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

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The study protocol was performed according to the 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.

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causes watery diarrhoea. *E. histolytica* and *Shigella* spp. cause bloody diarrhoea, and almost all enteropathogens are responsible for persistent diarrhoea[6,9,10].

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 dairrhoeal 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. lambia, 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 (χ^2 = 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* (χ^2 = 1.40, P = 0.591). The prevalence of cyclosporiasis was associated with children who did or did not attend hospital (χ^2 = 46.18, P = 0.013). 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 (χ^2 = 13.80, P = 0.269).

G. lambia (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 5). Statistical analysis showed significant association between the presence of bacterial pathogen and the age of children (χ^2 = 69.159, P = 0.001). Similar to parasitic infection, the highest (29.07%) of bacterial infection was also found in the month of May (Table 6). 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%). *Salmonela* spp. was most susceptible to ciprofloxacin (85.71%), and *V. cholerae* were 100% sensitive to tetracycline, gentamicin and ciprofloxacin.

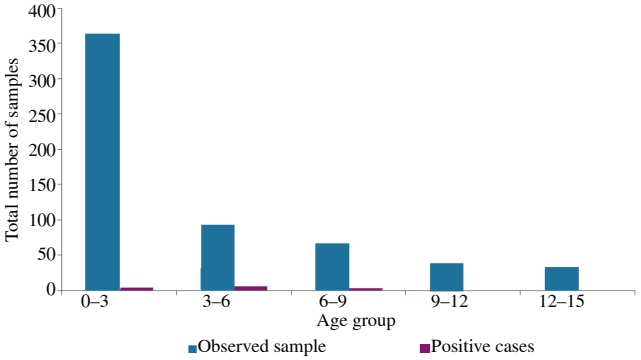

Figure 1. Distribution of *C. cayetanensis* according to different ages of children.

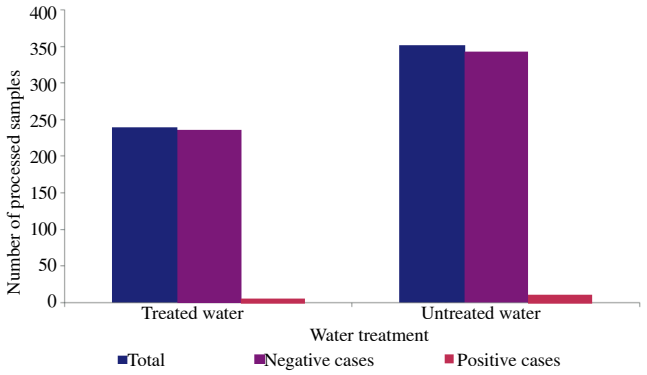

Figure 2. Frequency distribution of *C. cayetanensis* based on the treatment of water.

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* (%)]
		Male	Female	
April	41	0	0	0 (0.00)
May	102	2	1	3 (2.94)
June	93	3	1	4 (4.30)
July	82	3	1	4 (4.87)
August	61	1	0	1 (1.63)
September	53	0	0	0 (0.00)
October	58	0	0	0 (0.00)
November	52	0	0	0 (0.00)
December	46	0	0	0 (0.00)
Total	588	9	3	12 (2.04)

Table 3

Prevalence of *C. cayetanensis* according to collection sites.

Collection sites	No. of processed samples	Positive cases		Total [*n* (%)]
		Male	Female	
Nobel Hospital, Biratnagar	435	3	1	4 (33.33)
Pichara	66	2	1	3 (25.00)
Kesalia	51	2	1	3 (25.00)
Katahari	25	2	0	2 (16.67)
Baijnathpur	11	0	0	0 (0.00)
Total	588	9	3	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. lambia	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	7	5	139
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 [*n* (%)]	588	144 (83.72)	15 (8.72)	6 (3.49)	7 (4.07)	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
Ciprofloxacin	13 (86.66)	2 (13.34)	6 (85.71)	1 (14.29)	6 (100.00)	0 (0.00)
Gentamicin	11 (73.33)	4 (16.67)	5 (71.42)	2 (28.57)	6 (100.00)	0 (0.00)
Cotrimoxazole	9 (60.00)	6 (40.00)	2 (28.57)	5 (71.42)	0 (0.00)	6 (100.00)
Ampicillin	10 (66.66)	5 (33.33)	3 (42.85)	4 (57.14)	–	–
Norfloxacin	14 (93.33)	1 (6.33)	–	–	–	–
Nalidixic acid	12 (80.00)	3 (20.00)	–	–	0 (0.00)	6 (100.00)
Tetracycline	–	–	–	–	6 (100.00)	0 (0.00)
Erythromycin	–	–	–	–	4 (66.66)	2 (33.33)
Chloramphenicol	–	–	3 (42.85)	4 (57.14)	–	–

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 (χ^2 = 7.29,

P = 0.449).

Table 9

Month and gender-wise distribution of rotavirus.

