

# Cyclosporiasis Among School Going Children of Kathmandu Valley

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## Abstract

Intestinal parasitic infections among children possess a critical public health issue in Nepal. This study was conducted to determine cyclosporiasis among school going children of Kathmandu valley. A total of 187 collected stool samples were subjected to direct microscopy, formal-ether concentration technique, Sheather's sucrose floatation technique and modified acid fast staining technique. The incidence of cyclosporiasis, cryptosporidiosis and other intestinal parasitosis was 24.1%, 29.4% and 51.9% respectively. The cyclosporiasis was higher in female (28.4%) without any significance ( $p=0.190$ ). The cyclosporiasis was found highest in age group 5-8 (25.2%) but statistically insignificant ( $p=0.895$ ). Cyclosporiasis was higher in symptomatic case (25.6%) but found statistically insignificant ( $p=0.469$ ). Similarly, cyclosporiasis was higher in school children without following hygienic practice (28.0%) but without any significance ( $p=0.103$ ). There was significant difference between detection of cyclospora before and after sporulation by modified acid fast staining technique ( $p<0.001$ ) and highest association was seen between *Cyclospora cayetanensis* and *Cryptosporidium parvum*. The outcome of this study revealed that cyclosporiasis remain highly endemic in school children of Kathmandu valley. This suggests proper diagnosis and specific treatment is required to lower the parasitic burden.

**Key words:** *Cyclospora cayetanensis*, *Cryptosporidium parvum*, modified acid fast staining technique

## Introduction

The cyclosporiasis is an emerging disease found in both immunocompromised and immunocompetent person. *C. cayetanensis* is specific to humans (Ortega *et al.* 1993) and its infection leads to watery diarrhea with the absence of any leucocytes or blood (Powell 1995). The carriers may be temporary in immunocompetent hosts or chronic in immunocompromised hosts (Eberhard *et al.* 2000). Cyclosporiasis is found in patients from 12- 87 year old age. Indirect transmission through flies, fingers, fomites and food is the usual mode of transmission. Epidemiologic data indicate that the human-associated cyclospora is transmitted by water and food (Hoge *et al.* 1995). Sexual and racial predilection does not appear to exist for cyclosporiasis. The low socio-economic groups of people are more susceptible to cyclospora than the people of high socio-economic groups. People

with the occupation of sex work, farming, kitchen activities, hospital works, and school children are the vulnerable groups for cyclosporiasis (Ghimire *et al.* 2005). Current information on the epidemiology of cyclosporiasis comes from Nepal, Haiti and Peru (John & Petri 2006).

Immunity may not occur after infection however, some amount of immunity may be present in adults who are exposed to the infection as the infection is less prevalent in adults living in endemic areas. High oocysts frequency case has been observed in the stool of diarrheal persons and AIDS patients (Sherchand & Cross 2003). The unsporulated oocysts require some days for sporulation outside the human beings, however, the environment may trigger the development process in the oocysts (Smith *et al.* 1997). The present

study has been designed to analyze present situation of cyclosporiasis among school going children in Kathmandu valley and its relation with behavioral pattern and life style of the children relating to health.

### Methodology

A total of 187 stool samples were collected from school going children of Kathmandu valley in a screw capped plastic container. A questionnaire consisting age, sex, hygienic practices and gastrointestinal symptoms were filled. All the samples were processed in Microbiology Laboratory of National College, Kathmandu. Each fresh stool sample was examined macroscopically and microscopically for the detection of parasites. Microscopic examination of samples was done by standard formal-ether concentration method. Sheather's sucrose floatation method followed by modified acid fast staining was conducted for detecting oocysts of *Cryptosporidium* spp. and *Cyclospora* spp. Cyclospora positive specimens were stored at ambient temperature in 2.5% potassium dichromate for about 10 days and were examined for sporulation. The data obtained were analyzed by statistical software SPSS (version 11.5).

