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

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**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 campylobactoriosis 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-Barrés 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) \cite{man2011} and the isolates were identified by biochemical tests as described previously \cite{man2011,man2012}. 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 microaerophilic 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, indol, acetate hydrolysis tests and H$_2$S 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 using PCR by using designed primers in the study as forward primer (5’-TTCCATGACGACCTTCCTCC-3’) and reverse primer (5’-CTACCTCTTATTTGCTTGTGC-3’). The primers used for amplification of *MAP A* gene were forward primer(5’-CTATTTTATTTTGTAGTGCTGTG-3’)

and reverse primers (5’-GCTTATTGGCATTGGTTTATTATA-3’) \cite{takagi2019}. 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 \cite{takagi2019}. The annealing temperature for *Map* A gene was optimized as 52 °C for 1 min \cite{takagi2019}. 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 \cite{wong2012}. 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 \cite{kim2016}.

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$_2$S production (Table 1) while other samples were negative for H$_2$S production *C. jejuni* biotype 2 strains are H$_2$S positive, whereas *C. jejuni* biotype 1 strains are H$_2$S negative \cite{man2011}. In this study two isolates were positive for H$_2$S production belong to biotype 2 while other belong to biotype 1 of *C. jejuni*.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Samples/source</th>
<th>Isolates showed growth on mCCDA agar</th>
<th>Biochemical test</th>
<th>H$_2$S production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Samples Examined</td>
<td>Isolates</td>
<td>Catalase</td>
<td>Oxidase</td>
</tr>
<tr>
<td>3</td>
<td>Fecal swab (DF1-DF19)</td>
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<td>19</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>19</td>
<td>19</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
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Table 1: Result of biochemical test of *C. jejuni* isolated from different sources
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, 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 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).

![Fig1](image.png)

**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


