Study of Prevalence of Campylobacter Gastroenteritis among Pediatric Population Using a Multiplex PCR in a Tertiary Care Hospital in Puducherry, South India

Lakshmi Shanmugam1, Jharna Mandal2, Niranjan Biswal3, Noyal M Joseph4

ABSTRACT

Background and objectives: Campylobacter is one of the four common causes of diarrheal illness worldwide. Campylobacter infection is more common in developing than in developed countries. As conventional methods pose a great difficulty for the isolation and identification of this organism, molecular methods are much preferred.

Materials and methods: A total of 133 stool samples were collected from children less than 13 years presenting to pediatric outpatient department and the emergency department in a tertiary care hospital in Puducherry. The stool samples were extracted, and the DNA was subjected to multiplex PCR to detect Campylobacter species, followed by sequencing.

Results: Campylobacter species was detected in 13 children (9.7% of the study population) (95% CI: 5.5–16.4), with Campylobacter jejuni (11 of 13) being the predominant species. The prevalence was higher in children less than 24 months (18.18%) with a higher predisposition to girls (14.29%). The most common clinical presentation was found to be acute watery diarrhea (10%). Campylobacter detection was higher from August to November (62%), with the highest incidence in October (22.3%). Campylobacter was detected in six (21.4%) children who had contact with pets.

Conclusion: The study reveals that the prevalence of Campylobacter infection was high in Puducherry. There is an increased need to implement molecular assays for the routine detection of Campylobacter in all clinical pediatric stool samples.

Keywords: Campylobacter, Children, Gastroenteritis, Multiplex PCR.

Journal of Gastrointestinal Infections (2021): 10.5005/jp-journals-10068-3050

INTRODUCTION

Campylobacter is one of the four common causes of diarrheal illness worldwide. CDC estimates that about 1.5 million U.S. residents are infected by Campylobacter every year. In India, diarrhea is the third common cause of mortality, with a mortality rate of 13%, and is responsible for the death of nearly 300,000 under 5 years children in India every year. C. jejuni and C. coli are the primary agents of gastroenteritis in humans. Campylobacter is a slender, curved/spiral, “S”-shaped motile gram-negative, nonspore-forming microaerophilic organism with polar flagella. Ingestion of contaminated food and water remains the primary mode of transmission.

The traditional method for the isolation of Campylobacter species from stool samples is by inoculating the sample onto the selective media such as blood or charcoal-based media and incubating the plates at 42°C in a microaerophilic (5% O2, 10% CO2, 85% N2) environment. Despite the public health problems posed by this organism, the routine isolation and identification of this organism are often missed due to the inappropriate diagnostic modalities. Therefore, this study was conducted to utilize multiplex PCR to identify Campylobacter, thereby determining the prevalence of Campylobacter infection among the pediatric population in a tertiary care hospital in Puducherry.

MATERIALS AND METHODS

The study was a hospital-based cross-sectional descriptive study and was approved by the Institute Ethics committee (IEC No JIP/IEC/2018/0398). The duration of the study was from January 2019 to June 2020.

Study Population

Stool samples from children less than 13 years of age with acute diarrhea and dysentery were included in the study after obtaining informed written consent from the parents/guardians. Stool samples from children with hospital-acquired (the onset of loose or watery stools at least 48 hours after hospital admission), persistent, and chronic diarrhea were excluded.

Sample Size Calculation

The approximate sample size calculated was 133 using sample size for sensitivity specificity studies by Naing. This was calculated
considering the detection rate of Campylobacter using PCR to be 34% (0.34), the sensitivity of PCR to be 97% (i.e., 0.97) from a study conducted in New Delhi with a precision of 5% (0.05) and with a confidence level of 95% (0.95).

### Stool Processing
All the stool samples underwent routine processing as per our laboratory standard operating procedures upon reception at the laboratory. Briefly, each sample was examined macroscopically for the consistency, presence of obvious blood/mucus, and visible worms/proglottids. This was followed by microscopic examination of saline wet mount preparation to look for the presence of pus cells, red blood cells (RBCs), ova, cyst, and trophozoites. Subsequently, each sample was plated onto MacConkey agar (MAC), xylose lysine deoxycholate agar (XLD), and inoculated into selenite F enrichment broth. Watery samples were subjected to an additional plating on thiosulfate citrate bile salt sucrose agar (TCBS) and alkaline peptone water (APW). The plates and the broth were incubated aerobically at 37°C for 18 hours. After incubation the plates were read for the presence of any suspected colonies of Salmonella/Shigella/Vibrio and Aeromonas on XLD and MAC agar. Subculture from selenite F broth was done after 16–18 hours of incubation on MAC and XLD agar and incubated at 37°C for 18 hours. Subculture from APW was done within 6–8 hours of incubation on TCBS agar and incubated at 37°C for 18 hours. The remaining stool samples were preserved at 2–8°C till the DNA extraction is performed.

