

## Isolation and PCR Characterisation of Thermophilic *Campylobacter* Species in Dogs Presented to Selected Veterinary Clinics in Jos, Nigeria

Solomon N. Karshima<sup>1</sup>, Ahmadu A. Bobbo<sup>2</sup>

<sup>1</sup>Department of Veterinary Public Health and Preventive Medicine, University of Jos, PMB 2084, Jos, Nigeria, <sup>2</sup>Department of Animal Health, College of Agriculture, Jalingo, PMB 1025, Jalingo, Nigeria

### ABSTRACT

#### Key words:

Isolation, characterisation, thermophilic *Campylobacter*, multiplex PCR, food-borne zoonosis

*Campylobacter* species are responsible for majority of zoonotic food-borne gastroenteritis worldwide, posing more serious threats among the young, the aged and the immuno-compromised. This study determined infection rates and characterised *Campylobacter* species isolated from dogs presented to selected Veterinary clinics in Jos, Nigeria using a multiplex PCR. The study analyzed 341 faecal sample from 146 (42.8%) male and 195 (57.2%) female dogs, of which 81 were positive revealing an overall infection rate of 23.8%. Breed based infection rates showed significant variation ( $p < 0.05$ ) and ranged between 10.5% and 39.8%. Infection rates in relation to dog types also varied significantly ( $p < 0.05$ ) and ranged between 12.4% and 42.4%. Infection rates based on condition of faeces varied significantly ( $p < 0.05$ ) and ranged between 12.3% and 48.1%. Adults and puppies recorded infection rates of 25.8% and 16.4% respectively while the 30.4% and 15.0% recorded by females and males respectively varied significantly ( $p < 0.05$ , OR=0.4027, 95% CI=1.437-4.290). Clinic based infection rates varied significantly ( $p < 0.05$ ) between 9.0% and 45.8%. PCR identified *Campylobacter jejuni* in 41 (50.6%), *C. coli* in 31 (38.3%) and mixed infections in 9 (11.1%) of the 81 positive samples revealing overall species based infection rates of 12.0%, 9.1% and 2.6% respectively. Majority of *C. coli* (9.9%), *C. jejuni* (18.5%) and mixed infections (6.2%) were isolated from dogs with normal, mucoid and diarrhoeic faeces respectively. *Campylobacter* was more prevalent among indigenous, hunting as well as dogs with mucoid faeces. *Campylobacter coli*, *C. jejuni* and mixed infections were more commonly associated with dogs that had normal, mucoid and diarrhoeic faeces respectively.

Corresponding Author: Solomon N. Karshima, [torkarshima@yahoo.co.uk](mailto:torkarshima@yahoo.co.uk)

### 1. INTRODUCTION

*Campylobacter* is a generic name for bacteria that cause a group of infectious diseases referred to as campylobacteriosis. These bacteria are among the major causes of bacterial gastroenteritis worldwide and are associated with majority of childhood morbidity in resource-limited countries (EFSA, 2013). Risk factors for acquiring infections include; the consumption of undercooked meat and meat products, unpasteurised milk and milk products, untreated water as well as unhygienic practices like contact with animals and their faeces (Danborg et al., 2004; Minihan et al., 2004). Infection can be initiated with as few as 500 organisms with clinical symptoms manifested between 2-4 days post infection (Black et al., 1988).

Though infections due to *Campylobacter* species are self-limiting (Samuel et al., 2004); mild to severe diseases manifested by watery or bloody diarrhoea and abdominal cramps were reported in humans (Skirrow and Blaser, 2000; Janssen et al., 2008) and

animals (Bell and Manning, 1990; Young et al., 2000). *Campylobacter*s are now major public health issues worldwide because of their resistance to antibiotics especially tetracycline and fluoroquinolones (Ternhag et al., 2007; Fabrega et al., 2008) as well as their ability to trigger Guillain-Barre Syndrome and reactive arthritis (Hughes and Cornblath, 2005).

