**Cyclospora infection among the school children of Kathmandu, Nepal: prevalence and associated risk factors.**

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**Abstract**

**Background:** The intestinal coccidian protozoa *Cyclospora cayetanensis* has emerged as an important cause of parasitic diarrhea among the children living in developing countries. This study aimed to determine the prevalence of *Cyclospora* among the school children of Kathmandu with relation to various associated risk factors.

**Methodology:** A total of five hundred and seven stool samples from students between the age group 3-14 years, studying in 13 different schools of Kathmandu were collected during the study period (May- November, 2014) and processed in Public Health Research Laboratory, Institute of Medicine, Kathmandu, Nepal. A modified Acid Fast staining technique (Kinyoun’s method) was used to detect oocyst of *Cyclospora* from the formal-ether concentrated stool samples.

**Results:** *Cyclospora* was detected in 3.94% (20/507) of the stool samples examined. The prevalence was found to be highest among the students between the age group 3-5 years i.e. 10.15% (13/128), peaking during the rainy season (June-August). The detection rate of the parasite was found to be significantly higher (p<0.05) among the children presenting with diarrheal symptom, household keeping livestock(s) and consumers of raw vegetables/fruits with prevalence 10.57 % (11/104), 10.11% (9/89) and 7.25% (14/193) respectively.

**Conclusion:** Consumption of untreated drinking water, fresh produce (raw fruits/vegetables) without proper washing and livestock presence at home were found to be some of the predisposing factors for higher susceptibility of infection due to *Cyclospora*. This finding confirms a public-health issue with
potentially serious consequences whereby, children can be infected through the exposure to oocyst contaminated food, water and get ill.

**Keywords:** *Cyclospora cayetanensis*, School Children, Prevalence and Kathmandu.

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1. Introduction

The coccidian parasite *Cyclospora cayetanensis* has been recognized as a new cause of prolonged diarrhea among the children in developing countries[1, 2]. The organism produces environmentally resistant oocysts, which are excreted in the feces of the infected individuals[3]. The major risk factors associated with the transmission of the parasite have been identified as the consumption of oocysts contaminated water and contaminated food produce[3-6]. Direct person-to-person transmission is unlikely because the immature oocysts that are shed in feces require days to weeks in the environment to become infective[7]. Patients normally report symptoms of nausea, vomiting, anorexia leading to weight loss, abdominal cramping and pain with increased gas, flatulence, fatigue and watery diarrhea[8-10]. The modified acid fast staining technique and hot safranin staining technique can be used to detect the oocysts of the parasite from the stool samples[11, 12]. Children with lower age group are more susceptible to infection with *Cyclospora* due to less developed immune system and poor personal hygiene[1]. Less data is available on the prevalence of *Cyclospora* among the school children in Nepal[4, 13-16]. To the best our knowledge, this is one of the few studies carried out to assess the prevalence of *Cyclospora* infection under endemic condition with primary focus on school children. This study is expected to elucidate
a deeper insight regarding the prevalence of *Cyclospora* among apparently healthy school children thereby, encouraging more study in immunocompetent hosts.
2. Materials and methods

2.1 Study site and study population

It was conducted in Kathmandu district, the capital city of Nepal from May- November 2014. Kathmandu is situated at an average elevation of 1,400 meters (4,600 ft) above the sea level and has an approximate population density of 4,416 per km square within the total area of 50.67 square kilometers (19.56 sq mi).

The study population were school going children in between the age group 3-14 years. Thirteen different schools within Kathmandu district were selected randomly from among the total schools within the city for the study purpose. A total of five hundred and seven stool samples were collected from children of the selected schools during the school hour.

2.2. Study design, Sample collection and transportation

It is a cross-sectional type study, done among the school children of Kathmandu. Non-probability sampling technique was adopted for the study. Fresh stool specimens were collected in a clean, dry and screw capped leak proof plastic container. Students were advised to bring about 30 grams or nearly 30 ml of stool sample avoiding contamination with urine, water and other substances. Single stool specimen was collected from each student which was then transported maintaining cold chain, as soon as possible to Public Health Research Laboratory, Institute of medicine, Kathmandu for laboratory examination.

2.3. Fecal examination and microscopy

Macroscopic examination was done by observing the stool sample with naked eyes and the information obtained namely: consistency, presence/absence of mucus and blood were recorded before the microscopy of the samples. The fecal samples were then processed by
direct-smear technique, in both normal saline solution and 1% iodine solution after formalin ether concentration for the identification of protozoa and helminthes[17, 18]. Subjects whose fecal samples were suspected by direct wet mount to contain *Cyclospora*-liked organisms were selected for further laboratory investigation with a modified Acid Fast staining technique (Kinyoun’s method) for the identification of oocysts of *Cyclospora* through microscopy[11, 18]. A commercially available staining reagent (HI Media K005 for ZN Acid Fast Stains) was used for staining of the stool samples. The morphology of the stained oocysts was then observed and dimension measured using cell sensation software version 1.12 for DP73 camera installed to Olympus BX53 microscope used for the microscopy. Positive control slide provided by CDC was observed side by side during the microscopy of stool samples to prevent any discrepancy in the identification. No additional confirmatory testing such as *Cyclospora* direct fluorescent antibody staining was performed for the purpose of this study.