### Stool DNA Extraction and PCR
Genomic DNA was extracted directly from the feces using QIAamp® Fast DNA Stool Mini Kit obtained from (Qiagen, Germany). The steps of extraction were done as per the manufacturer’s protocol. Every extraction set was carried out along with the extraction of nuclease-free water, which served as the negative extraction control, and a positive control. The primers used in the reactions was used as the internal control. The target genes for the multiplex PCR were 16S rRNA (genus Campylobacter), mapA (C. jejuni), ceuA (C. coli), and actb for internal control. β-actin was used as the internal control to rule out the presence of PCR inhibitors in the stool samples. Amplification reactions were as follows: one cycle of 10 minute at 95°C followed by 35 cycles each consisting of 30 seconds at 95°C, 90 seconds at 59°C, and 60 seconds at 72°C. The PCR was terminated by a final extension step of 10 minutes at 72°C. Amplification was expected to generate 857, 589, 462, and 619 base pair DNA fragments specific for the genus Campylobacter and for C. jejuni, C. coli, and β-actin genes, respectively. Post PCR, the PCR products were subjected to 1.5% gel electrophoresis and were visualized under ultraviolet light. A 100 bp DNA ladder was used in gel electrophoresis that aided in determining the basepair size of the bands that are formed. Reference strain (ATCC C. jejuni 33291) was used as the positive control. Each set of multiplex PCR reactions was carried out along with a no template control, negative extraction control, and a positive control. The primers used in the study are mentioned in Table 1.

### Sequencing of PCR Products
The products of PCR amplification were subjected to sequencing using an ABI 3730 XL sequencer (Applied Biosystem, Foster, California, USA) at Eurofins Genomics Private Limited, India. The sequences generated were assembled and compared using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information. Translation of DNA sequences into protein sequences was done using Expert Protein Analysis System translate. The translated protein sequences were assembled and compared using the protein BLAST of the NCBI.

### Statistical Analysis
All the data were entered and analyzed using STATATA 2.0 statistical software. Continuous variables, like age, were summarized as median depending on the distribution of the data. Categorical variables like gender, duration of symptoms, blood, or mucus in stools, duration of antibiotic use were expressed as a percentage. The Chi-square test was used to compare the two groups. A p-value of <0.05 was considered statistically significant. Outcome variable like detection of Campylobacter was expressed in percentage with 95% confidence interval.

### RESULTS
A total of 133 children were included in our study. Out of this, 77 (57.8%) were boys, and 56 (42.1%) were girls. The age distribution of these 133 children were 66 children (49.6%) less than 24 months, 22 (16.5%) between 25 and 60 months, 34 (25.5%) between 61 and 120 months and 11 children (8.2%) between 121 and 156 months. Of these 133, 111 (83.4%) presented with acute watery diarrhea and 22 (16.5%) with dysentery. 105 children (79%) had no contact with pets, and 28 (21%) had contact with pets. Of them, 96 (72.1%) presented in 3–5 days, 32 (24%) presented in 2 days, 5 (3.7%) presented after 5 days. Among the 133 children, 11 (8.3%) were already started on antibiotics, of which nearly nine were under antibiotic coverage for 3–5 days. Among these 133, 111 (83.4%) presented with acute watery diarrhea and 22 (16.5%) with dysentery. 105 children (79%) had no contact with pets, and 28 (21%) had contact with pets. Of them, 96 (72.1%) presented in 3–5 days, 32 (24%) presented in 2 days, 5 (3.7%) presented after 5 days. Among the 133 children, 11 (8.3%) were already started on antibiotics, of which nearly nine were under antibiotic coverage for 3–5 days. Among these 133 children were 66 children (49.6%) less than 24 months, 22 (16.5%) had contact with pets. Of them, 96 (72.1%) presented in 3–5 days, 32 (24%) presented in 2 days, 5 (3.7%) presented after 5 days. Among the 133 children, 11 (8.3%) were already started on antibiotics, of which nearly nine were under antibiotic coverage for 3–5 days. Among these 133, 111 (83.4%) presented with acute watery diarrhea and 22 (16.5%) had contact with pets. Of them, 96 (72.1%) presented in 3–5 days, 32 (24%) presented in 2 days, 5 (3.7%) presented after 5 days.