### Results

Among 187 stool samples, the prevalence of cyclosporiasis was 24.1%. The genderwise distribution of cyclosporiasis in school going children revealed that the prevalence was higher among females (28.4%) than males (20.2%) (Table 1). However, the genderwise occurrence of cyclosporiasis was found statistically insignificant. The prevalence of cyclosporiasis was found highest in the age group 5-8 (25.2%) (Table 2). The occurrence of cyclosporiasis in different age group was statistically insignificant. The occurrence of *C. cayetanensis* in symptomatic case (25.6%) was higher than asymptomatic case (20.7%) but it was found statistically insignificant (Table 3). The occurrence of *C. cayetanensis* in school going children without maintaining hygienic practice (28.0%) was higher than with maintaining hygienic practice (17.4%) but, the result was found statistically insignificant (Table 4). There was high significant difference between detection of cyclospora before and after sporulation by modified acid fast staining technique (Table 5). Highest association was between *C. cayetanensis* and *Cryptosporidium parvum* (67.3%) (Table 6).

**Table 1.** Genderwise distribution of *C. cayetanensis*

Sex	Frequency (n)	Positive(n)	%	p value
Male	99	20	20.2	p=0.190
Female	88	25	28.4	
<b>Total</b>	<b>187</b>	<b>45</b>	<b>24.1</b>	

**Table 2.** Frequency distribution of *C. cayetanensis* with age

Age	Frequency (n)	Positive (n)	%	p value
3-5 years	46	10	21.7	p=0.895
5-8 years	107	27	25.2	
8-12 years	34	8	23.5	
<b>Total</b>	<b>187</b>	<b>45</b>	<b>24.1</b>	

**Table 3.** Symptom wise distribution of *C. cayetanensis*

Symptoms	Frequency (n)	Positive (n)	%	p value
Symptomatic	129	33	25.6	p=0.469
Asymptomatic	58	12	20.7	
<b>Total</b>	<b>187</b>	<b>45</b>	<b>24.1</b>	

**Table 4.** Distribution of *C. cayetanensis* with hygiene practice

Hygiene Practice	Frequency (n)	Positive (n)	%	p value
Following	69	12	17.4	p=0.103
Not Following	118	33	28.0	
<b>Total</b>	<b>187</b>	<b>45</b>	<b>24.1</b>	

**Table 5.** Comparison of *C. cayetanensis* detected from different techniques

Modified acid fast staining after sporulation in 2.5% potassium dichromate	First modified acid fast staining before sporulation	Total	p value
Positive (n)	11	19	p<0.001
Negative (n)	14	143	
<b>Total</b>	<b>25</b>	<b>162</b>	

**Table 6.** Association of *C. cayetanensis* with other intestinal parasites

Parasites detected	<i>Cyclospora cayetanensis</i> (n)	%
<i>Cryptosporidium parvum</i>	41	67.3
<i>Giardia lamblia</i>	9	14.7
<i>Entamoeba histolytica</i>	8	13.2
<i>Entamoeba hartmani</i>	1	1.6
<i>Ascaris lumbricoides</i>	1	1.6
<i>Trichuris trichiura</i>	1	1.6
<i>Entamoeba coli</i>	0	0
<i>Strongyloides stercoralis</i>	0	0
<b>Total</b>	<b>61</b>	<b>100</b>

## Discussion

The occurrence of cyclosporiasis was 24.1%. This finding was higher than the findings of Kimura *et al.* (2005) and Ghimire *et al.* (2005). The finding was similar to the finding of Sherchand *et al.* (1999) where prevalence of cyclosporiasis was 29.8%.

In endemic countries like Nepal, the annual attack rate for *C. cayetanensis* has been reported to be as high as 40% (Sherchand *et al.* 1999). These differences may be due to different geographical areas, seasons, time, climatic condition, socioeconomic factors of the population, non-specific treatment, lack of proper diagnosis, etc. (Adam *et al.* 2000). This high prevalence can also be correlated with the epidemiological routes of food borne and water borne transmission of intestinal parasites. In rainy season, the seepage of water from the distribution pipe or rain water may contaminate vegetables either when they are openly kept on the ground for sale or in the fields just before harvesting. Similarly, the use of human waste as fertilizer for plants or indirectly via contaminated sewage water used for crop irrigation and during washing of products might be other reasons for high prevalence of cyclosporiasis (Ghimire *et al.* 2008).

