease (Acke et al. 2006; Iannino et al. 2017; Acke 2018). Feeding homemade and commercial diets, compost exposure, and outdoor water access have all been linked to *Campylobacter* colonization in dogs (Leonard et al. 2011; Procter et al. 2014; Karama et al. 2019). The infection has also been linked to purebred dogs, concurrent enteric disease, and antibiotic treatment (Carbonero et al. 2012; Santaniello et al. 2021). Additionally, canine puppies are more susceptible to infection with *Campylobacter* species than adult dogs (Holmberg et al. 2015).

Despite reports of puppies serving as important reservoirs for *Campylobacter* pathogens, current data on *Campylobacter* species epidemiology in Kenyan puppies is limited. Therefore, the study aimed to determine the prevalence and associated risk factors of *Campylobacter* species in puppies in the Nairobi Metropolitan Region, Kenya.

MATERIALS AND METHODS

Ethical approval

The Biosecurity, Animal Use, and Ethics Committee (BAUEC) of the Faculty of Veterinary Medicine, University of Nairobi, Kenya, approved this research (FVM BAUEC/2019/237; 02-Sep-2019). Fecal collection from the puppies was done in accordance with the BAUEC guidelines, which require animal welfare and the use of biosecurity measures when handling biological materials.

Study area and design

From January 2021 to August 2021, a cross-sectional study was conducted in Kenya's Nairobi Metropolitan Region, which includes Nairobi County, where the capital city, Nairobi, is located, and the surrounding counties of Kajiado, Kiambu, Machakos, and Murang'a (Fig. 1). This region is Kenya's main economic center, as well as one of Africa's largest and fastest growing cities (Mundia 2017). The Nairobi Metropolitan Region is home to a large number of kennels with a wide range of dog breeds and management practices.

Study population

Study puppies were randomly selected from breeding kennels, shelters and those presented to the University of Nairobi Teaching and Referral Hospital for treatment, vaccinations, routine check-up, and boarding. The breeding kennels were conveniently chosen based on the breeders' willingness to participate in the study as well as the presence of puppies in the kennel. The sample size of 245 puppies was calculated using Dohoo et al. (2009)'s formula, with an expected *Campylobacter* species prevalence of 20%, a 5% margin of error, and a 95% confidence level.

A detailed questionnaire was administered to collect puppy-level factors (age, breed, sex, vaccination status and deworming status), and management factors (type of

feed, type of housing, kennel hygiene and environmental hygiene). The Waltham Size, Health, and Physical Examination (SHAPE) Score™ was used to provide a body condition score (BCS) to each puppy, with values ranging from A (underweight) to G (obesity) (German et al. 2006). The Canine Inflammatory Bowel Disease Activity Index (CIBDAI) clinical scoring approach described by Jergens et al. (2003) was used to assess puppy health for the presence of gastrointestinal disease. The severity of a gastrointestinal infection is measured by assigning numerical values to six cardinal signs of gastrointestinal infection. Clinical significance was assigned to the infection as a whole, with scores ranging from 0 to 3 for clinically insignificant to 4-5 for mild, 6-8 for moderate, and 9 or greater for severe.

Sample collection

A total of 260 rectal swabs were collected from puppies: 210 from breeding kennels, 6 from animal shelters, and 44 from the Veterinary Hospital. Sterile cotton tipped swabs were rotated inside the rectum of the puppy for 10 seconds and then placed in screw-capped test tubes containing 10mL of sterile buffered peptone water (BPW) (Himedia) and transported to the bacteriology laboratory in the Department of Public Health, Pharmacology and Toxicology, University of Nairobi within three hours of sample collection.

Isolation and phenotypic characterization of *Campylobacter* species

The rectal swabs were streaked directly onto modified-charcoal-cefoperazone-dexoycholate agar (mCCDA) (Oxoid, CM0935), which contained CCDA selective supplement (polymyxin B 2500IU, rifampicin 5mg, trimethoprim 5mg and cycloheximide 50mg) (Oxoid, SR0167E). The plates were then microaerophilically incubated at 42°C for 48 hours. The growth of *Campylobacter* colonies was described as greyish, flat, and moist, with a proclivity to spread. Four suspect colonies were subcultured onto mCCDA (Oxoid, CM0935) and incubated at 42°C for 48 hours for each mCCDA plate that showed growth. Each agar plate's presumed positive colonies were tested for oxidase and peroxidase breakdown.

Data analysis

The prevalence of *Campylobacter* species and other demographic parameters were calculated with descriptive statistics. The Chi-square test was utilized to compare the *Campylobacter* species carriage ratios of distinct categorical groups. Using univariable logistic regression, potential factors associated with *Campylobacter* species carriage in puppies were investigated. Statistically significant ($P < 0.05$) covariates were retained in the model using a backward step-wise elimination procedure. In the final mixed effects logistic regression analysis, all variables that showed an association with the outcome variable in the univariable logistic regression analysis ($P < 0.05$) were considered. Potential clustering of puppies within kennels was checked by including kennels as a random effect in the modelling. Model fit was assessed through checking for multi-collinearity, overall goodness of fit of the model, influential data points, and outliers.

