

Isolation, Molecular Detection, and Risk Factors of *Campylobacter* Infection From Companion Dogs



Darioush Gharibi^{1*}, Bahman Mosallanejad², Reza Avizeh², Mahboobeh Feyzabadi³

¹Department of Pathobiology, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

²Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

³Graduated of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

*Corresponding Author:

Darioush Gharibi,
Department of Pathobiology,
Faculty of Veterinary Medicine,
Shahid Chamran University,
Ahvaz, Golestan Bulevar, Postal
code: 6135714333, Iran.
Tel: +989132831841,
Email: d.gharibi@scu.ac.ir

Published Online December 30,
2020

Keywords: *Campylobacter*, Dog,
Culture, PCR, Ahvaz, Iran



Abstract

Background: *Campylobacter* is an organism that is usually associated with diarrhea in pet animals and humans, as well as other domestic, wild, and laboratory animals.

Objective: The aim of the present survey was the isolation, molecular detection, and risk factors of *Campylobacter* infection from companion dogs referred to the Veterinary Hospital of Ahvaz district, the South-West of Iran.

Materials and Methods: Rectal swabs were examined by culture and polymerase chain reaction (PCR) methods from 122 companion dogs (52 diarrheic and 70 clinically healthy). Several risk factors were reviewed, including age, gender, breed, nutrition status, and lifestyle.

Results: The results showed that only five samples (4.1%) were positive for *Campylobacter* spp. in the culture method. *Campylobacter* spp. was detected in 18 out of 122 dogs by the PCR, yielding an overall prevalence of 14.8%. The most prevalent species of *Campylobacter* among the referred dogs were *C. coli* (38.89%) and *C. jejuni* (33.33%). A lower prevalence was found for *C. upsaliensis* (11.11%) and *C. lari* (5.55%). Concurrent infections were observed in two cases of *C. upsaliensis* + *C. lari* (5.55%) and *C. coli* + *C. lari* (5.55%). No significant difference was noted between healthy (11.43%) and diarrheic (19.23%) dogs ($P > 0.05$). Eventually, age, gender, breed, nutrition status, and lifestyle had no significant effect on *Campylobacter* infection ($P > 0.05$).

Conclusion: Although the prevalence of *Campylobacter* was moderate in the dog population of Ahvaz district, these bacteria can constitute a public health hazard because of the frequent presence of *Campylobacter* species in the feces.

Received September 18, 2020; Revised December 10, 2020; Accepted December 20, 2020

Background

Campylobacter spp. are thin, curved, Gram-negative rods that are found in singular, pairs, or chains with three to five spirals. The cells may be S- or gull-shaped when joined together. *Campylobacter* species are microaerophilic and have a single, none sheathed polar flagellum.

There are more than 30 different species and subspecies of *Campylobacter* in humans and animals.¹ *Campylobacter jejuni* is the organism which is typically associated with diarrhea in dogs, cats, and humans, as well as other domestic, wild, and laboratory animals. *C. coli* and other intestinal *Campylobacters*, *C. upsaliensis*, *C. helveticus*, and *C. lari* have been isolated from asymptomatic and diarrheic dogs and cats.^{2,3}

Dogs may be more sensitive to clinical diseases when stressed by hospitalization, simultaneous disease, pregnancy, traveling, or surgery.^{2,4} The majority of dogs show subclinical infection but some of them will develop

mild to moderate enteritis. Acute *Campylobacteriosis*, which extends in puppies and some adult dogs, is present by mucus-laden, watery, bloody or bile-streaked diarrhea, anorexia, dehydration, abdominal pain, and occasional vomiting. In many cases, dogs are asymptomatic carriers of *Campylobacter* species and play a significant role in the epidemiology of *Campylobacteriosis* and *Campylobacter* spp. in animals and humans. Particularly, many dogs live as free in urban and rural areas and have access to other animals that increases the risk of public health.²

The prevalence of different species of *Campylobacter* spp. in animals varies and depends on the age, animal species, housing (e.g., kennel or shelter), and the presence of associated disease or infection with other enteropathogenic bacteria, the sampling season, geographic region, and the study design.⁵⁻⁷ For example, the prevalence of *C. jejuni* is significantly greater in dogs younger than six months old and those living in high-

density regions for long periods, and in the autumn months. *C. jejuni* has been isolated from 29% and 21% of diarrheic dogs and cats, respectively, compared with 4% of clinically healthy dogs and cats. In other studies, the isolation rate varies from zero to 50%.^{2,7} The results of the conducted surveys in Iran showed a relatively high prevalence of *Campylobacteriosis* in dogs and cats. For example, from a total of 100 dogs and cats, 39 cases were infected with *Campylobacter* in a research study in Tehran.⁸