*Campylobacter* infections have been reported in both healthy and diarrhoeic dogs (Workman et al., 2005). High prevalence rates of up to 58% and 76% were reported in cats and dogs in Switzerland respectively (Wieland et al., 2005). The risk of transmission between companion animals and their owners have been reported (Emborg and Heuer, 2003). Although the actual role of pets as sources of human *Campylobacter* infections is uncertain, Wolf et al. (2001) showed evidence of transmission of *Campylobacter jejuni* between a man and a dog living in the same household by the use of amplified fragment length polymorphism.

In Nigeria, *Campylobacter* infections have been reported in man (Adekunle et al., 2009; Okolo et al., 2013), various animals like birds (Salihu et al., 2012), small ruminants (Salihu et al., 2009a; Uaboi-Egbenni et al., 2011), cattle (Ngulukun et al., 2011) as well as animal products (Salihu et al., 2009b; Salihu et al., 2010) and water (Ugboma et al., 2013). However there is dearth of information on the prevalence of *Campylobacter* in dogs despite the documented evidence that companion dogs can serve as reservoirs and transmit the bacteria to their owners (Wolfs et al., 2001; Workman et al., 2005). This study was aimed to isolate and characterize thermophilic *Campylobacter* species from dogs presented to five selected veterinary clinics in Jos, Nigeria between January and December, 2014.

## 2. MATERIALS AND METHODS

### 2.1. Study Area

This work was carried out in Jos; the capital city of Plateau State which is located in North-central Nigeria between latitudes 10° 00' and 9° 50'N and longitudes 9° 00' and 8° 55'E. It has annual temperatures ranging between 16 and 27 °C and an annual average rain fall of 1314.8 mm. Jos is located at about 1290 m above sea level and the mean relative humidity varies between 14% and 74%.

### 2.2. Study Design

The study was a randomised cross sectional study. All Veterinary Clinics in Jos were identified and five were randomly selected for the study where permissions were sought for the collection of samples. Systematic sampling was employed for the sampling of dogs. All even numbered fresh cases of dogs presented on Mondays, Wednesdays and Fridays to the selected veterinary clinics between January and December, 2014 were included in the study while all cases presented on Tuesdays, Thursdays, Saturdays and Sundays as well as all odd numbered, follow up cases of dogs and other animal species presented to the selected veterinary clinics during this period were excluded in the study.

### 2.3. Sample Collection

A total of 341 dog faecal samples were aseptically collected directly from the rectum using sterile swab sticks. These swabs were immediately returned into their holders and transported in cooled ice box to the Centre for Biotechnology, Ahmadu Bello University, Zaria, Nigeria for analysis. Full descriptions of breed, dog type, condition of faeces, age, sex and study clinics were recorded.

### 2.4. Isolation of *Campylobacter* on CCDA

The swabs were streaked onto Charcoal Cefoperazone Desoxycholate Agar (CCDA) plates and then incubated at 42°C for two days under micro-aerophilic conditions as previously described by Hendriken *et al.* (2003). Culture plates with suspected *Campylobacter* were then sub-cultured onto fresh CCDA plates to get pure culture free of contaminants.

### 2.5. Genomic DNA Extraction

DNA extraction was done with Chelex 100 resin (CAT No.142-1253; Bio-Rad Laboratories, CA, USA) using the protocol earlier described by Gomley *et al.* (2008) and the DNA obtained was stored at -80 °C until needed for PCR.

### 2.6. PCR Amplification of *Campylobacter* DNA

PCR was carried out using the protocol and primer set  
Cam 220F- 5'  
GGTGTAGGATGAGACTATATA 3' and Cam  
659R- 5' TTCCATCTGCCTCTCCCY 3' (Moreno et al., 2001) in a 25.0 µl reaction volume containing 0.75 µl of 25 mM MgCl<sub>2</sub> (Fermentas, Canada), 2.5 µl of 5x Green GoTaq reaction buffer (Promega, USA), 0.25 µl of 10 mM dNTP mix (Applied Biosystems, UK), 0.25 µl of GoTaq DNA polymerase (Promega, USA), 0.5 µl of both forward and reverse primers, and 3.0 µl of the extracted *Campylobacter* DNA to amplify a 439 bp 16S rRNA fragment from thermotolerant campylobacters.