RESULTS

Prevalence of *Campylobacter* species infection and analysis of factors associated with *Campylobacter* species infections among puppies in the Nairobi Metropolitan Region. The results from conventional culture and biochemical tests revealed 150 *Campylobacter* species isolates giving a prevalence of 57.7%. The distribution of various kennel management and puppy factors associated with *Campylobacter* species infections based on culture are described in Table 1.

Puppies from shelters (100%) had the highest *Campylobacter* species isolation rate compared to puppies from breeding kennels at 57.6% and from the veterinary hospital at 52.3%. At 61.4%, puppies fed homemade diets had a higher rate of *Campylobacter* species isolation than those fed commercial diets at 57.1%. The prevalence of *Campylobacter* species in female puppies was higher at 63.5% compared to the male puppies at 51.2%. The univariable logistic regression analysis identified 10 factors associated with positive *Campylobacter* species culture status ($P<0.05$) (Table 1). Two of the factors were associated with higher *Campylobacter* species carriage. They include: puppies that are housed together (OR: 2.4; $P=0.025$) and kennels that are washed daily (OR: 6.8; $P=0.0001$).

Eight factors were associated with lower *Campylobacter* species carriage: household puppies (OR: 0.03; $P=0.005$), puppies 2 to 5 months of age (OR: 0.2; $P=0.0001$), puppies more than five months of age (OR: 0.3; $P=0.044$), breed of puppies (OR: 0.3; $P=0.0001$), puppies kept for security (OR: 0.2; $P=0.045$), puppies with an ideal body condition or are moderately obese (OR: 0.5; $P=0.045$), puppies exposed to poultry (OR: 0.3; $P=0.024$), and puppies exposed to livestock (OR: 0.3; $P=0.043$).

Multivariable mixed effects logistic regression analysis of significantly associated explanatory variables for *Campylobacter* species carriage in puppies ($P<0.05$)

Multivariable logistic regression analysis revealed that the factors significantly associated with higher *Campylobacter* species positivity at $P<0.05$ were reasons for keeping puppies (OR: 14; $P=0.013$), deworming status of puppies (OR: 9.1; 95% CI: 3-27.5; $P=0.0001$), and puppies with a recent history of vomiting (OR: 4.7; 95% CI: 1.7-12.9; $P=0.003$). Protective factors identified were: household puppies (OR: 0.02; 95% CI: 0.0009-0.3; $P=0.005$), puppies more than 2 months of age (OR: 0.2; 95% CI: 0.06-0.4, $P=0.0001$), puppies kept for security (OR: 0.2; 95% CI: 0.04-0.7; $P=0.017$), and puppies with ideal body condition or are moderately obese (OR: 0.2; 95% CI: 0.1-0.5; $P=0.0001$).

Table 1: Descriptive statistics of variables and univariate logistic regression analysis of factors associated with *Campylobacter* species carriage in puppies ($P<0.05$)

Variables	Level	No. sampled	No. positive (%)	Odds ratio	P-value
Type of facility	Veterinary Hospital	44	23(52.3)	Ref	
	Breeding kennels	210	121(57.6)	1	0.736
	Shelters	6	6(100)	1	NA
Type of housing	Kenneled	249	149(59.8)	Ref	
	Household	11	1(9.1)	0.03	0.005*
Nature of housing	Individual	41	16(39)	Ref	
	Grouped	219	134(61.2)	2.4	0.025*
Daily washing of the kennels	Yes	229	140(61.1)	6.8	0.0001*
	No	31	10(32.3)	Ref	
Type of feed	Commercial	56	32(57.1)	Ref	
	Homemade	88	54(61.4)	0.9	0.91
	Others	116	64(55.2)	0.7	0.651
Sex of the puppy	Male	123	63(51.2)	Ref	
	Female	137	87(63.5)	1.6	0.168
Age of the puppy	<2 months	90	65(72.2)	Ref	
	2-5 months	108	52(48.1.1)	0.2	0.0001*
	>5months	62	33(53.2)	0.3	0.044*
Breed of the puppy	Local	52	35(67.3)	Ref	
	GSD	96	60(93.8)	0.6	0.225
	Others	112	55(49.1)	0.3	0.0001*
Reason for puppy	Commercial	171	106(62)	Ref	
	Pet	37	23(62.1)	0.6	0.276
	Breeding	17	8(47.1)	0.4	0.321
	Security	35	13(37.1)	0.2	0.045*
Deworming status	Not upto date	105	55(52.4)	Ref	
	Upto date	155	95(61.3)	1.4	0.635
Vaccination status	Not upto date	63	35(55.6)	Ref	
	Upto date	197	115(58.4)	1.3	0.458
Body condition (SHAPE)	Thin and lean	117	73(62.4)	Ref	
	Ideal and moderately obese	143	77(53.8)	0.5	0.045*
Recent diarrhea	Yes/No	36/224	19(52.8)/131(58.5)	1.25/Ref	0.677
Recent vomiting	Yes/No	14/246	10(71.4)/140(57)	2.1/Ref	0.077
Diagnosed with parvoviral enteritis	Yes/No	12/248	6(50)/144(58.1)	0.9/Ref	0.494
Concurrent bacterial infections	Yes/No	18/242	9(50)/141(58.3)	1.2/Ref	0.818
Recent treatment with antibiotics	Yes/No	42/218	24(57.1)/126(57.8)	1.6/Ref	0.561
Exposure to poultry	Yes/No	43/217	16(37.2)/134(61.8)	0.3/Ref	0.024*
Exposure to livestock	Yes/No	16/244	6(37.5)/144(59)	0.3/Ref	0.043*

