Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
www.entomoljournal.com
JEZS 2020; 8(2): 1357-1360

Received: 14-01-2020
Accepted: 16-02-2020

Sumedha Bobade
Department of Animal Biotechnology, Madras Veterinary College, Tanuvas Chennai, Tamil Nadu, India

K Vijayarani
Department of Animal Biotechnology, Madras Veterinary College, Tanuvas Chennai, Tamil Nadu, India

KG Tirumurugaan
Department of Animal Biotechnology, Madras Veterinary College, Tanuvas Chennai, Tamil Nadu, India

A Thangavelu
Department of Animal Biotechnology, Madras Veterinary College, Tanuvas Chennai, Tamil Nadu, India

S Vairamuthu
Department of Animal Biotechnology, Madras Veterinary College, Tanuvas Chennai, Tamil Nadu, India

Corresponding Author:
Sumedha Bobade
Department of Animal Biotechnology, Madras Veterinary College, Tanuvas Chennai, Tamil Nadu, India

Phenotypic and genotypic characterization of *Campylobacter jejuni* from fecal sample of dog suffer from diarrhea

Sumedha Bobade, K Vijayarani, KG Tirumurugaan, A Thangavelu and S Vairamuthu

Abstract
Campylobacter has emerged as an important zoonotic food borne pathogen of human and animals worldwide. *Campylobacter* spp. is frequently isolated from animal, poultry and environmental samples. *Campylobacter* is one of the most common bacterial enteropathogens of food borne origin in industrialized countries with *C. jejuni* being the most common species followed by *C. coli*. The aim of this study was to investigate the incidence of *Campylobacter jejuni* from dog fecal sample suffer from diarrhea. A total of 19 diarrheal fecal swabs from dog were collected. The samples were screened by cultural examination and studied for biochemical and molecular characterization for confirmation. Total 14 (73.68%) isolates showed typical morphological characteristics on the basis of cultural examination. The isolates were further subjected to phenotypic characterization using biochemical test and genotypic characterization using Polymerase Chain Reaction. The result showed that 8 (42.10%) isolates were found to be positive for *C.jejuni*. Suggested that close contact with dogs can be recognised as risk factor for human campylobacteriosis from this zoonotic organism.

Keywords: mCCDA, *Hip O*, *MAP*, hippurate, *C.jejuni*

Introduction
Campylobacter jejuni is a leading cause of bacterial diarrheal disease worldwide and a frequent commensal organism of the gastrointestinal tract of poultry and many wild animals (birds such as ducks and gulls), agriculturally-important farm animals (Cattle and Pigs) and companion animals (such as dogs and cats) and it is responsible for zoonoses [1]. Campylobacter considered to be the most common bacterial cause of human gastroenteritis in the world also is 1 of 4 key global causes of diarrheal diseases [2]. Dogs, especially puppies, are a known source of sporadic *Campylobacter* infections in humans, but are uncommonly reported to cause outbreaks.

The disease is predominantly food borne but many sources of transmission of zoonotic infection of campylobacteriosis have been described including close contact with pets and dog owners are at high risk of Campylobacter infection [3, 4]. Also, Campylobacter can spread to person by direct contact with animals such as pets [5, 6]. Campylobacter species majorly colonized in the intestine of cats and dogs [7] and shed in the faeces of these animals into the environment [8].

Dogs are significant reservoirs of Campylobacter and contribute to human enteric infections [9]. In humans, clinical signs of Campylobacteriosis include diarrhea, abdominal pain, fever, headache, nausea and vomiting. Most of *Campylobacter* are sporadic and self-limiting, but there are post-infection complications, for example, Guillain-Barres syndrome [10]. Thermo tolerant *Campylobacter* which has a clinical significance, *C. jejuni* and its closely connected *C. coli* represents more than 90% of human infections [11]. *Campylobacter* is one of the most common pathogen-related causes of diarrheal illnesses globally and has been recognized as a significant factor of human disease for more than three decades [12].

Campylobacter is difficult to isolate, grow and identify. Hence this study was attempted to detect the presence of *C. jejuni* using cultural, biochemical and PCR technique and compare these techniques for detection of *C.jejuni* from dog fecal samples.

Materials and methods

Collection of samples

A total of 19 fecal swabs of dogs suffer from diarrhea were collected from Department of Clinics Madras Veterinary College. All the samples were collected using sterile cotton swabs (HiMedia, India), transported immediately to the laboratory under cold conditions for microbiological analysis.

Processing of samples

The isolation was performed according to Man (2011) [13] and the isolates were identified by biochemical tests as described previously [14, 15]. The reference strain *Campylobacter jejuni* (ATCC 33291) was used as standard culture.