Amplification was done using a Gene Amp PCR system 9700 (Applied Biosystems, UK). The amplification conditions were as follows; pre-denaturation at 95 °C for 5 minutes, 30 cycles of denaturation at 95°C for 1 minute, annealing at 58 °C for 1 minute, extension at 72 °C for 2 minutes and a final extension at 72 °C for 2 minutes. Amplified products were analyzed by electrophoresis in a 1.5% agarose gel at 140 volts for 50 minutes and UV illumination after ethidium bromide staining.

### 2.7. Data Analysis

Data obtained during the study were analyzed using the Statistical Package for Social Sciences (SPSS Version 20.0) and Graph-Pad Prism 4.0. Prevalence rates were calculated by multiplying the ratio between number of infected dogs and the number of total dog analysed. This was employed for different variables such as breed, dog type, condition of faeces, age, sex and study clinics. The Chi Square ( $\chi^2$ ) test and Odds ratio were employed where appropriate at 95% confidence interval to determine statistical association between the prevalence rates of

the different variables measured and values of  $p < 0.05$  were considered significant.

### 3. RESULTS

The study analysed 341 faecal samples of which 146 (42.8%) were male and 195 (57.2%) were female dogs for the presence of thermophilic *Campylobacter* species. Of this, 81 samples were positive revealing an overall prevalence of 23.8%. Prevalence rates in relation to dog breeds were 10.5% (6/57), 12.9% (4/31), 18.2% (12/66), 29.2% (7/24), 39.8% (47/118), 11.5% (3/26) and 10.5% (2/19) for Alsatians, Bull mastiffs, Caucasians, Rottweilers, Indigenous dogs, Neapolitan mastiffs and Terriers respectively and varied significantly ( $p < 0.05$ ) as shown in Table 1.

Prevalence rates in relation to dog types varied significantly ( $p < 0.05$ ) and were 12.4% (14/113), 20.6% (28/136) and 42.4% (39/92) for companion, guard and hunting dogs respectively (Table 2). Based on conditions of faeces, prevalence rates were 12.3% (9/73), 29.6% (29/98), 48.1% (26/54) and 14.7% (17/116) for dogs with bloody, diarrhoeic, mucoid and normal faeces respectively and this

showed significant association ( $p < 0.05$ ) as in Table 3.

Adult dogs and puppies revealed prevalence rates of 25.8% (69/268) and 16.4% (12/73) respectively while the 30.4% (59/194) and 15.0% (22/147) observed for females and males respectively showed significant variation ( $p < 0.05$ , OR=0.4027, 95% CI=1.437-4.290) as shown in Table 4. There was also significant association ( $p < 0.05$ ) between the prevalence rates of 15.4% (12/78), 45.8% (33/72), 30.9% (21/68), 9.0% (6/67) and 16.1% (9/56) recorded by clinics I, II, III, IV and V respectively (Table 5).

PCR identified *Campylobacter jejuni* in 41 (50.6%), *C. coli* in 31 (38.3%) and mixed infections in 9 (11.1%) of the 81 positive samples revealing overall species based infection rates of 12.0% (41/341), 9.1% (31/341) and 2.6% (9/341) respectively (Fig 1). Seven *C. coli*, 2 *C. jejuni* and 1 mixed infection were associated with bloody faeces, 5 *C. coli*, 12 *C. jejuni*, and 5 mixed infections were associated with diarrhoea, 11 *C. coli*, 15 *C. jejuni* and 3 mixed infections were associated with mucoid faeces as well as 8 *C. coli* and 12 *C. jejuni* were associated with normal faeces as shown in Figure 2.