### Table 1: Details of multiplex PCR for the detection of members of the genus and of the thermophilic species of Campylobacter

| Target gene | Sequence 5′–3′ of primers | Amplicon length specificity | References |
|-------------|---------------------------|-----------------------------|------------|
| 16S rRNA    | ATCTAA TGG CTT AAC CAT TAA AC | 857 bp Campylobacter        | Denis et al., 1999¹² |
|             | GGA CGG TAA CTA GTC TAG TT T |                     |            |
| mapA        | CTATTATTATTATTATGCTTGTG     | 589 bp Campylobacter jejuni | Denis et al., 1999¹²¹ |
|             | GCTTTATTATTATTAGTTTATTA     |                     |            |
| ceuA        | TGTATTATTATTATATGAGCA CGC  | 462 bp Campylobacter coli  | Denis et al., 1999¹²¹ |
|             | AAATGGAATATTGTCCAA ACTAG    |                     |            |
| actb        | GCACCA CACCTTCTACAATG TGCTTGCTGATCCACACTCTG | 619 bp beta actin | Glare et al., 2002¹³ |

**Campylobacter Prevalence in Puducherry**

---

1. Denis et al., 1999
2. Denis et al., 1999
3. Denis et al., 1999
4. Glare et al., 2002
to be *Salmonella* species (10.3%), followed by *Shigella* species (6.7%) in the stool culture.

All these stool samples were subjected to multiplex PCR, of which 13 children were tested positive for campylobacteriosis, among which 8 (14.3%) were girls and 5 (6.5%) were boys \( (p = 0.13) \). The majority (18.9%) were less than 24 months which was found to be statistically significant. \( (p \leq 0.05) \). Acute watery diarrhea was found to be the common clinical presentation accounting for about 10%.

Distribution and association of campylobacteriosis with demographic and clinical variables is depicted in Table 2.