Campylobacter spp. is one of the most prevalent bacteria causing gastroenteritis in humans. Poultry and pet animals are the most important reservoirs for human infection. Risk factors for infection include nutrition with undercooked or contaminated meat products (especially poultry), consuming contaminated or unpasteurized milk and dairy products, drinking water from contaminated supplies, foreign travel, and contact with contaminated pets.^{5,9,10} The people who are in close contact, living or working with pet animals (especially young children), and the immunocompromised patient must be aware of the risks of *Campylobacteriosis*. It is recommended that these people comply with sanitary measures when dealing with puppies or kittens and the pets with gastroenteritis signs and obtaining a new pet from a centralized housing environment.^{11,12} Moreover, people using raw meat-based diets for pet animals must consider the potential risk of infection which is associated with this manner.^{7,13}

There are many different methods for the diagnosis of *Campylobacteriosis*, including direct microscopic examinations, culture, serologic test (e.g., enzyme-linked immunosorbent assay), and molecular identification (e.g., polymerase chain reaction, PCR). Direct microscopic examination (dark-field or phase-contrast microscopy) alone is insufficient and thus further examination is needed to confirm *Campylobacteriosis*.⁶ *Campylobacter* spp. are fastidious and slow growth, hence, their isolation heavily relies on the applied procedure in this regard.⁶ Additionally, the phylogenetic alliance of *Campylobacter* with *Arcobacter* and *Helicobacter* spp., as well as the recognition of several *Campylobacter* spp. has limited the discriminatory power of culture and phenotypic methods, and thus they are not considered suitable for true *Campylobacter* spp. Diagnosis.¹⁴ On the other hand, molecular assays (PCR) are becoming increasingly available for diagnosing *Campylobacter* infections in dogs and cats since they are easy, rapid, and sensitive and allow the speciation of isolates from culture or infected tissues. Molecular methods permit rapid identification and prevail problems with culture, growth situation, reduced viability of bacteria, and bacterial contamination. Molecular techniques are regarded as the gold standard for *Campylobacter* genus and species identification and are valuable in epidemiological research, especially in *Campylobacter* strain-typing.^{10,15} Specific primers can be used for 23SrRNA as internal controls for recognizing

closely related genera *Campylobacter*, *Arcobacter*, and *Helicobacter*. These organisms can also be rapidly identified on clinical isolates using the PCR- restriction fragment length polymorphism analysis of the 16 SrRNA gene.¹⁶

Unfortunately, based on our knowledge, no survey has so far investigated *Campylobacteriosis* in the dogs of Ahvaz district. Furthermore, epidemiological research with the molecular diagnostic method is needed to determine the range and role of *Campylobacter* spp. in pet animals, and their zoonotic significance, especially in this area. Therefore, the present study aimed to focus on the isolation and molecular characterization of *Campylobacter* spp. in companion dogs in the Ahvaz region, South-West of Iran.

Materials and Methods

Sample Population

A cross-sectional survey was conducted in the Ahvaz region, South-West of Iran from November 2016 to September 2017. One hundred twenty-two dogs (52 diarrheic and 70 clinically healthy dogs) were sampled, and only those dogs not recently been treated with antibiotics or glucocorticoids were included in the study. The companion dogs were referred to the Veterinary Teaching Hospital of Shahid Chamran University of Ahvaz for vaccination, health care, and different diseases including diarrhea as requested by their owners. All parameters were recorded including age, gender, breed, clinical signs, nutrition status, lifestyle (open or close environment), and vaccination history. The studied dogs were divided into two groups based on their age (less and higher than one year old). In some cases, sedative drugs such as ketamine with a dosage of 15 mg/kg and acepromazine 0.15 mg/kg were injected intramuscularly. Fresh fecal specimens from the dogs were examined by culture and PCR methods. The swab samples were collected from the rectum of the studied dog population. Two simultaneous swabs were taken of every dog. A swab was used for culture and transported in an Amies transport medium and was sent on ice to the laboratory of veterinary microbiology as soon as possible (less than 1 hour). The other sample was kept at -20°C for DNA extraction from the stool.

Culture Method

Supplemented Preston enrichment broth (Himedia, India) including 5% (v/v) defibrinated lysed horse blood, sodium pyruvate (0.125 g/500 mL), ferrous sulfate (0.125 g/500 mL), Polymyxin B (250 000 units/500 mL), rifampicin (0.005 g/500 mL), trimethoprim (0.005 g/500 mL), and amphotericin B (0.005 g/500 mL) was used for the enrichment of the specimens for 24 hours at 37°C under microaerophilic conditions (5% O₂, 10% CO₂, and 85% N₂).¹⁷ Then, a loop full of enriched broth was streaked onto Preston *Campylobacter* selective agar (Himedia;

India) including 7% (v/v) defibrinated lysed horse blood, sodium pyruvate (0.125 g/500 mL), ferrous sulfate (0.125 g/500 mL), polymyxin B (250 000 units/500 mL), rifampicin (0.005 g/500 mL), trimethoprim (0.005 g/500 mL), and amphotericin B (0.005 g/500 mL). Next, they were incubated for 48-72 hours under microaerophilic conditions.¹⁷ Suspect colonies were purified by streaking onto blood agar and presumptively identified by Gram- and dilute carbol fuchsin staining (DCF), biochemical tests (catalase and oxidase), and phase-contrast microscopy according to standard procedures.³ Compatible colonies were confirmed by the PCR.