**Table 1:** Breed based prevalence of *Campylobacter* in dogs presented to veterinary clinics in Jos.

Breed	Number examined	Males (%)	Females (%)	Number positive	Prevalence (%)
Alsatian	57	45 (78.9)	12 (21.1)	6	10.5
Bull mastiff	31	23 (74.2)	8 (25.8)	4	12.9
Caucasian	66	49 (74.2)	17 (25.8)	12	18.2
Rottweiler	24	17 (70.8)	7 (29.2)	7	29.2
Indigenous	118	22 (18.6)	96 (81.4)	47	39.8
Neapolitan mastiff	26	24 (92.3)	2 (7.7)	3	11.5
Terrier	19	15 (78.9)	4 (21.1)	2	10.5
<b>Total</b>	<b>341</b>	<b>195 (57.2)</b>	<b>146 (42.8)</b>	<b>81</b>	<b>23.8</b>
$\chi^2$	-	-	-	29.86	-
P-value	-	-	-	<0.0001	-

**Table 2:** Prevalence of *Campylobacter* species in dogs presented to veterinary clinics in Jos in relation to dog type.

Dog type	Number examined	Males (%)	Females (%)	Number positive	Prevalence (%)
Companion	113	51 (45.1)	62 (54.9)	14	12.4
Guard	136	63 (46.3)	73 (53.7)	28	20.6
Hunting	92	33 (35.9)	59 (64.1)	39	42.4
<b>Total</b>	<b>341</b>	<b>147 (43.1)</b>	<b>194 (56.9)</b>	<b>81</b>	<b>23.8</b>
$\chi^2$	-	-	-	26.46	-
p-value	-	-	-	<0.0001	-

**Table 3:** Prevalence of *Campylobacter* species in dogs presented to veterinary clinics in Jos in relation to condition of faeces.

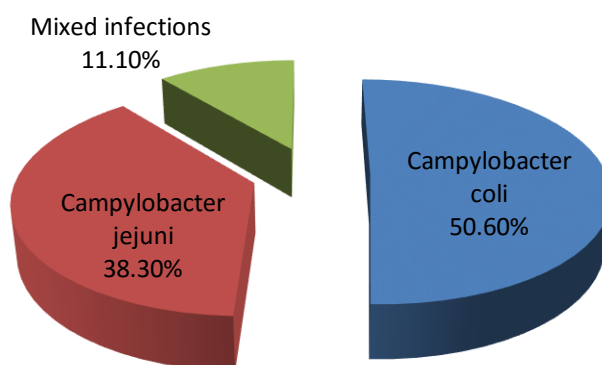
Condition of faeces	Number examined	Males	Females	Number positive	Prevalence (%)
Normal	116	68 (58.6)	48 (41.4)	17	14.7
Diarrhoeic	98	41 (41.8)	57 (58.2)	29	29.6
Mucoid	54	23 (42.6)	31 (57.4)	26	48.1
Bloody	73	15 (20.6)	58 (79.4)	9	12.3
<b>Total</b>	<b>341</b>	<b>147 (43.1)</b>	<b>194 (56.9)</b>	<b>81</b>	<b>23.8</b>
$\chi^2$	-	-	-	30.15	-
P-value	-	-	-	<0.0001	-