The genderwise distribution of cyclosporiasis in school going children revealed higher prevalence in female school going children (28.4%) than in male school going children (20.2%) but the result was statistically insignificant ( $p=0.190$ ). But the higher prevalence in females can be correlated with their working habit in kitchen, consumption of different raw items such as panipuri, Chana Chatpatae, fruits and vegetables which may be contaminated with oocysts of *C. cayetanensis*. Other factors for higher prevalence of cyclosporiasis with females can be due to less active immune system of females followed by the loss of

antibody through bleeding/menstruation and parturition. But, all the children were below 12 years so this may not be the reason in our case (Ghimire *et al.* 2008). Literature has shown that there is no sexual predilection (Chacin-Boralla *et al.* 2001). This insignificant result has clearly given significant message that there is no discrimination between sex of the person and chances of being infected by the parasites.

In the present study, the prevalence of cyclosporiasis was found highest in age group 5-8 (25.2%) but it was statistically insignificant ( $p=0.895$ ). In contrary to our research, the research conducted by Charles (1995) showed that higher prevalence of cyclosporiasis in age less than 18 months. But, our result was similar to the result conducted by Kimura *et al.* (2005). Many researchers throughout the world have also suggested that the children and elderly people are more vulnerable towards cyclosporiasis. This might be due to the low immunity power of these groups. Besides, young children play here and there, they don't bother much about hygienic practice, eat and drink whatever they get, so, their chances of acquiring parasitic infestation is very high. The current finding was similar to Sherchand and Sharma (2003) where cyclosporiasis was found highest in the age group 25-60 months (12%).

The occurrence of *C. cayetanensis* in symptomatic case (25.6%) was higher than asymptomatic case (20.7%) but it was found statistically insignificant ( $p=0.469$ ). The occurrence of *C. cayetanensis* in school going children without maintaining hygienic practice (28.0%) was higher than with maintaining hygienic practice (17.4%) but the result was found statistically insignificant ( $p=0.103$ ). The person maintaining good

hygiene is less vulnerable to the parasitic infestation than those not maintaining hygienic practice. But, current insignificant result might be due to the limitation of questionnaire.

The oocysts of *C. cayetanensis* must undergo sporulation as unsporulated oocysts are not infective to man. So the clean environment will certainly provide unfavorable condition for the sporulation of oocysts and there is less chance of being infected with it. This is due to the influx of people in the valley, unplanned housing, poor sewage systems, street flooding and environmental contamination with fecal matter. Chlorination is virtually ineffective in municipal water supply of Kathmandu due to the high level of contamination with organic matter. Oocysts of *C. cayetanensis* is resistant to chlorination compared with diarrheagenic bacteria, and can be present even in coliform free water. Therefore, it appears that water and vegetables plays a major role in transmitting *C. cayetanensis* infection in Nepal (Adhikari *et al.* 2006). Oocysts of *C. cayetanensis* have also been detected in sewage water, vegetables, and feces of animals and birds which may be an additional source of environmental contamination (Sherchand *et al.* 1999). But, the result of present study does not show any sort of relationship between cyclosporiasis and good hygiene practice, which may be due to small sample size, biasness during questionnaire filling, shyness of school going children, lack of proper knowledge etc. There was significant difference between detection of cyclospora before and after sporulation by modified acid fast staining technique ( $p < 0.001$ ). Highest association was between *C. cayetanensis* and *Cryptosporidium parvum* (67.3%). This result clearly demonstrates the high prevalence of *C. cayetanensis*.

Thus, cyclosporiasis is one of the most prevalent intestinal protozoal parasite. Effective control strategies as well as knowledge and proper hygienic education are necessary to combat the parasitic infection of this parasite.

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