DNA Extraction and PCR

DNA was extracted from stool samples by using a DNA extraction kit (AccuPrep® Stool DNA Extraction Kit, Bioneer) according to the manual of the kit. In the present survey, specific primers were used for the detection of *Campylobacter* genus and the species of *C. jejuni*, *C. coli*, *C. lari*, and *C. upsaliensis* in the obtained fecal samples and the isolates in the culture by the multiple PCR technique. The sequences of primers are presented in Table 1.

The multiplex PCR was performed in a 50 µL reaction mixture containing master mix (25 µL, including Tris-HCl pH: 8.5, (NH₄)₂SO₄, 2 mM Mg Cl₂, 0.2% Tween 20, 0.4 mM dNTPs, 0.2 units/µL Ampliqon Taq DNA polymerase, and inert red dye; Ampliqon, Denmark), each primer (1 µL = 0.4 mM; Bioneer, South Korea), distilled water (13 µL), and template DNA (2 µL). A reaction with DNA of *C. jejuni* and *C. coli* and a reaction without the template DNA were used as positive and negative controls, respectively. The PCR program was performed in a thermal cycler (Eppendorf, Germany) as initial denaturation at 95°C for 15 minutes, 35 cycles of 30 seconds at 95°C, 90 seconds at 58°C, and 60 seconds at 72°C, followed by a final extension at 72°C for 7 minutes.¹⁸ Amplification products were separated on 1.5% agarose gel containing safe stains (Cinnagen, Iran).

Statistical Analysis

The studied dogs were grouped by age, gender, breed, nutrition status, clinical signs (diarrheic and non-diarrheic), and lifestyle (open or close environment) to

determine whether these factors were associated with *Campylobacter* infection by Chi-square test, Fisher’s exact test, and Z test. Statistical analyses were performed using SPSS (Version 16.0; SPSS Inc., Chicago, USA). Logistic regression was applied to calculate odds ratios, and differences were considered statistically significant when P≤0.05.

Results

In the present study, 122 dogs were tested for the presence of *Campylobacter* spp. using culture and PCR techniques. The breed distribution of the studied dogs was mixed (30.3%), Terrier (25.4%), Doberman Pinscher and German Shepherd (9.8%), Spitz (5.7%), Siberian Husky and Shitzu (4.9%), Rottweiler (4.1%), and other breeds (4.9%), respectively (Table 2). The age of the studied dogs was between one month and five years (median 1.9 years). Overall, fewer samples were positive by culture compared to the direct PCR. Among the 122 samples, only 5 samples (4.1%) were positive in the culture. All the suspected *Campylobacter* isolates were confirmed as *Campylobacter* by the PCR and were confirmed as *C. coli* by specific primers for the species. *Campylobacter* spp. was detected in 18 of the 122 dogs in the PCR, yielding an overall prevalence of 14.8%. The most prevalent species of *Campylobacter* among the dogs were *C. coli* (38.89%) and *C. jejuni* (33.33%). A lower prevalence was observed for *C. upsaliensis* (11.11%) and *C. lari* (5.55%). Concurrent infections were observed in two cases, namely, *C. upsaliensis* + *C. lari* (5.55%) and *C. coli* + *C. lari* (5.55%). Figure 1 displays representative bands of the *Campylobacter* genus and species by the multiplex PCR method.

The prevalence of *Campylobacter* in the studied dogs by PCR was 14.8% (95% CI: 21.3-8.%, 18 out of 122 cases).

There was no significant relationship between age and infection (P>0.05). The average age and standard deviation of infected and non-infected dogs with *Campylobacter* were 9.06±6.49 and 10.55±7.78, respectively, which were not statistically significant (P>0.050). The relative frequency of positive cases was 12.30% and 2.5% in dogs under and above one year old, respectively (Table 2). The odd of infection in 1-year-old dogs and younger was 1.75

Table 1. Nucleotide Sequence of Primer, Target Gene, and Product Size for Detecting *Campylobacter* Genus and Species