**Table 4:** Age and sex based prevalence of *Campylobacter* species in dogs presented to veterinary clinics in Jos.

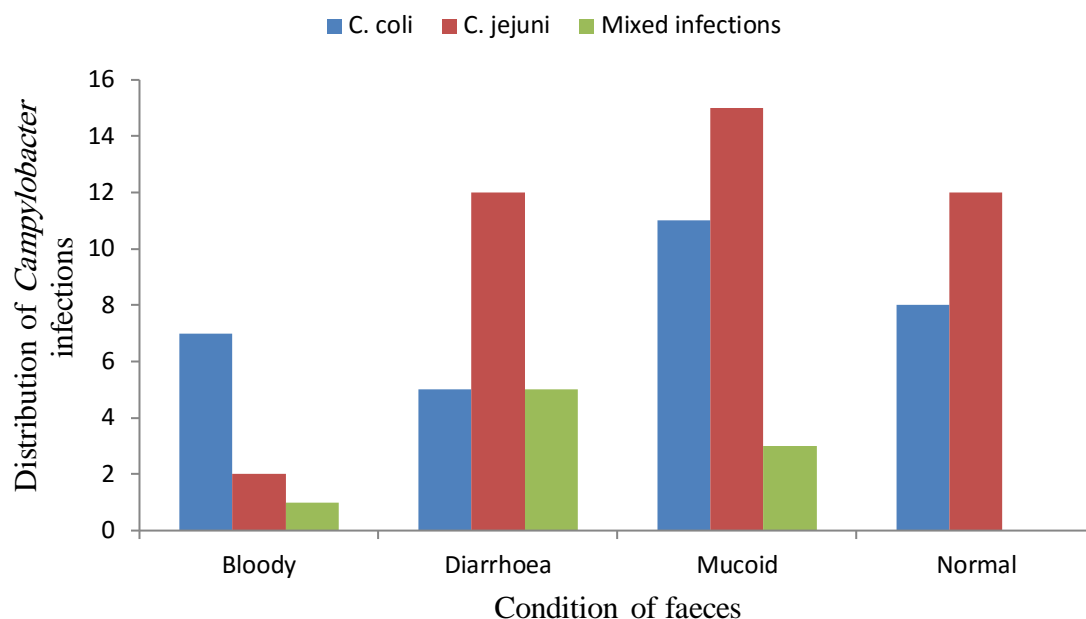
AGE	Number examined	Number positive	Prevalence (%)	P- value ( $\chi^2$ )	Odds ratio (95% CI)
Adults	268	69	25.8	0.0976	1.7630
Puppies	73	12	16.4	(2.7440)	(0.8956-3.469)
<b>Total</b>	<b>341</b>	<b>81</b>	<b>23.8</b>	-	-
<b>SEX</b>					
Females	194	59	30.4	0.0009	0.4027
Males	147	22	15.0	(11.02)	(1.437-4.290)
<b>Total</b>	<b>341</b>	<b>81</b>	<b>23.8</b>	-	-

**Table 5:** Prevalence of *Campylobacter* species in dogs in relation to veterinary clinics

Clinics	Number examined	Males (%)	Females (%)	Number positive	Prevalence (%)
I	78	34 (43.6)	44 (56.4)	12	(15.4)
II	72	41 (56.9)	31 (43.1)	33	(45.8)
III	68	23 (33.8)	45 (66.2)	21	(30.9)
IV	67	36 (53.7)	31 (46.3)	6	(9.0)
V	56	13 (23.2)	43 (76.8)	9	(16.1)
<b>Total</b>	<b>341</b>	<b>147 (43.1)</b>	<b>194 (56.9)</b>	<b>81</b>	<b>(23.8)</b>
$\chi^2$	-	-	-	34.23	-
P-value	-	-	-	<0.0001	-



**Fig 1:** Distribution of *Campylobacter* species among the 81 dogs positive for campylobacteriosis.



**Fig 2:** Distribution of *Campylobacter* species in relation to condition of faeces

#### 4. DISCUSSION

This study revealed the occurrence of *Campylobacter* infections in dogs presented to five selected veterinary clinics in Jos, Nigeria. These infections may be associated with poor hygienic practices by these dog owners, consumption of unclean water, consumption of uncooked chicken (Workman et al., 2005) and human stool as these are possible sources of *Campylobacter*. The two species; *Campylobacter coli* and *Campylobacter jejuni* identified by this study are of zoonotic importance and were earlier reported by Gras et al. (2013).

The overall prevalence rate revealed by this study is within the range of 23-41% documented by Acke et al. (2006) for household dogs and those attending Veterinary facilities and lower than the 37.0% reported by Holmberg et al. (2015) in Sweden, however, it was higher than the 11.0% recorded by Danborg et al. (2004) in Denmark. These variations may be associated with factors such as stress, poor sanitation, and overcrowding, new arrival in kennels, pregnancy and concomitant infections with other enteric pathogens.

