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Prevalence of *Cyclospora cayetanensis* and other enteropathogen among children under the age of 15 years in Biratnagar, Nepal

Manish Dahal¹, Ram Hari Dahal², Dhiraj Kumar Chaudhary³*

¹Department of Microbiology, Mahendra Morang Adarsha Multiple Campus, Tribhuvan University, Biratnagar, Nepal
²Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal
³Department of Soil Science, Prithu Technical College, Institute of Agriculture and Animal Science, Tribhuvan University, Lamahi, Dang, Nepal

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**ABSTRACT**

**Objective:** To find out the prevalence of *Cyclospora cayetanensis* (*C. cayetanensis*) and other enteropathogen among children under the age of 15 years from different areas of Biratnagar Municipality and those who visited Nobel Medical College and Teaching Hospital, Biratnagar, Nepal.

**Methods:** A total of 588 stool samples were collected from children with diarrhoeal illness. Samples were processed to observe parasites using normal saline, iodine and potassium dichromate. Bacterial enteropathogens were detected through bacterial cultures and biochemical analysis. Serological tests and antibiotic susceptibility tests were performed for pure bacterial isolates. ELISA was done to find the prevalence of rotavirus.

**Results:** Among 588 processed samples, 12 (2.04%) cases showed potential *C. cayetanensis* infection which was highly distributed in the age group of 3–6 years during the month of July. *Giardia lambia* was found to be more prevalent than *C. cayetanensis* and *Escherichia coli* and was the highest bacterial enteropathogen detected among children suffering from diarrhoeal illness. The overall prevalence of rotavirus was 22.89% of total children under 5 years age and found the highest in the month of May.

**Conclusions:** The present study concluded that the prevalence of *C. cayetanensis* was relatively low as compared to other parasite. A wide range of other parasite was involved as causative agents of diarrhoea other than *C. cayetanensis*. Rotavirus has been found as a major causative agent of childhood diarrhoea in children less than 5 years and this study showed that parasites and other enteropathogen were more prevalent in the months of May, June and July.

1. **Introduction**

*Cyclospora cayetanensis* (*C. cayetanensis*) is an intracellular intestinal protozoan responsible for uncontrolled debilitating diarrhoea in developing countries[1,2]. Diarrhoea is a serious problem in developing countries where death of children occurs due to diarrhoeal dehydration. An individual having diarrhoea loses a large quantity of water from the body in the form of liquid stool three times or more frequently than normal in a day[3]. In developing countries, diarrhoeal disease accounts for approximately 19% of the total deaths in children under the age of 5 years[4]. Not only *C. cayetanensis* but other protozoan like *Entamoeba histolytica* (*E. histolytica*), *Cryptosporidium parvum*, *Giardia lambia* (*G. lambia*), *Ascaris lumbricoides* (*A. lumbricoides*), *Trichuris trichura* (*T. trichura*), *Blastocystis hominis* (*B. hominis*), *Hymenolepis nana* (*H. nana*) and *Balantidium coli* as well as pathogenic bacteria including *Escherichia coli* (*E. coli*), *Shigella* spp., *Vibrio cholerae* (*V. cholerae*), *Aeromonas* spp., *Campylobacter* spp., *Yersinia enterocolitica* and *Salmonella* spp. play a vital role in causing diarrhoea[5,6]. Rotavirus remains the most common cause of severe diarrhoea and adenovirus causes acute gastroenteritis under the age of 5 years[7,8]. Three forms of diarrhoea (watery diarrhoea, bloody diarrhoea and persistent diarrhoea) are potentially life-threatening diarrhoea during childhood. *Cyclospora* infection
causes watery diarrhoea. *E. histolytica* and *Shigella* spp. cause bloody diarrhoea, and almost all enteropathogens are responsible for persistent diarrhoea[9,10].