Table 2: Descriptive statistics of the occurrence *Campylobacter* species infection based on the Canine Inflammatory Bowel Disease Activity Index (CIBDAI) clinical scoring system

Infection status (CIBDAI)	No. assessed	Culture positive (%)
Clinically insignificant	22	8(34.8)
Mild	3	1(33.3)
Moderate	18	14(77.8)
Severe	11	7(63.6)

Prevalence of *Campylobacter* species infection based on the Canine Inflammatory Bowel Disease Activity Index (CIBDAI) clinical scoring system

Fifty-four out of 260 puppies exhibited one or more of the six cardinal signs of gastrointestinal infection used to assess the degree of illness. Of the puppies whose infection status was classified as severe, 63.6% were positive for *Campylobacter* species while 36.4% of the puppies whose infection status was classified as clinically insignificant were positive for *Campylobacter* species (Table 2).

DISCUSSION

The study of zoonotic diseases such as campylobacteriosis is important as people are increasingly keeping dogs in their homes. Given that dogs, particularly puppies, can be reservoirs of pathogenic *Campylobacter*, more information on the epidemiology of this disease in these animals is required.

According to the current study, 57.7% of the puppies tested positive for *Campylobacter* species. This proportion was within the reported range of 8.58 to 75.7% in studies (Giacomelli et al. 2015; Leahy et al. 2017; Torkan et al. 2018; Ma Socorro Edden and Gil 2018; Thepault et al. 2020; Gharibi et al. 2020). The differences in *Campylobacter* species prevalence among puppies observed in these studies may be attributable to variations in study methodology, management systems, and hygiene practices.

When age was investigated as a risk factor for *Campylobacter* carriage in puppies, we found that puppies less than 2 months old were more likely to carry *Campylobacter* species (72.2%), similar to previous studies (Verma et al. 2014; Holmberg et al. 2015; Ahmed et al. 2018; Santaniello et al. 2021). Puppies under the age of two months are more likely to harbor *Campylobacter* species, which is most likely due to their naïve immune systems and an underdeveloped gut microbiota that is incapable of competitively excluding pathogens (Karama et al. 2019).

Higher positivity of *Campylobacter* species carriage was found in puppies deworming status was upto date (61.3%) compared with those whose deworming status was not upto date (52.4%). This could be explained by the fact that younger puppies are more likely to have their deworming status upto date due to their increased risk of suffering the negative effects from helminthosis and as mentioned above, have naïve immune systems hence increased risk of campylobacteriosis.

This study revealed that the reason for keeping puppies had a significantly higher risk for *Campylobacter* species. Puppies kept as pets are more likely fed with homemade cooked food which may be source of

Campylobacter species (Leonard et al. 2011). Poor food handling practices may also increase the chances of infection and fecal shedding of the microorganism, spreading it to other pets, domestic animals and humans, especially those who are vulnerable due to age or immunosuppression (Mbindyo et al. 2021; Santaniello et al. 2021).

Clinical manifestations of gastroenteritis include diarrhea and vomiting (Bhat et al. 2013). Consistent with previous research (Parsons et al. 2010; Giacomelli et al. 2015), this study found no significant association between diarrhea occurrence and *Campylobacter* positive status. This study found a statistically significant association between vomiting in puppies and the isolation of *Campylobacter* species. This result contradicts the findings of Verma et al. (2014), who found no correlation between *Campylobacter* species infection and the incidence of vomiting in dogs. Our findings, however, concur with those of Guest et al. (2007), who found a link between gastrointestinal signs and *Campylobacter* species infection in puppies.

Conclusion

The present study shows that *Campylobacter* species are present in Kenyan puppies. Significant risk factors that predispose puppies to *Campylobacter* species infections were identified as reason for keeping puppy, deworming status of puppies, and puppies with a recent history of vomiting. Understanding the risk factors for *Campylobacter* species carriage in puppies will help develop awareness and management strategies to improve puppy health and welfare and reduce the risk of pathogen transmission from puppies to humans.

Author's contributions

SNM, JMAK, TOA, GOA, and CMM designed the study, SNM collected data, SNM and BWM performed the experiments. SNM and DWM analyzed the data, all the authors were involved in the drafting of the manuscript and approved the final submitted manuscript.

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Uncorrected Proof