### Table 2: Distribution and association of campylobacteriosis with demographic and clinical variables

| Variables                  | Total no. of samples (n/%) | No. of campylobacter positive cases, n (%) | 95% CI | \( p \) value |
|----------------------------|----------------------------|-------------------------------------------|--------|--------------|
| **Gender**                 |                            |                                           |        |              |
| Boys                       | 77 (57.8%)                 | 5 (6.5)                                   | 2.1–14.5 | 1            |
| Girls                      | 56 (42.1%)                 | 8 (14.3)                                  | 6.4–26.2 | 0.13         |
| **Age**                    |                            |                                           |        |              |
| <24 months                 | 66 (49.6%)                 | 11 (16.7)                                 | 8.6–27.9 | 0.12         |
| 25–60 months               | 22 (16.5%)                 | 0                                         | —       | —            |
| 61–120 months              | 34 (25.5%)                 | 2 (5.9)                                   | 0.7–19.7 | 1            |
| 121–156 months             | 11 (8.2%)                  | 0                                         | —       | —            |
| **Clinical presentations** |                            |                                           |        |              |
| Acute watery diarrhea      | 111 (83.4%)                | 11 (9.9)                                  | 5.1–17.0 | 0.9          |
| Dysentery                  | 22 (16.5%)                 | 2 (9.1)                                   | 1.1–29.2 | 1            |
| **Fever**                  |                            |                                           |        |              |
| Yes                        | 37                         | 6 (16.2)                                  | 6.2–32.0 | 0.12         |
| No                         | 96                         | 7 (7.2)                                   | 3.0–14.4 | 1            |
| **Abdominal pain**         |                            |                                           |        |              |
| Yes                        | 51                         | 5 (9.8)                                   | 3.3–21.4 | 0.99         |
| No                         | 82                         | 8 (9.8)                                   | 4.3–18.3 | 1            |
| **Nausea/vomiting**        |                            |                                           |        |              |
| Yes                        | 27                         | 5 (18.5)                                  | 6.3–38.1 | 0.08         |
| No                         | 106                        | 8 (7.5)                                   | 3.3–14.3 | 1            |
| **Duration of symptoms**   |                            |                                           |        |              |
| <2 days                    | 32 (24%)                   | 3 (9.4)                                   | 2.0–25.0 | 0.7          |
| 3–5 days                   | 96 (72.1%)                 | 8 (8.3)                                   | 3.7–15.8 | 1            |
| >5 days                    | 5 (3.7%)                   | 0                                         | —       | —            |
| **Contact with pets**      |                            |                                           |        |              |
| Yes                        | 28 (21%)                   | 6 (21.5)                                  | 8.3–40.1 | 0.9          |
| No                         | 105 (79%)                  | 7 (6.7)                                   | 2.7–13.3 | 1            |
| **Under antibiotic coverage** |                         |                                           |        |              |
| Yes                        | 11 (8.3%)                  | 0                                         | —       | —            |
| <48 hours                  | 9 (6.8%)                   | 0                                         | —       | —            |
| 48–120 hours               | 2 (1.5%)                   | 0                                         | —       | —            |
| No                         | 122 (91.7%)                | 13 (10.7)                                 | 5.8–17.5 | —            |
| **Stool macroscopy**       |                            |                                           |        |              |
| Watery                     | 55 (41.4%)                 | 8 (14.5)                                  | 6.5–26.7 | 0.16         |
| Semi-formed                | 49 (36.8%)                 | 3 (6.1)                                   | 1.3–16.9 | 1            |
| Formed                     | 29 (21.8%)                 | 2 (6.9)                                   | 8.5–22.8 | 0.89         |
| **Stool microscopy**       |                            |                                           |        |              |
| Pus cells+                 | 26 (19.6%)                 | 3 (11.5)                                  | 2.4–30.2 | 0.56         |
| RBC+                       | 3 (2.3%)                   | 2 (66.7)                                  | 9.4–99.2 | <0.001       |
| Pus cells and RBC+         | 4 (3%)                     | 0                                         | —       | —            |
| Nil                        | 100 (75.2%)                | 8 (8.0)                                   | 3.5–15.2 | 1            |
| **Stool culture**          |                            |                                           |        |              |
| *Salmonella* species       | 13 (10.3%)                 | 0                                         | —       | —            |
| *Shigella* species         | 10 (6.7%)                  | 1 (10.0)                                  | 0.2–44.5 | 1            |
| Nil                        | 110                        | 12 (10.9)                                 | 5.8–18.3 | 0.92         |
Of 133 samples, 13 (9.8%) were positive for 16S rRNA gene, which corresponds to the genus Campylobacter, and 11 (8.4%) among the 13 samples were positive for the mapA gene confirming the species jejuni. The two remaining samples detected Campylobacter species other than C. jejuni and C. coli. The internal control, β actin gene, had flagged positively in all the stool samples ruling out the presence of PCR inhibitors.

Aligned sequences were searched in NCBI-BLAST (megablast) for the similarity of significant matches in the database. The sequences of 16S rRNA showed 99.75% similarity with C. jejuni strain BFR-CA-12970 chromosome (Accession ID-CP054848.1) and mapA sequences showed 99.45% homology with C. jejuni strain 129108 chromosome (Accession ID-CP053854.1).

**Discussion**

Campylobacteriosis is common in developing countries. The overall burden was estimated to be 9.8% in the present study (95% CI: 5.5–16.4). This is in concordance with an earlier study from our institute in 2011, which showed a prevalence of 10% and with the study in Assam and Nagaland in 2014–2016. A report from Bhubaneswar from 2016 to 2017 found that 16.77% were positive for Campylobacter PCR. A study conducted in Kolkata in 2008–2010 observed campylobacteriosis was 7% by culture and 16.2% by real-time PCR. The prevalence of campylobacter was observed to be 8.5% in Meghalaya, Assam in 2010. In 2012, the prevalence of campylobacter reported from Vellore was 4.5%. In 2015, a low prevalence of campylobacteriosis (2.6%) from 1,145 diarrheal samples was reported from North India with C. jejuni as the most common species detected by culture and molecular investigation.

In a study from South India, the prevalence of C. fetus subsp. jejuni was found to be 14.8% isolated from the faeces of healthy individuals.

Asian studies showed an isolation rate of 14.9% from China, 17.7% from Bangladesh, 8% from Tehran, 12% from Lahore, and 18% from Rawalpindi. European studies revealed an isolation rate of 71.4% in the Netherlands, 31.9% in Portugal, 4.7% in Southern Ireland, 9.6% in North Poland. Moreover, African studies stated a prevalence of 21% in Malawi, 8.9% in Madagascar, 5.8% in Kenya, and 0.5% in Nigeria.