Gene	Genus/Species	Sequence	Size	Reference
16SrRNA	<i>Campylobacter</i>	F:5'- GGATGACACTTTTCGGAGC -3' R: 5'- CATTGTAGCACGTGTGTC -3'	816	
Aspartokinase	<i>C. coli</i>	F:5'- GGTATGATTTCTACAAAGCGAG -3' R: 5'- ATAAAAGACTATCGTCGCGTG -3'	502	
<i>C. jejuni</i>	oxidoreductase	F:5'- CAAATAAAGTTAGAGGTAGAATGT-3' R: 5'- CCATAAGCACTAGCTAGCTGAT -3'	161	18
<i>C. lari</i>	glyA	F:5'- TAGAGAGATAGCAAAGAGA -3' R: 5'- TACACATAATAATCCCACCC -3'	251	
lpxA	<i>C. upsaliensis</i>	F:5'- CGATGATGTGCAAATTGAAGC -3' R: 5'- TTCTAGCCCCCTTGCTTGATG -3'	86	

Table 2. Distribution of Absolute and Relative Frequency of *Campylobacter* Infection in the Population of Dogs Referred to the Veterinary Hospital of Ahvaz Based on Age, Gender, Breed, Health Conditions, Living-, and Nutrition status

Parameter		Negative	Positive	Total
		Absolute (Relative %)	Absolute (Relative %)	Absolute (Relative %)
Age	≤ 1 year	77 (63.1)	15 (12.3)	92 (75.4)
	> 1 year	27 (22.1)	3 (2.5)	30 (24.6)
	Total	104 (85.2)	18 (14.8)	122 (100)
Gender	Female	53 (85.5)	9 (14.5)	62 (50.8)
	Male	51 (85)	9 (15)	60 (49.2)
	Total	104 (85.2)	18 (14.8)	122 (100)
Breed	Mixed	30 (24.59)	7 (5.75)	37 (30.3)
	Terrier	25 (20.5)	6 (4.92)	31 (25.4)
	Doberman Pinscher	11 (9)	1 (0.82)	12 (9.8)
	German Shepherd	12 (9.84)	0 (0)	12 (9.8)
	Spitz	7 (5.74)	0 (0)	7 (5.7)
	Siberian Husky	5 (4.1)	1(0.82)	6 (4.9)
	Shitzu	6 (4.92)	0 (0)	6 (4.9)
	Ruth Weiler	2 (1.64)	3 (2.49)	5 (4.1)
	unknown	6 (4.92)	0 (0)	6 (4.9)
	Total	104 (85.2)	18 (14.8)	122 (100)
Health conditions	Diarrheic	42 (34.4)	10 (8.2)	52 (42.6)
	Healthy	62 (50.8)	8 (6.6)	70 (57.4)
	Total	104 (85.2)	18 (14.8)	122 (100)
Living status	Indoor	45 (36.9)	8 (6.6)	53 (43.4)
	Outdoor	59 (48.3)	10 (8.2)	69 (56.6)
	Total	104 (85.2)	18 (14.8)	122 (100)
Nutrition status	Cooked food	70 (57.3)	12 (9.9)	82 (67.2)
	Raw food	34 (27.9)	6 (4.9)	40 (32.8)
	Total	104 (85.2)	18 (14.8)	122 (100)

times greater compared to dogs older than 1 year (95% CI: 0.15-2.12). Furthermore, 1.1% of fluctuation in infection was justified by age.

The relative frequency of positive cases in male and female dogs was approximately equal (Table 2). The infection was not associated with gender ($P > 0.05$). Based on the univariate logistic regression, the odds of infection in males was 1.039 compared to that in females (95% CI: 0.38-2.82). Further, 0.008% of fluctuation in infection was justified by gender.

The prevalence of infection was higher in mixed breeds (5.75%) in comparison with other breeds (Table 2), nevertheless, the difference was not significant ($P > 0.05$). In addition, 6.6% of fluctuation in infection was justified by the breed.

The prevalence of infection in diarrheal and healthy dogs was 8.2% and 6.6%, respectively (Table 2), and no significant difference was noted between the dogs in this regard ($P > 0.05$). The univariate logistic regression demonstrated that the odds of infection in diarrheic dogs was 1.85 compared to none-diarrheic (95% CI: 0.67-5.06). Furthermore, 2.1% of fluctuations in infection were

justified by gastrointestinal status (diarrheic or none-diarrheic).

The relative frequency of positive cases in dogs living in open environments was higher than that of those living indoors, which was not statistically significant ($P > 0.05$, Table 2). The odds of infection in dogs living outdoors was 1.04 times greater than that of those living indoors (95% CI: 0.38-2.87) and it justified 0.01% of fluctuation in infection.

The relative frequency of positive cases in dogs fed with raw food was higher compared to those fed with cooked foods, which was not statistically significant ($P > 0.05$, Table 2). The odds of infection in dogs fed with raw food was 1.029 times greater in comparison with those fed with cooked food (95% CI: 0.356-2.978). Moreover, 0.008% of fluctuation in infection was justified by nutrition status.