2.4. Data Analysis

The data obtained were entered in MS Excel version 2007 and analyzed using SPSS version 16 for window program. Two tailed Pearson's Chi-square test was used to test significance of attributes between the study variables. A value of \( \alpha < 0.05 \) was assumed where ever applicable and 95% confidence interval along with the exact p-values was represented. The p-value < 0.05 was considered statistically significant.

2.5 Ethical considerations

This study was approved by Institutional review board, Institute of medicine, research department, Kathmandu, Nepal [Ref: 282(6-11-E)]\(^2\). Prior to sample collection, a questionnaire on various demographic, socio-economic and health related parameters was filled with the help of
teachers and in case of minors it was sent to parents in order to gather information related to
subject after taking written/oral informed consent from teachers and parents as per the necessity.

3. Results

Among the total 507 participants, 236 were male and 271 were female. *Cyclospora* oocysts
were detected in 3.94% (20/507) of the children examined (8 males and 12 females, gender
ratio 1:1.5). *Cyclospora, Giardia* co-infection was seen in 35% (7/20) and *Cyclospora, Cryptosporidium* co-infection in 5% (1/20) of total *Cyclospora* positive cases.

**Age-Wise distribution of the children with *Cyclospora* infection:**

Age-wise distribution of the *Cyclospora* infected cases showed the highest prevalence of
10.15% (13/128) among children between the age-group 3-5 years, followed by the children
between the age-group 6-8 years i.e., 2.53% (4/158) as shown in figure.1.

**Month-wise detection pattern of *Cyclospora* oocysts:**

The monthly distribution pattern of *Cyclospora* infection showed the highest infection rate
during the month of August i.e., 9.8% (9/97) followed by July i.e., 9% (9/100) and June i.e.,
2.04 % (2/98) with no infection detected during the month of May, September, October and
November (figure 2).

**Clinical characteristics of children with *Cyclospora* infection:**

Out of the total 507 participants, 20.51% (104/507) were symptomatic and remaining 79.49%
(403/507) were asymptomatic. Among the 20 cases detected positive for *Cyclospora* oocyst
in the stool sample, 11 had diarrhea like symptoms compared to 9 without diarrhea related
symptoms (table 1). The diarrheal episode among the cases ranged from 5 days to 9 days.
Mode of drinking water consumption and *Cyclospora* infection:

Out of the total participants, 1.2 % (3/244) of the children consuming treated water were found to be positive for *Cyclospora* oocyst compared to 6.46 % (17/263) of them consuming untreated water for drinking purpose (table 2).

Livestock(s) presence at home and *Cyclospora* infection:

Among the total 20 cases detected positive for *Cyclospora* oocysts, family of 9 children had reared livestock(s) at their home compared to 11 families who didn’t have any form of livestock(s) reared at home (table 3).

Fresh produce (Raw fruits/Vegetables) consumption practice among the children and *Cyclospora* infection:

As far as fresh produce consumption practice is concerned *Cyclospora* infection was found to be present in 7.25% (14/193) of the children consuming raw vegetables/fruits without proper washing compared to 1.91% (6/314) of those not consuming raw vegetables/fruits (table 4).
Table 1: Distribution of *Cyclospora* infection among the children with and without diarrhea related symptoms.

<table>
<thead>
<tr>
<th>Case type</th>
<th>Cyclospora positive</th>
<th>Cyclospora negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of Diarrhea related symptoms</td>
<td>11 (10.57%)</td>
<td>93</td>
<td>104</td>
</tr>
<tr>
<td>Absence of diarrhea related symptoms</td>
<td>9 (2.23%)</td>
<td>394</td>
<td>403</td>
</tr>
<tr>
<td>Grand Total</td>
<td>20 (3.94%)</td>
<td>487</td>
<td>507</td>
</tr>
</tbody>
</table>

$\chi^2_{cal} = 15.18, \ p = 9.7 \times 10^{-5}$

Table 2: Distribution of *Cyclospora* infection on the basis of mode of drinking water consumption.

<table>
<thead>
<tr>
<th>Mode of water consumption</th>
<th>Cyclospora positive</th>
<th>Cyclospora negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumers of treated water</td>
<td>3 (1.22%)</td>
<td>241</td>
<td>244</td>
</tr>
<tr>
<td>Consumers of untreated water</td>
<td>17 (6.46%)</td>
<td>246</td>
<td>263</td>
</tr>
<tr>
<td>Grand Total</td>
<td>20 (3.94%)</td>
<td>487</td>
<td>507</td>
</tr>
</tbody>
</table>

$\chi^2_{cal} = 9.152, \ p = 0.002484$

Table 3: Distribution of *Cyclospora* infection on the basis of livestock presence at home.