Phenotypic characterization

Cultural examination

Samples were enriched in modified Charcoal Cefoperazone Deoxycholate (mCCDA) broth with CCDA supplement (FD 135) under microaerophillic conditions (candle jar method) by using internal gas generation system using (Microaerophilic gas pack CampyPack-BD oxoid) and streaked on mCCDA agar.

Biochemical test

The isolates were identified as *C. jejuni* based on their morphological and biochemical tests. The isolates were processed for phenotypic characterization and identified by biochemical tests, viz. oxidase, catalase, indoxyl acetate hydrolysis tests and H_2S production in triple sugar iron test.

Molecular confirmation of *Campylobacter jejuni*

The biochemically identified isolates were further employed for molecular confirmation as *C. jejuni* by polymerase chain reaction amplifying specific target gene using species-specific oligonucleotide primers. DNA were extracted by Phenol-Chloroform extraction method and the DNA concentration was quantified by nanodrop and stored at -20°C until further processing.

Genotypic confirmation of isolates by polymerase chain reaction for *Hip O* gene and *MAP A* gene

The isolates were subjected to PCR targeting hip O and MAP A genes. Polymerase chain reaction was carried out using primers for species specific genes. The PCR was performed in a thermal cycler (Applied Biosystem). The *hipO* gene region is the hippuricase gene, specific for *C. jejuni*. Primers for specific identification were designed using the *hipO* gene sequences of *C.jejuni* based on the sequences available in the GenBank.

The isolates were confirmed by PCR using designed primers in the study as forward primer (5-TTCCATGACCACCTCTTCC-3) and reverse primer (5-CTACTTCTTTATTGCTTGCTGC -3).

The primers used for amplification of *MAP A* gene were forward primer(5-CTATTTTATTTTTGAGTGCTTGTG-3) and reverse primers (5-GCTTTATTTGCCATTTGTTTTATTA-3) [16].

The PCR reactions were performed in 25 µl reaction mixture, containing 12.5 µl PCR master mix (2X-Ampliqon), 1µl of each primer of a 10 µM primer concentration, 1µl $MgCl_2$ (25mM), 3 µl template DNA and 6.5 µl nuclease-free water making atotal volume of 25 µl. The amplification conditions consisted of initial denaturation at 94 °C for 3 min, 35 cycles with denaturation at 94 °C for 1 min, annealing for *Hip* O gene at 53°C for 1 min, and extension at 72 °C for 1 min, followed by a final extension at 72 °C for 5 min respectively [17].The annealing temperature for *Map* A gene was optimized as 52 °C for 1 min [16]. The DNA from *C. jejuni* (ATCC 33291) was included as positive control for PCR identification of the isolates and without sample DNA used as negative control. The amplified products were observed and photographed using gel documentation System.

Results and discussion

Campylobacter spp. is a major cause of gastroenteritis, there is an urgent need to control these pathogens with zoonotic and public health point of view. In the present study a total of 19 samples were studied for presence of *Campylobacter* from fecal samples of dogs. The Campylobacter species are difficult to isolate but the results from inoculation studies showed that plates with either blood or charcoal had a better recovery rate than other media used for isolation. Modified blood free Charcoal cefoperazone deoxycholate agar is commonly used worldwide [18]. On selective agar, Blood free modified charcoal cefoperazone deoxycholate (mCCDA), the isolates showed typical grey/white or creamy grey in colour and moist spreading type colonies with sticky nature were confirmed phenotypically as Campylobacter. The suspected colonies were examined for morphological Gram's staining. In current study a total of 19 samples were processed for isolation and overall incidence of *Campylobacter* was found to be 14 (73.68%) by cultural examination. *Campylobacter jejuni* was the most commonly identified species in dogs (51.5%), the high incidence of Campylobacter in dogs and the predominance of *C. jejuni*, including strains that were identical to human isolates, suggest that dogs are a more important source of *C. jejuni* enteritis than chickens [9].

Biochemical characterization

The test for hippurate hydrolysis is critical for separation of *Campylobacter jejuni* and *C. coli* strains. All 8(42.10%) isolates were positive for catalase, oxidase, nitrate and hippurate hydrolysis, Ninhydrin test. The samples from Dog fecal swab (2) were positive for H_2S production (Table 1) while other samples were negative for H_2S production *C. jejuni* biotype 2 strains are H_2S positive, whereas *C. jejuni* biotype 1 strains are H_2S negative [19]. In this study two isolates were positive for H_2S production belong to biotyope 2 while other belong to biotype 1 of *C. jejuni*.