Discussion

Campylobacteriosis is an important zoonotic disease, and the companion and stray dogs are important in the epidemiology of the disease. The infected dogs can be concerned with disease transmission to other

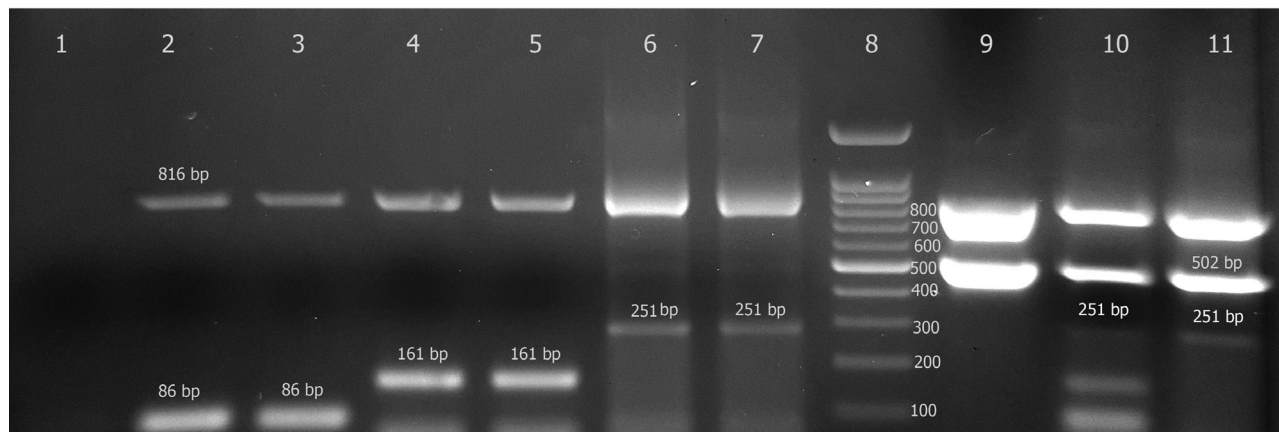


Figure 1. PCR Detection of the *Campylobacter* Genus and Species. Note: PCR: Polymerase chain reaction. The well No. 1 negative control. The wells No. 2 and 3: Positive sample and positive control of *C. upsaliensis*, respectively. The wells No. 4 and 5: Positive sample and positive control of *C. jejuni*, respectively. The wells No. 6 and 7: Positive sample and positive control of *C. lari*, respectively. The well No. 8: 100 bp ladder. The well No. 9: Positive sample of *C. coli*. The well No. 10: multiplex PCR on four positive controls of *Campylobacter upsaliensis*, *jejuni*, *lari*, and *coli*. The well No. 11: The sample had concurrent infection with two species of *C. lari* and *C. coli*.

animals while there is a risk of infection transmission to humans. The study on infectious diseases such as *Campylobacteriosis* is compulsory due to the increasing tendency of people to keep dogs in the home.² Given that the dogs can be actually as one of the main reservoirs of pathogenic *Campylobacter* spp., it is necessary to increase information about the epidemiology of the disease in these animals. The present study revealed that 14.8% of companion dogs were positive for *Campylobacter* infection by the PCR in the Ahvaz region, South-West of Iran. Rectal swab samples were examined by culture and PCR methods. Molecular techniques are considered to be the gold standard for the trustworthy identification of the species of *Campylobacter* and are valuable in epidemiological studies in humans and animals² although there have been a few studies based on molecular methods for *Campylobacter* species in pets in Iran. The direct PCR was found to be more sensitive compared to the culture method for the detection of *Campylobacter* species. The findings are consistent with those of other studies.¹⁹⁻²¹

The prevalence of infection in diarrheal and healthy dogs was 8.2% and 6.6%, respectively. The other studies reported *Campylobacter* infection rates of 4.81-51.1%.^{5,22-27} The variation in the rate of infection may rely on the sample size, age, diet, and gastrointestinal status (diarrheal or healthy) of the studied dogs, different diagnosis methods, and geographical location, as well as the season of sampling. The most prevalent species of *Campylobacter* among the dogs were *C. coli* (38.89%) and *C. jejuni* (33.33%). A lower prevalence was observed for *C. upsaliensis* (11.11%) and *C. lari* (5.55%). Dogs and cats are the main reservoirs of *C. jejuni* and *C. upsaliensis*.²⁸ Engvall et al, Hald et al, and Wieland et al reported that the majority of bacterial isolates from dogs were *C. upsaliensis*.^{22,29,30} Workman et al detected a predominant prevalence for *C. jejuni* in dogs.³¹ The importance of these *Campylobacter* species, especially *C. jejuni* in humans

is transmission to humans through sick or carrier dogs. Domestic dogs are usually in close contact with family, especially children. The infection of 11.42% of healthy dogs with *Campylobacter* showed that healthy dogs (even without diarrhea) could be a threat to humans as a carrier of *Campylobacter* spp. Concurrent infections were observed in two cases (*C. upsaliensis* + *C. lari* and *C. coli* + *C. lari*). Similar mixed infections of *Campylobacter* species were reported of dogs in previous studies.^{22,30}