The median age of presentation in our study was 36 months. Campylobacteriosis was higher (18.9%) in children less than 24 months which is in concordance with the study conducted earlier in our institute. Similar findings were observed in Odisha, Israel, and Bangladesh and was also in line with the World Health Organization, indicating that the Campylobacter infection is common in children less than 2 years in the developing countries.

Another study from Denmark found a higher incidence in 1–4 years and young adults (15–24 years). Kappareud et al. found a higher incidence of Campylobacter in 0–4 years of age. This increased incidence in infants and toddlers could be because of the immature immune system, poor hand hygiene practices and contact with soil, water, and pets.

Campylobacteriosis was found to be more in girls (14.3%) than boys (6.5%). This is in concordance with the study from Odisha, which showed a female preponderance (20%), whereas in an earlier study conducted in our institute males had a higher prevalence. This difference is not attributable to any host-specific or pathogen-specific factors related to virulence or manifestations of the disease.

The clinical presentation varies between developing countries and developed countries. In the former, it was watery diarrhea, whereas in the latter, it was bloody diarrhea. The most common presentation was found to be acute watery diarrhea (10%) in the present study. This is in concordance with the earlier study conducted in our institute. The duration of symptoms was 3–5 days in 13.2% of the children. The mean duration of symptoms was 3 ± 1.1 days which is in line with the WHO data, suggesting that the symptoms typically last for 3–6 days. In our study, the detection of Campylobacter was higher in watery stools (61.5%), followed by semi-formed stools (23%) and the least in formed stools (15%). Nearly 70% of the stools were devoid of pus cells and RBC.

The positivity rate was found to be higher (21.4%) in children who had contact with pets, similar to a Denmark study which found 52% isolation in children with pets. A study in North Poland found an isolation rate of 8.1% from pets. Pintar et al. found that the prevalence of Campylobacter in pet animals and petting zoo animals was 24.7 and 6.5%, respectively. Increased contact with pets has been associated with increased disease manifestations because the pets are known to serve as reservoirs.

Campylobacteriosis was highly detected during August–November (62%), with the highest in October (22.3%) as depicted in Figure 1. This is in concordance with the study from Denmark where the highest prevalence was from June to October. In China, the isolation rate was the highest (6.29%) during June–August, and in Beijing, it was in June. This could be attributed to the monsoon changes as diarrheal episodes are relatively higher in the rainy season because of possible contamination of the ingested food and water with potential enteric pathogens.

None of the children with Campylobacter infection was started on antibiotics before sample collection. This is because fluid and electrolyte replacement is of prime importance for treating all forms of acute diarrhea. Antibiotics were not routinely recommended for all diarrhea cases in our hospital. This is in line with the existing guidelines for treating diarrheal diseases where antibiotics are indicated for dysentery and immunocompromised patients.

Only 1 (7.6%) out of the 13 samples had grown Shigella sonnei in culture. There was no co-infection of other diarrheal disease-causing bacteria with Campylobacter observed in our study. Co-infection of Campylobacter with Shigella 0157 (2.2%) and...
Fig. 2: Gel electrophoresis image showing bands for genus Campylobacter (857 bp) and species jejuni (589 bp) β actin gene (619 bp). Lanes: L1, 100 bp DNA ladder; L2, No template control (NTC); L3, Negative extraction control (NEC); L4, L6, Samples positive for Campylobacter jejuni; L8, Sample negative for Campylobacter species with detection of internal control; L5, L7, L9, Blank; L10, Positive control (ATCC C. jejuni 33291) without internal control

Rotavirus (2.2%) was observed in an earlier study.16 Rotavirus was not investigated in the present study.

Multiplex PCR detected the genus Campylobacter in 13 (9.8%) samples, of which 11 (84%) were C. jejuni. Figure 2 depicts the Gel electrophoresis image showing bands for genus Campylobacter (857 bp) and species jejuni (589 bp) β actin gene (619 bp). Species identification could not be made in the other two samples. The reason for this could be attributed to issues with multiplexing, where amplification of one target could have had a suppressive effect on the amplification of the other target. C. coli or other species were not detected in any of the samples. A similar picture was observed in an earlier study from Assam, where 80% (n = 41) of the positive samples had detectable C. jejuni.15 Nadeem et al. in Kolkata and Chen et al. in China observed 70% (n = 142) and 89% (n = 142) of the positives were C. jejuni, respectively.17,21 In a study from North India, 27 (90%) out of 30 positive samples were C. jejuni and 3 (10%) were C. coli.20 This finding suggests that C. jejuni is the most predominant species in humans than the other species.