Nepal is a developing nation. The major obstacles in the development of Nepal are illiteracy and poverty which are the most important predisposing factors for the prevalence of infectious disease. Diarrhoea is a principal cause of childhood mortality and morbidity in Nepal[3]. *C. cayetanensis* is an emerging pathogen that is responsible for cyclosporiasis. There is an emerging public health concern about cyclosporiasis and other parasitic infection around the world, but these infections are not routinely sought in the laboratory[11,12]. It is extremely necessary to find out the prevalence of *C. cayetanensis* in children who are more susceptible to the risk of diarrhoea. To the best of our knowledge, only a few studies on cyclosporiasis have been done in Nepal, and none of the studies have been conducted in the eastern region of Nepal. Hence, we carried this study in order to find out the prevalence of *C. cayetanensis* and other enteropathogen among children with diarrhoeal symptoms under the age of 15 years. This study could be helpful in diagnosing the parasites which are less found in a general hospital laboratory and also puts a light on *C. cayetanensis* as an emerging pathogen among the children in Nepal.

2. Materials and methods

This study was conducted among the children under the age of 15 years having a diarrhoeal illness attending the Paediatric Department of Nobel Medical College and Teaching Hospital and different rural areas of Biratnagar Municipality, Nepal during April 2015 to December 2015. Stool samples were collected from children of different regions in Biratnagar Municipality and from children visiting Paediatric Department of Nobel Medical College and Teaching Hospital, Nepal having diarrhoeal illness as described by guidelines of Centers for Disease Control and Prevention[13]. Samples were processed in the central research laboratory of Nobel Hospital, Biratnagar, Nepal. Parasites were observed using normal saline, iodine and potassium dichromate[14]. Bacterial cultures and biochemical tests were performed for the identification of bacterial isolates[15]. Serological tests and antibiotic susceptibility tests for the isolates were done according to the procedure described by World Health Organization[16]. The ELISA was used for detection of rotavirus[17]. Verbal informed consent was taken from study population. Data were analyzed using statistical package of SPSS version 17.0. Chi-square test was performed and a *P*-value was considered significant when it was less than 0.05.

2.1. Ethical consideration

The study protocol was performed according to Helsinki declaration and approved by Research Ethics Committee of Nobel Medical College and Teaching Hospital, Kathmandu University, Nepal. Informed written consent was obtained from all the patients included in the study.

3. Results

During April to December 2015, a total of 588 cases were taken into study. Among them, 59% were male and 41% were female children. The most number of the total parasites (*C. cayetanensis*, *E. histolytica*, *G. lamblia*, *A. lumbricoides*, *T. trichura*, *B. hominis*, and *H. nana*) was distributed to children in the age group of 0–3 years and the least number of parasites was distributed to children in the age group of 12–15 years (Table 1). The prevalence of *C. cayetanensis* infection was found to be 2.04% and the highest positive cases occurred in the month of June and July (Table 2). There was no significant association between months and the presence of *C. cayetanensis* in children (χ² = 32.03, *P* = 0.182). Age wise distribution of cyclosporiasis showed that the parasite was distributed more (5.49%) in the age group of 3–6 years and most infections (33.33%) were found in Nobel Hospital (Figure 1 and Table 3). Statistically, there was no significant association between the age of children and the presence of *C. cayetanensis* (χ² = 1.40, *P* = 0.591). The prevalence of cyclosporiasis was associated with children who did or did not attend hospital (χ² = 46.18, *P* = 0.015). Frequency distribution on cyclosporiasis based on water treatment system revealed that the highest number of cases (9) was distributed to children using untreated water (Figure 2). Statistical analysis using Chi-square test showed that there was no significant association in presence of *C. cayetanensis* between children using untreated water and those using treated water (χ² = 13.80, *P* = 0.269).

*G. lamblia* (12.07%) and *E. histolytica* (9.86%) were the most prevalent parasites and most parasites were detected in the month of May (35.29%) excluding *C. cayetanensis* (Table 4). Age wise distribution of bacterial pathogens revealed that the highest bacterial enteropathogens (41.86%) were distributed to age group of 0–3 years and *E. coli* (83.72%) was found to be dominant pathogen (Table 4). Similar to parasitic infection, the highest (29.07%) of bacterial infection was also found in the month of May (Table 5). Seropositivity of *V. cholerae* O1 was found to be 100% among 6 isolates of *V. cholerae* and none of *Shigella sonnei* was found among *Shigella* spp. (Table 7). Antibiotic susceptibility pattern (Table 8) showed that *Shigella* spp. was most susceptible to norfloxacin (93.33%) followed by ciprofloxacin (86.66%) and gentamicin (73.33%). *Salmonella* spp. was most susceptible to ciprofloxacin (85.71%), and *V. cholerae* were 100% sensitive to tetracycline, gentamicin and ciprofloxacin.
Figure 2. Frequency distribution of C. cayetanensis based on the treatment of water.