Table 1: Result of biochemical test of *C. jejuni* isolated from different sources

S. No.	Samples/source	Samples Examined	Isolates showed growth on mCCDA agar	Biochemical test						
				Catalse	Oxidase	Nitrate	Glysin	Ninhydrin	H_2S production	
									Positive	Negative
3	Fecal swab (DF1-DF19)	19	19	8	8	8	8	8	2	6
Total		19	19	8	8	8	8	8	2	6

Genotypic characterization

The isolates were confirmed by polymerase chain with species specific primers for *Hip* O and *MAP* A gene. The size of amplified PCR product for *Hip* O gene was 270 bp and the size of the PCR product for MAP A gene was 589 bp.

The prevalence of *Campylobacter* from younger and adult dogs with or without clinical symptoms were found to be 56.58% and 33.33% respectively [20] while in this study the diarrheic samples from young dogs was found to be 8 (42.10%). A total of 46 thermophilic *Campylobacter* were isolated comprising 33 *C. jejuni* (81.25%) [21]. Presence of *Campylobacter* was found to be 67 (64.42%) out of which six isolates belong to *C. jejuni* species, 5 (18.51%) were from chicken and 1(4.17%) from dog was recorded [22] in current study incidence was found to be 8(42.10%) from diarrheal fecal swab of dogs on basis of *MapA* gene. The reported prevalence of Campylobacter spp. and *C. jejuni* was 13% and 5%, respectively from environmental dog faeces collected at Palmerston North dog-walking areas [23].Campylobacter spp. were isolated from client-owned dogs and cats with an overall Campylobacter spp. prevalence of 36% and 16%, respectively, the most common species identified being *C. jejuni* [3].

A total of 19 *Campylobacter* isolates, two *C. jejuni and* one *C. coli* were recovered from dogs and cats faecal samples. The prevalence rates of *Campylobacter* spp. were 16.0% (8 out of 50) in dogs and 22.0% (11 out of 50) in cats [24]. The overall prevalence of 62% (31 of 50) of Campylobacter spp. was confirmed in the dogs based on genus-specific PCR following bacterial isolation and 9 (18%) were positive for *C. jejuni* [25] while in this study 8 (42.10%) isolates from diarrheal fecal samples from dog were found to be positive (Figure 1).

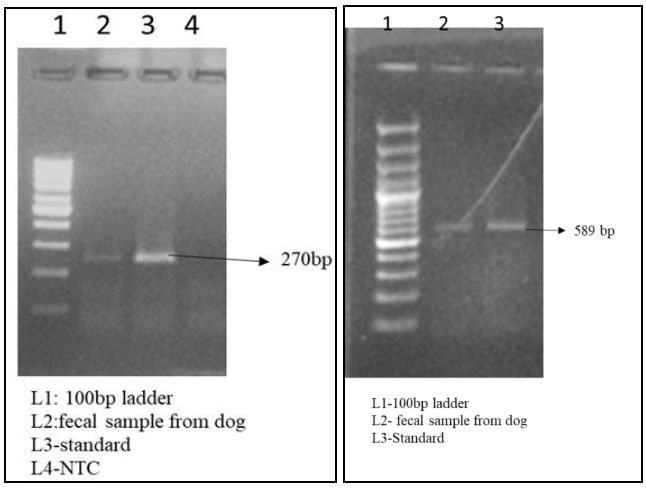

Fig 1: Agarose gel showing the amplified product of *Hip O* and *MAP A* gene from dog fecal sample of *Campylobacter jejuni* (270 bp and 589 bp)

Conclusion

Campylobacter spp. are well-recognized human pathogens, and the species most commonly causing diarrheal disease in humans include *C. jejuni*. Campylobacter spp. are potentially zoonotic from dogs to humans, individuals exposed to young dogs are most likely to become infected from contact with dogs shedding Campylobacter. However, other sources of Campylobacter, the most common means by fecal contamination for acquisition of this pathogen recognised as risk factor for human Campylobacteriosis causes diarrhoea.