Months	Positive			Negative			Total
	Male	Female	Total [*n* (%)]	Male	Female	Total [*n* (%)]	
April	7	8	15 (31.25)	19	14	33 (68.75)	48
May	12	7	19 (31.66)	25	16	41 (68.34)	60
June	6	8	14 (30.43)	12	20	32 (69.57)	46
July	9	1	10 (20.40)	27	12	39 (79.60)	49
August	6	4	10 (17.85)	26	20	46 (82.15)	56
September	3	0	3 (12.50)	15	6	21 (87.50)	24
October	2	2	4 (16.00)	11	10	21 (84.00)	25
November	4	3	7 (17.94)	19	13	32 (82.06)	39
December	4	1	5 (15.15)	20	8	28 (84.85)	33
Total		87			293		380

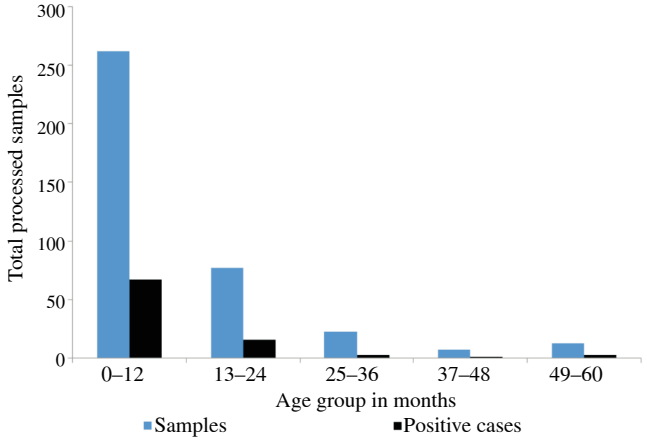

Figure 3. Distribution of rotavirus according to age of children under 5 years.

4. Discussion

C. cayetanensis is a rapidly emerging pathogen usually found in children and traveller's traveling in the endemic region[11,18]. It is a major diarrhoeal pathogen causing prolonged and severe diarrhoea and even resulting in death. *C. cayetanensis* is not commonly sought in the common clinical laboratory and this makes the pathogen even more dangerous potentially[19]. The diarrhoeal outbreak in Nepal occurs due to poor socio-economic status, the persistence of traditional and social ills, lack of hygienic practices and poor health conditions[20].

The main findings of present study help to understand about *C. cayetanensis* as emerging pathogens in causing diarrhoea among children of the rural area of Nepal. The overall prevalence of *Cyclospora* infection among all age-group children was 2.04%. The higher cases of cyclosporiasis were accounted during June and July and no case of infection was found during August to December and April. This indicates that summer and rainy season are the major periods of *Cyclospora* infection in rural region of Nepal. These findings are in agreement with the previous studies[11,19,21-23]. In this study, high cases of parasitic infection including *C. cayetanensis* were found in the age group of 3–6 years. This finding was in agreement with previous studies conducted by Sherchand and Cross[11] and Madico *et al.*[24]. The comparison of cyclosporiasis based on water treatment system showed that the highest number of infection (9) was found among children consuming untreated water and fewer (3) cases were found to be infected with *C. cayetanensis* among children consuming chlorinated water. This may be due to the high transmission rate of oocysts of *Cyclospora* in sewage water which may contaminate tap water during the month of June and July. The positive case which resulted from children consuming chlorinated water may be due to the resistance of oocysts to chlorination[11].

Distribution of parasites other than *C. cayetanensis* revealed high cases with *G. lambia* and *E. histolytica*. This pattern is similar to other studies[21,25]. *E. coli* was accounted to be the highest bacterial enteropathogens and was highly distributed in the age group of 0–3 year children. These data are comparable with the study made by Ono *et al.*[26] and Ben Salem-Ben Nejma *et al.*[27]. Seropositivity pattern of *V. cholerae* with 100% *V. cholerae* O1 serotype provides clear insight of possible cholera disease among the children enrolled in the study population. Distribution of serotype of *Shigella* and *Salmonella* suggests the probable occurrence of shigellosis and typhoid fever. Antibiotic susceptibility pattern of bacterial isolates revealed effective antibiotics: norfloxacin for *Shigella* spp., ciprofloxacin for *Salmonella* spp. and tetracycline, gentamicin, and ciprofloxacin for *V. cholerae*.

Among children under 5 years of age, rotavirus remains the most frequent causative agents of diarrhoeal outbreak. The high prevalent rate of rotavirus found in this study under 5 years of age may be due to underdeveloped immune system of children, lack of hygienic practices and consumption of raw food and untreated water. In several literatures, rotavirus is considered as the most common cause of viral gastroenteritis[7,8].

This study showed *C. cayetanensis* as an emerging pathogen causing diarrhoea in rural area of Nepal. A wide range of other parasites was also involved as causative agents of diarrhoea other than *C. cayetanensis*. Rotavirus has been found as a major causative agent of childhood diarrhoea in children under 5 years of age. The picture of the study does not bear the burden of the whole country, so similar studies should be carried out in other area of the country. A wide range of another organism such as *Campylobacter* spp., *Yersinia* spp., *Clostridium perfringens* are not 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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