In this study, the relative frequency of positive cases was more in dogs under one-year-old. Although there was no significant relationship between age and infection, several surveys have emphasized that dogs less than one year old were most likely to be infected with *Campylobacter*.^{2,16,22,24-26,32} It may be because of the weakness of the puppies and the low level of immunity in young dogs compared to adults.^{22,30}

Although *Campylobacter* was more prevalent in male compared to female dogs, the statistical analysis showed no significant difference, implying that *Campylobacter* exposure has no gender predilection in dogs, which is consistent with the results of a previous study.³³ In the case of the breed, although the prevalence of infection was higher in mixed breeds in comparison with other breeds, the difference was not significant, which corroborates with the findings of another study.³⁴

The prevalence of *Campylobacter* infection in dogs living indoor or outdoor is a risk factor for infection. In addition, its incidence in dogs living in kennels is higher compared to single-household pets due to the high density of dogs and increased communication between the dogs.^{5,22,31} Nevertheless, in the present study, lifestyle was not significant between the two groups, which is consistent with the results of Andrzejewska et al.²⁶

The previous research revealed that the probability of *Campylobacter* infection in dogs fed with raw meat is more than those fed with cooked food. The dogs can

be infected with raw meat from poultry, wild birds, pigs, and other animals.^{2,26} However nutrition status was not statistically significant in the studied dogs.

Campylobacter species can be found in dogs and cats with gastrointestinal symptoms as an opportunistic infection and may act as a primary or secondary pathogen. Several studies reported that the infection rate to *Campylobacter* was higher in diarrheic dogs^{24,25,27,35} while the true relation between gastrointestinal status in animals and the presence of *Campylobacter* remains uncertain.^{23,36} However, no significant difference was noted among healthy and diarrheic dogs in this study, which matches the findings of Harrus et al² and Sandberg et al.³⁷ Systemic signs of fever, lethargy, vomiting, and weight loss were not observed in the infected dogs.

Campylobacter coli and *C. jejuni* were commonly isolated from the feces of companion dogs in the Ahvaz district. Veterinarians should encourage good hand hygiene for the owners of pets because carriage may be prolonged. In conclusion, the PCR is a sensitive and specific procedure for the detection of *Campylobacter*. This study highlights the necessity of using rapid and effective diagnostic techniques for screening healthy and diarrheic dogs.

Conclusion

Our results showed that *Campylobacter* is a specific infection and appears to be endemic in dogs in this area. In addition, it is a zoonotic pathogen, thus the prompt treatment of the infected dogs is suggested to prevent human disease. Testing programs for the diagnosis and the prohibition of contact are the most effective preventative ways between sick and healthy animals. Considering that the vaccine is unavailable for *Campylobacter* infection, the only ways for the prevention of the disease are observing the principle of hygiene and avoiding contact with feces, especially stray dogs. Finally, further studies will be necessary for various areas to survey the overall epidemiological status of campylobacteriosis in dog populations.

Authors' Contributions

Experiment design, experiment conduct, data interpretation and manuscript writing - review & editing by DG and BM; Visualization, Investigation and Methodology by RA; literature review, Investigation, laboratory performance and practice by MF.

Ethical Approval

We hereby declare that all ethical standards have been respected in the experiment and preparation of the article.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

Acknowledgments

The authors would like to acknowledge the Research Vice-chancellor of the Shahid Chamran University of Ahvaz for financial support.