The higher prevalence rate recorded by the indigenous dogs may not be unconnected with the poor management practices observed by their owners as most of these dogs scavenge for food on waste dumping sites thereby acquiring infections. Their higher representation in the sample size may be another possible explanation. The lower infection rate recorded by the exotic breeds on the other hand may be due to the adequate attention paid to these

breeds which are usually kept for commercial purposes and companionship.

Prevalence rates of *Campylobacter* species in relation to dog types showed significant variation with higher infection rate among hunting dogs. Continuous exposure to wildlife reservoirs during hunting may be a possible explanation for the higher prevalence rate among hunting dogs. Guard dogs are commonly left to scavenge exposing them to the risk of acquiring infections from dumping sites. Companion dogs might have acquired infections through relating with their human owners or consumption of contaminated food, water and raw meat especially chicken.

Female dogs recorded significantly higher prevalence than the males probably due to the stress associated with hormonal imbalances during pregnancy and lactation which usually increases female susceptibility to infections. Though there was no any significant variation between the infection rates in adult dogs and puppies, the higher infection rate observed in the adults may not be unconnected with the increased scavenging activities associated with the adults. Holmberg et al. (2015) reported higher prevalence in younger dogs in Denmark in contrast with the present finding.

This study also detected higher prevalence of *Campylobacter* among dogs with muroid and diarrhoeic faeces which was not unexpected since it has been reported by several workers elsewhere (Burnens et al., 1992; Engvall et al., 2003; Acke et al., 2006). These bacteria cause gastroenteritis thereby interfering with the absorption of fluid

resulting in diarrhoea. *Campylobacter* infections are also said to occur concomitantly with parvovirus (Dillon et al., 1987), this may explain the isolation of the organism in dogs with bloody faeces.

Clinic based prevalence rates showed very high level of statistical association. These variations may not be unconnected with differences in management practices. All the clinics sampled were also assessed for levels of sanitation which varied significantly according to clinics. The higher infection rates observed in clinics II and III was not unexpected because there levels of sanitation were very poor. Nosocomial infections usually associated with unhygienic practices in veterinary clinics may be another possible explanation for the variations in the infection rates in different clinics.

PCR identified *Campylobacter jejuni* in 41, *Campylobacter coli* in 31 and mix infections in 9 of the 81 positive samples giving species-based infection rates of 12.0%, 9.1% and 2.6% respectively. This is in line with earlier reports by Danborg *et al.* (2004) and Holmberg *et al.* (2015) who also reported higher prevalence of *Campylobacter jejuni* in dogs suggesting that *Campylobacter jejuni* may be the commonest *Campylobacter* species infective to dogs. The majority of mixed infections of *C. coli* and *C. jejuni* were observed among dogs with severe cases of diarrhoea suggesting possibility of synergistic effects by these bacteria. The role of *C. jejuni* in the formation of mucoid faeces needs further investigations as majority of our isolates in dogs with mucoid faeces were *C. jejuni*.

The epidemiological significance of this finding is the risk of spreading these zoonotic pathogens among other dogs and dog owners especially children, old people and the immuno-compromised. This finding is also of serious public health importance especially considering the fact that *Campylobacter jejuni* is incriminated in triggering Guillain-Barre syndrome and immune-mediated myelitis in humans. Although this study did not conduct drug sensitivity testing on the isolates, the risk of these dogs transmitting multi-drug resistant *Campylobacter* species to their owners is also of great public health concern (Ternhag et al., 2007; Fabrega et al., 2008).

In conclusion, *Campylobacter* infections were more prevalent among indigenous, hunting as well as dogs with mucoid faeces. *Campylobacter coli*, *Campylobacter jejuni* and mixed infections were more commonly associated with dogs that had normal, mucoid and diarrhoeic faeces respectively. There is the risk of humans acquiring zoonotic *Campylobacter* infections from their dogs especially

the companion dogs. Dog owners, Veterinarians as well as Veterinary assistants must observe strict hand hygiene after handling dogs to protect themselves from *Campylobacter* infections of canine origin.

## 5. CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this paper.

## 6. ACKNOWLEDGEMENTS

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