References

1. Facciola A, Riso R, Avventuroso E, Visalli G, Delia SA, Lagana P. Campylobacter: from microbiology to prevention. Journal of Preventive Medicine and Hygiene. 2017; 58:79-92.
2. WHO. Campylobacter, 2018, 1-5 https://www.who.int/news-room/fact-sheets/detail/.
3. Bojanic 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; 64:438-449.
4. Gras LM, Smid JH, Wagenaar JA, Koene MGJ, Havelaar AH, Friesema IHM *et al*. Increased risk for *Campylobacter jejuni* and *C. coli* infection of pet origin in dog owners and evidence for genetic association between strains causing infection in humans and their pets. Epidemiology and Infection. 2013; 141(12):2526-2535.
5. ESR. The Institute of Environmental Science and Research Ltd. Notifiable diseases New Zealand: annual report 2015. Porirua, New Zealand. ISSN, 2016, 1179-3058.
6. Westermarck E. Chronic diarrhea in dogs: what do we actually know about it? Topics in Companion Animal Medicine. 2016; 31(2):78-84.
7. Dearlove BL, Cody AJ, Pascoe B, Meric G, Wilson DJ, Sheppard SK. Rapid host switching in generalist Campylobacter strains erodes the signal for tracing human infections. The ISME Journal. 2016; 10:721-729.
8. Goni MD, Muhammad J, Goje M, Abatcha MG, Bitrus AA, Abbas MA. Campylobacter in dogs and cats; its detection and public health significance: a Review. Advances in Animal and Veterinary Sciences. 2017; 5(6):239-248.
9. Workman SN, Mathison GE, Lavoie MC. Pet Dogs and Chicken Meat as Reservoirs of Campylobacter spp. in Barbados. Journal of Clinical Microbiology, 2005, 2642-2650.
10. Hansson I, Sandberg M, Habib I, Lowman R, Engval EO. Knowledge gaps in control of Campylobacter for prevention of Campylobacteriosis. Transboundary and Emerging Diseases. 2016; 65(1):30-48.
11. Mikulic M, Humski A, Njari B, Ostovic M, Duvnjak S, Cvetnic Z. Prevalence of Thermotolerant Campylobacter spp. in Chicken Meat in Croatia and Multi locus Sequence Typing of a Small Subset of *Campylobacter jejuni* and Campylobacter coli isolates. Food Technology and Biotechnology. 2016; 54(4):475-481.
12. Magana M, Chatzipanagiotou S, Burriel AR, Ioannidis A. Inquiring into the gaps of Campylobacter surveillance methods. Veterinary Sciences. 2017; 4(36):1-14.
13. Man SM. The clinical importance of emerging Campylobacter species. Nature Reviews Gastroenterology & Hepatology. 2011; 8:669-685.
14. Fitzgerald C, Nachamkin I. Campylobacter and Arcobacter. In Murray, P. R. (Ed.). Manual of Clinical Microbiology. Washington D.C.:ASM Press, 2007, 933-946.
15. Lastovica AJ, Allos MB. Clinical significance of Campylobacter and related species other than *Campylobacter jejuni* and *Campylobacter coli*. In Campylobacter, 3rd edn, eds. Nachamkin, I,; Szymanski, C. M.; Blaser, M. J. Washington, D.C.: American Society

for Microbiology, 2008, 123-50.
16. Khoshbakht RM, Tabatabaei, Hosseinzadeh S, Aski HS, Seifi S. Genetic Characterization of *Campylobacter jejuni* and *C. coli* Isolated from Broilers Using flaA PCR-Restriction Fragment Length Polymorphism Method in Shiraz, Southern Iran. Jundishapur Journal of Microbiology. 2015; 8(5):e18573.
17. Al Amri A, Senok AC, Ismaeel AY, Al-Mahmeed AE, Botta GA. Multiplex PCR for direct identification of Campylobacter spp. in human and chicken stools. Journal of Medical Microbiology. 2007; 56(10):1350-1355.
18. Hutchinson DN, Bolton FJ. Improved blood-free selective medium for the isolation of *Campylobacter jejuni* from fecal specimens. Journal of Clinical Pathology. 1984; 37:956-957.
19. Penner JL. The Genus Campylobacter: a Decade of Progress. Clinical microbiology reviews. 1988; 1(2):157-172.
20. Kumar R, Verma AK, Kumar A, Srivastava M, Lal HP. Prevalence of Campylobacter spp. in Dogs attending Veterinary Practices at Mathura, India and Risk Indication Associated with Shedding. Asian Journal of Animal and Veterinary Advances. 2012; 7(8):754-760.
21. Kumar SM, Ramees TP, Dhanze H, Anjay S, Gupta ZB, Dubal M *et al*. Occurrence of Thermophilic Campylobacter in Foods of Animal Origin, Animal Faeces and Human Stool. Journal of Veterinary Public Health. 2015; 13(2):105-109.
22. Begum S, Sekar M, Gunaseelan L, Gawande M, Suganya G, Annal Selva Malar P *et al*. Molecular identification of *Campylobacter jejuni* and *coli* from chicken, calves and dogs to determine its potential threat on human being Veterinary World. 2015; 8(12):1420-1423.
23. Mohan V. Faeco-prevalence of *Campylobacter jejuni* in urban wild birds and pets in New Zealand. BMC Research Notes. 2015; 8:1.
24. Torkan S, Vazirian B, Khamesipour F, Dida GO. Prevalence of thermotolerant Campylobacter species in dogs and cats in Iran. Veterinary Medicine and Science. 2018; 4:296-303.
25. Bojanic K, Midwinter AC, Marshall JC, Biggs PJ, Acke E. Isolation of emerging Campylobacter species in working farm dogs and their frozen home-killed raw meat diets. Journal of Veterinary Diagnostic Investigation. 2019; 31(1):23-32.