References

1. Parte AC. LPSN--list of prokaryotic names with standing in nomenclature. *Nucleic Acids Res.* 2014;42(Database issue):D613-616. doi:10.1093/nar/gkt1111
2. Harrus S, Waner T, Neer T, Greene C. *Infectious Diseases of the Dog and Cat* (No. Ed. 3). WB Saunders\Elsevier Science; 2012.
3. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. *Clinical Veterinary Microbiology E-Book*. Elsevier Health Sciences; 2013.
4. Szczepanska B, Andrzejewska M, Spica D, Klawe JJ. Prevalence and antimicrobial resistance of *Campylobacter jejuni* and *Campylobacter coli* isolated from children and environmental sources in urban and suburban areas. *BMC Microbiol.* 2017;17(1):80. doi:10.1186/s12866-017-0991-9
5. Acke E, McGill K, Golden O, Jones BR, Fanning S, Whyte P. Prevalence of thermophilic *Campylobacter* species in household cats and dogs in Ireland. *Vet Rec.* 2009;164(2):44-47. doi:10.1136/vr.164.2.44
6. Marks SL, Rankin SC, Byrne BA, Weese JS. Enteropathogenic bacteria in dogs and cats: diagnosis, epidemiology, treatment, and control. *J Vet Intern Med.* 2011;25(6):1195-1208. doi:10.1111/j.1939-1676.2011.00821.x
7. Bojanić K, Midwinter AC, Marshall JC, Rogers LE, Biggs PJ, Acke E. Isolation of *Campylobacter* spp. from client-owned dogs and cats, and retail raw meat pet food in the Manawatu, New Zealand. *Zoonoses Public Health.* 2017;64(6):438-449. doi:10.1111/zph.12323
8. Mahzounieh M, Ghorbani M, Zahraei Salehi T. Identification of *Campylobacter* spp. in apparently healthy dog's and cat's stool by multiplex PCR. *Journal of Comparative Pathobiology Iran.* 2014;10(4):11-1-1106.
9. Altekruze SF, Tollefson LK. Human campylobacteriosis: a challenge for the veterinary profession. *J Am Vet Med Assoc.* 2003;223(4):445-452. doi:10.2460/javma.2003.223.445
10. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, Man SM. Global epidemiology of *Campylobacter* infection. *Clin Microbiol Rev.* 2015;28(3):687-720. doi:10.1128/cmr.00006-15
11. Peña A, Abarca K, Weitzel T, et al. One Health in practice: a pilot project for integrated care of zoonotic infections in immunocompromised children and their pets in Chile. *Zoonoses Public Health.* 2016;63(5):403-409. doi:10.1111/zph.12241
12. Campagnolo ER, Philipp LM, Long JM, Hanshaw NL. Pet-associated campylobacteriosis: a persisting public health concern. *Zoonoses Public Health.* 2018;65(3):304-311. doi:10.1111/zph.12389
13. Fredriksson-Ahomaa M, Heikkilä T, Pernu N, Kovanen S, Hielm-Björkman A, Kivistö R. Raw meat-based diets in dogs and cats. *Vet Sci.* 2017;4(3):33. doi:10.3390/vetsci4030033
14. Engvall EO, Brändström B, Gunnarsson A, Mörner T, Wahlström H, Fermér C. Validation of a polymerase chain reaction/restriction enzyme analysis method for