Table 1
Age and gender distribution of children in terms of the total parasites.

| Age group (years) | Male | Female | No. of children | Total parasite positive cases [n (%)] |
|-------------------|------|--------|-----------------|-------------------------------------|
| 0–3               | 225  | 138    | 363             | 65 (43.04)                          |
| 3–6               | 43   | 48     | 91              | 36 (23.84)                          |
| 6–9               | 35   | 30     | 65              | 27 (17.88)                          |
| 9–12              | 27   | 11     | 38              | 12 (7.94)                           |
| 12–15             | 17   | 14     | 31              | 11 (7.28)                           |
| Total             | 347  | 241    | 588             | 151 (25.68)                         |

Table 2
Month wise distribution of C. cayetanensis.

| Months     | No. of processed samples | Positive cases | Total [n (%)] |
|------------|--------------------------|----------------|---------------|
| April      | 41                       | 0              | 0 (0.00)      |
| May        | 102                      | 2              | 3 (2.94)      |
| June       | 93                       | 3              | 4 (4.30)      |
| July       | 82                       | 3              | 4 (4.87)      |
| August     | 61                       | 1              | 1 (1.63)      |
| September  | 53                       | 0              | 0 (0.00)      |
| October    | 58                       | 0              | 0 (0.00)      |
| November   | 52                       | 0              | 0 (0.00)      |
| December   | 46                       | 0              | 0 (0.00)      |
| Total      | 588                      | 9              | 12 (2.04)     |

Table 3
Prevalence of C. cayetanensis according to collection sites.

| Collection sites         | No. of processed samples | Positive cases | Total [n (%)] |
|--------------------------|--------------------------|----------------|---------------|
| Nobel Hospital, Biratnagar| 435                      | 3              | 4 (33.33)     |
| Pichara                  | 66                       | 2              | 3 (25.00)     |
| Kesalia                  | 51                       | 2              | 3 (25.00)     |
| Kathari                  | 25                       | 2              | 2 (16.67)     |
| Bajaiathpur              | 11                       | 0              | 0 (0.00)      |
| Total                    | 588                      | 9              | 12 (2.04)     |

Table 4
Distribution of parasites except C. cayetanensis.

| Parasites             | April | May  | June | July | August | September | October | November | December | Total |
|-----------------------|-------|------|------|------|--------|-----------|---------|----------|----------|-------|
| Processed samples     | 41    | 102  | 93   | 82   | 61     | 53        | 58      | 52       | 46       | 588   |
| E. histolytica        | 5     | 15   | 11   | 9    | 7      | 3         | 2       | 4        | 2        | 58    |
| G. lamblia            | 9     | 19   | 13   | 11   | 4      | 5         | 5       | 3        | 3        | 71    |
| A. lumbricoides       | 0     | 0    | 0    | 1    | 0      | 0         | 0       | 0        | 0        | 1     |
| T. trichura           | 0     | 0    | 1    | 1    | 0      | 0         | 0       | 0        | 0        | 2     |
| B. hominis            | 0     | 2    | 2    | 1    | 0      | 0         | 0       | 0        | 0        | 5     |
| H. nana               | 0     | 0    | 1    | 1    | 0      | 0         | 0       | 0        | 0        | 2     |
| Total                 | 14    | 36   | 28   | 24   | 11     | 8         | 7       | 5        | 139      | 1848  |
| Percentage            | 34.14 | 35.29| 30.10| 29.26| 18.03  | 15.09     | 12.06   | 13.46    | 10.86    | 23.63 |

Table 5
Distribution of bacterial pathogens according to age groups.