The strength of our study is that multiplex PCR was performed where genus and species level identification of Campylobacter was done in a single step. However, there were some limitations in the present study, i.e., (i) lesser sample size, (ii) inability to retrieve detailed history as the population comprised largely of infants and toddlers, and (iii) real-time PCR could have been a better choice for detecting pathogens in clinical samples because of its increased sensitivity and least contamination rate. We could not do that in the present study because of the limited financial resources.

Conclusion
As there are a subtitle number of children with undiagnosed Campylobacter infection, there is a need for the implementation of molecular tests for diagnosis. The advantage of this multiplex PCR is that the detection and species differentiation can be done in a single step with a relatively less turnaround time. This aids in the early diagnosis of the infection and better patient management. This study highlights the prevalence of Campylobacter as a common agent of gastroenteritis and a need to employ sensitive and robust PCR-based tools for its detection.
18. Rizal A, Kumar A, Vidyarthi AS. Prevalence of pathogenic genes in *Campylobacter jejuni* isolated from poultry and human. Internet J Food Safety 2010;12:29–34.

19. Rajendran P, Babji S, George AT, et al. Detection and species identification of *Campylobacter* in stool samples of children and animals from Vellore, South India. Indian J Med Microbiol 2012;30(1):85. DOI: 10.4103/0255-0857.93049.

20. Vaishnavi C, Singh M, Thakur JS, et al. Low prevalence of campylobacteriosis in the Northern region of India. Adv Microb Biol 2015;5(03):155. DOI: 10.4236/aim.2015.53015.

21. Rajan DP, Mathan VI. Prevalence of *Campylobacter fetus* subsp. *jejuni* in healthy populations in southern India. J Clin Microbiol 1982;15(S):749–751. DOI: 10.1128/jcm.15.S.749-751.1982.

22. Jie CH, Sun XT, Zheng ZE, et al. *Campylobacter enteritis* in adult patients with acute diarrhea from 2005 to 2009 in Beijing, China. Chin Med J 2011;124(10):1508–1512. PMID: 21740807.

23. Szczepanska B, Andrzejevska M, Spica D, et al. 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.

24. Kaakoush NO, Castaño-Rodríguez N, Mitchell HM, et al. Global epidemiology of *Campylobacter* infection. Clin Microbiol Rev 2015;28(3):687–720. DOI: 10.1128/CMR.00006-15.

25. Adekunle O, Coker A, Kolawole D. Incidence, isolation and characterisation of *Campylobacter* species in Osogbo. Biol Med 2009;1(1):24–27.

26. Weinerberger M, Lerner L, Valinsky L, et al. Increased incidence of *Campylobacter* spp. infection and high rates among children, Israel. Emerg Infect Dis 2013;19(11):1828–1831. DOI: 10.3201/eid1911.120900.

27. Glass RI, Stoll BJ, Huq MI, et al. Epidemiologic and clinical features of endemic *Campylobacter jejuni* infection in Bangladesh. J Infect Dis 1983;148(2):292–296. DOI: 10.1093/infdis/148.2.292.

28. Nielsen HL, Ejlersen T, Engberg J, et al. High incidence of *Campylobacter concisus* in gastroenteritis in North Jutland, Denmark: a population-based study. Clin Microbiol Infect 2013;19(5):445–450. DOI: 10.1111/j.1469-0691.2012.03852.x.

29. Kapperud G, Lassen J, Ostroff SM, et al. Clinical features of sporadic *Campylobacter* infections in Norway. Scand J Infect Dis. 1992;24(6):741–749. DOI: 10.3109/00365549209062459.

30. Pintar KD, Christidis T, Thomas MK, et al. A systematic review and meta-analysis of the *Campylobacter* spp. prevalence and concentration in household pets and petting zoo animals for use in exposure assessments. PLoS One 2015;10(12):e0144976. DOI: 10.1371/journal.pone.0144976.

31. Huang JL, Xu HY, Bao GY, et al. Epidemiological surveillance of *Campylobacter jejuni* in chicken, dairy cattle and diarrheapatiants. Epidemiol Infect 2009;137(8):1111–1120. DOI: 10.1017/S0950268809002039.

32. Kasper, Fauci, Hauser, Longo, Jameson, Loscalzo. *Harrison’s principles of internal medicine*. 19th ed. New York: McGraw Hill; 2015. p. 264.