- species identification of thermophilic campylobacters isolated from domestic and wild animals. *J Appl Microbiol.* 2002;92(1):47-54. doi:10.1046/j.1365-2672.2002.01491.x
15. On SL. Isolation, identification and subtyping of *Campylobacter*: where to from here? *J Microbiol Methods.* 2013;95(1):3-7. doi:10.1016/j.mimet.2013.06.011
 16. Holmberg M, Rosendal T, Engvall EO, Ohlson A, Lindberg A. Prevalence of thermophilic *Campylobacter* species in Swedish dogs and characterization of *C. jejuni* isolates. *Acta Vet Scand.* 2015;57(1):19. doi:10.1186/s13028-015-0108-0
 17. Bolton FJ, Robertson L. A selective medium for isolating *Campylobacter jejuni/coli*. *J Clin Pathol.* 1982;35(4):462-467. doi:10.1136/jcp.35.4.462
 18. Yamazaki-Matsune W, Taguchi M, Seto K, et al. Development of a multiplex PCR assay for identification of *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter hyointestinalis* subsp. *hyointestinalis*, *Campylobacter jejuni*, *Campylobacter lari* and *Campylobacter upsaliensis*. *J Med Microbiol.* 2007;56(Pt 11):1467-1473. doi:10.1099/jmm.0.47363-0
 19. Houg HS, Sethabutr O, Nirdnoy W, Katz DE, Pang LW. Development of a *ceuE*-based multiplex polymerase chain reaction (PCR) assay for direct detection and differentiation of *Campylobacter jejuni* and *Campylobacter coli* in Thailand. *Diagn Microbiol Infect Dis.* 2001;40(1-2):11-19. doi:10.1016/s0732-8893(01)00251-6
 20. Maher M, Finnegan C, Collins E, Ward B, Carroll C, Cormican M. Evaluation of culture methods and a DNA probe-based PCR assay for detection of *Campylobacter* species in clinical specimens of feces. *J Clin Microbiol.* 2003;41(7):2980-2986. doi:10.1128/jcm.41.7.2980-2986.2003
 21. Bullman S, O'Leary J, Corcoran D, Sleator RD, Lucey B. Molecular-based detection of non-culturable and emerging campylobacteria in patients presenting with gastroenteritis. *Epidemiol Infect.* 2012;140(4):684-688. doi:10.1017/s0950268811000859
 22. Wieland B, Regula G, Danuser J, et al. *Campylobacter* spp. in dogs and cats in Switzerland: risk factor analysis and molecular characterization with AFLP. *J Vet Med B Infect Dis Vet Public Health.* 2005;52(4):183-189. doi:10.1111/j.1439-0450.2005.00843.x
 23. Acke E, Whyte P, Jones BR, McGill K, Collins JD, Fanning S. Prevalence of thermophilic *Campylobacter* species in cats and dogs in two animal shelters in Ireland. *Vet Rec.* 2006;158(2):51-54. doi:10.1136/vr.158.2.51
 24. Moyaert H, Ceelen L, Dewulf J, Haesebrouck F, Pasmans F. PCR detection of *Campylobacter* species in feces from dogs. *Vlaams Diergeneeskd Tijdschr.* 2008;77(2):92-96.
 25. Selwet M, Cłapa T, Galbas M, Słomski R, Porzucek F. The prevalence of *Campylobacter* spp. and occurrence of virulence genes isolated from dogs. *Pol J Microbiol.* 2015;64(1):73-76.
 26. Andrzejewska M, Szczepańska B, Klawe JJ, Spica D, Chudzińska M. Prevalence of *Campylobacter jejuni* and *Campylobacter coli* species in cats and dogs from Bydgoszcz (Poland) region. *Pol J Vet Sci.* 2013;16(1):115-120. doi:10.2478/pjvs-2013-0016
 27. Rodrigues CG, Melo RT, Fonseca BB, et al. Occurrence and characterization of *Campylobacter* spp. isolates in dogs, cats and children. *Pesqui Vet Bras.* 2015;35(4):365-370. doi:10.1590/s0100-736x2015000400009
 28. Vandenberg O, Dediste A, Vlaes L, et al. Prevalence and Clinical features of non *jejuni/coli* *Campylobacter* species and related organisms in stool specimens. 2001. [Abstract P-22]. In: Hacker J, editor. Abstracts of scientific presentations of the 11th International Workshop on *Campylobacter*, *Helicobacter* and Related Organisms, Freiburg, Germany, Sept 1-5, 2001. *Int J Med Microbiol*;291(Suppl 31):144.
 29. Engvall EO, Brändström B, Andersson L, Båverud V, Trowald-Wigh G, Englund L. Isolation and identification of thermophilic *Campylobacter* species in faecal samples from Swedish dogs. *Scand J Infect Dis.* 2003;35(10):713-718. doi:10.1080/00365540310014558
 30. Hald B, Pedersen K, Wainø M, Jørgensen JC, Madsen M. Longitudinal study of the excretion patterns of thermophilic *Campylobacter* spp. in young pet dogs in Denmark. *J Clin Microbiol.* 2004;42(5):2003-2012. doi:10.1128/jcm.42.5.2003-2012.2004
 31. Workman SN, Mathison GE, Lavoie MC. Pet dogs and chicken meat as reservoirs of *Campylobacter* spp. in Barbados. *J Clin Microbiol.* 2005;43(6):2642-2650. doi:10.1128/jcm.43.6.2642-2650.2005
 32. Kumar R, Verma AK, Kumar A, Srivastava M, Lal HP. Prevalence of *Campylobacter* sp. in dogs attending veterinary practices at Mathura, India and risk indicators associated with shedding. *Asian J Anim Vet Adv.* 2012;7(8):754-60. doi:10.3923/ajava.2012.754.760
 33. Salihu MD, Magaji AA, Abdulkadir JU, Kolawale A. Survey of thermophilic *Campylobacter* species in cats and dogs in north-western Nigeria. *Vet Ital.* 2010;46(4):425-430.
 34. Lazou T, Fragkou F, Gelasakis A, et al. Prevalence, antimicrobial resistance and risk factors for *Campylobacter* colonising dogs and cats in Greece. *Bulg J Vet Med.* 2017;20(3):244-254. doi:10.15547/bjvm.1003
 35. Chaban B, Ngeleka M, Hill JE. Detection and quantification of 14 *Campylobacter* species in pet dogs reveals an increase in species richness in feces of diarrheic animals. *BMC Microbiol.* 2010;10:73. doi:10.1186/1471-2180-10-73
 36. López CM, Giacoboni G, Agostini A, Cornero FJ, Tellechea DM, Trinidad JJ. Thermotolerant campylobacters in domestic animals in a defined population in Buenos Aires, Argentina. *Prev Vet Med.* 2002;55(3):193-200. doi:10.1016/s0167-5877(02)00093-4
 37. Sandberg M, Bergsjø B, Hofshagen M, Skjerve E, Kruse H. Risk factors for *Campylobacter* infection in Norwegian cats and dogs. *Prev Vet Med.* 2002;55(4):241-253. doi:10.1016/s0167-5877(02)00095-8