| Age group (years) | E. coli | Shigella spp. | V. cholerae | Salmonella spp. | Total [n (%)] |
|-------------------|---------|---------------|-------------|-----------------|---------------|
| 0–3               | 68      | 2             | 1           | 1               | 72 (41.86)    |
| 3–6               | 42      | 4             | 3           | 3               | 52 (30.23)    |
| 6–9               | 23      | 7             | 2           | 2               | 34 (19.77)    |
| 9–12              | 6       | 1             | 0           | 1               | 8 (4.65)      |
| 12–15             | 5       | 1             | 0           | 0               | 6 (3.49)      |
| Total             | 144     | 15            | 6           | 7               | 172 (100.00)  |

Table 6
Distribution of enteropathogens according to months.

| Months     | Total samples | E. coli | Shigella spp. | V. cholerae | Salmonella spp. | Total [n (%)] |
|------------|---------------|---------|---------------|-------------|-----------------|---------------|
| April      | 41            | 11      | 1             | 0           | 1               | 13 (7.56)     |
| May        | 102           | 47      | 2             | 0           | 1               | 50 (29.07)    |
| June       | 93            | 26      | 5             | 2           | 2               | 35 (20.35)    |
| July       | 82            | 14      | 3             | 4           | 1               | 22 (12.79)    |
| August     | 61            | 10      | 2             | 0           | 1               | 13 (7.56)     |
| September  | 53            | 7       | 1             | 0           | 0               | 8 (4.65)      |
| October    | 58            | 12      | 0             | 0           | 0               | 12 (6.98)     |
| November   | 52            | 9       | 1             | 0           | 1               | 11 (6.40)     |
| December   | 46            | 8       | 0             | 0           | 0               | 8 (4.65)      |
| Total      | 588           | 144     | 15            | 6            | 7               | 172 (100.00)  |

Table 7
Frequency distribution of serotypes of Shigella spp., V. cholerae and Salmonella spp.

| Bacterial enteropathogens | Number | Percentage |
|---------------------------|--------|------------|
| Shigella dysenteriae      | 9      | 60.00      |
| Shigella flexneri         | 5      | 33.33      |
| Shigella boydii           | 1      | 6.66       |
| Shigella sonnei           | 0      | 0.00       |
| V. cholerae O1            | 6      | 100.00     |
| Salmonella typhi          | 4      | 57.14      |
| Salmonella paratyphi-A    | 2      | 28.57      |
| Salmonella paratyphi-B    | 1      | 14.28      |

Table 8
Antibiotic susceptibility distribution of bacterial isolates

| Antibiotics | Shigella spp. | Salmonella spp. | V. cholerae |
|-------------|---------------|-----------------|-------------|
|             | Susceptible   | Resistant       | Susceptible | Resistant | Susceptible | Resistant |
| Chloramphenicol | – –          | – –             | – –        | – –      | – –        | – –      |
| Norfloxacin  | 14 (93.33)    | 1 (6.67)        | 3 (60.00)  | 2 (40.00) | 6 (100.00) | 0 (0.00) |
| Gentamicin   | 11 (73.33)    | 4 (26.67)       | 3 (50.00)  | 3 (50.00) | 6 (100.00) | 0 (0.00) |
| Ciprofloxacin| 13 (86.66)    | 2 (13.34)       | 6 (85.71)  | 1 (14.29) | 6 (100.00) | 0 (0.00) |

Prevalence of rotavirus was 22.89% of the total children under 5 years of age and found the highest in the month of May (31.66%). Age wise distribution of rotavirus that showed the highest infection was found in the age group of 0–1 year (Table 9, Figure 3). Statistically, there was not any significant association between the presence of rotavirus and age of children ($\chi^2 = 7.29$, $\chi^2 = 7.29$).
These findings are in agreement with the previous studies [11,19,21-23]. The main findings of present study help to understand about major periods of transmission, treatment and challenges must be raised. Such organisms should be taken into consideration. Such organisms should be taken into consideration to get a more precise scenario of the diarrhoeal condition. Besides C. cayetanensis, Cryptosporidium spp. and Isospora belli are also greatly emerging parasites. So research must include consideration of these parasites. Diarrhoea which is mostly caused by parasites is due to lack of poor hygiene and sanitation. So awareness regarding transmission, treatment and challenges must be raised.

### Conflict of interest statement

We declare that we have no conflict of interest.

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