<table>
<thead>
<tr>
<th>Livestock</th>
<th>Cyclospora positive</th>
<th>Cyclospora negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Livestock presence at home</td>
<td>9 (10.11%)</td>
<td>80</td>
<td>89</td>
</tr>
<tr>
<td>Livestock absence at home</td>
<td>11 (2.63%)</td>
<td>407</td>
<td>418</td>
</tr>
<tr>
<td>Grand Total</td>
<td>20</td>
<td>487</td>
<td>507</td>
</tr>
</tbody>
</table>

$\chi^2_{cal} = 10.837, \ p = 0.000995$

Table 4: Distribution of *Cyclospora* infection on the basis of fresh produce (raw vegetable/fruits) consumption habit.

<table>
<thead>
<tr>
<th>Fresh produce consumption</th>
<th>Cyclospora positive</th>
<th>Cyclospora negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw vegetables/fruits consumers</td>
<td>14 (7.25%)</td>
<td>179</td>
<td>193</td>
</tr>
<tr>
<td>Non consumers of raw vegetables/fruits</td>
<td>6 (1.91%)</td>
<td>308</td>
<td>314</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>487</td>
<td>507</td>
</tr>
</tbody>
</table>

$\chi^2_{cal} = 9.0057, \ p = 0.002691$
4. Discussion

*Cyclospora* infection is generally associated with diarrhea among children in developing countries while traveler’s diarrhea, food and waterborne outbreaks usually occur in developed countries[1, 19]. It is endemic to Nepal, remaining as asymptomatic form with annual reported cases of cyclosporiasis as high as 40%[20]. In context of Nepal poor hygiene of children and lack of proper sanitary practice favor the infection of *Cyclospora*, which has been the case in our study as well. The infection rate of *Cyclospora* in our study is lower compared to another study conducted among the diarrheal children in Nepal which reported a higher prevalence rate of 7.9 %[16].The prevalence rate of 2.22 % of *Cyclospora* infection which is similar to our finding was reported in a study conducted among the school children of Thailand[21].The highest risk of infection of *Cyclospora* occurs in the first five years of life[1, 22-25], which is in concordance with our finding of higher infection rate among the children between the age group 3-5 years. Diarrhea is one of the important symptoms of cyclosporiasis[8]. Parameters like diarrheal complain and frequency, abdominal pain, vomiting and fever were taken as indices to determine the symptomatic cases and asymptomatic cases, accessed through questionnaire during the study. The higher detection rate of *Cyclospora* oocysts among children presenting with diarrheal symptoms in our study was statistically significant (p<0.05). *Cyclospora* infection markedly increases during the warm and rainy season which reflects the increased oocyst contamination of surface and domestic water supplies [4, 5, 14, 26]. In our study the prevalence of *Cyclospora* infection was found to be higher during the month of June, July and August which mark the peak of monsoon in this part of the world. This finding is in harmony with the reports of previous studies conducted in Nepal [4, 14, 27]. Waterborne oocysts are a common source of infection for *Cyclospora* [3]. Oocysts of *Cyclospora* have been reported to be resistant to chlorination[28, 29]. We considered water consumed after implementing any of these
methods including roll boiling for 5 minutes, filtration using conventional ceramic candle filter (pore size 1-5 um), Euroguard™ and bottled mineral water as treated water. The higher detection rate of *Cyclospora* oocysts among the children consuming untreated water in our study was found to be statistically significant (p<0.05). In context of Kathmandu, municipal supply of tap water is the major source of drinking water. However, there is a flaw in the supply system. Since, the sewage drainage and water supply pipeline run parallel to each other in a very close proximity, the water supply gets contaminated through the seepage into the pipes from sewage[16]. Thus, the fecal contamination of drinking water could be the reason behind higher prevalence of *Cyclospora* among the children drinking municipal tap water without adopting any measure of treatment. Meanwhile, role of animals as a natural reservoir of *C. cayetanensis* is uncertain but of increasing concern[2]. Domestic animals have been implicated as a risk factor for *Cyclospora* infection[25, 30]. Oocysts resembling those of *C. cayetanensis* have been described in several animals, including chickens, ducks, mice, rats, dogs and primates from different parts of Nepal[4]. This study endeavored to find out the relation between livestock(s) rearing and the risk of infection with *Cyclospora*. A significant relation (p<0.05) between *Cyclospora* infection among the children and presence of livestock(s) at home was seen in this study. Contamination of food has long been proposed as a possible route for transmission of *Cyclospora* [10]. The detection rate of *Cyclospora* oocysts was significantly higher (p<0.05) among the students who consumed fresh produce (raw vegetables/fruits) without proper washing. In Kathmandu use of human excreta as manure in crops is practiced. Likewise, open defecation by children in field and free grazing of cattle in riverside is seen. These practices can lead to contamination of river water which in turn is used to irrigate fields, wash the harvest. Thus, the contamination of vegetables by the oocysts occurs resulting in the higher infection rate among consumers of raw vegetables and fruits.
Furthermore, the oocysts of *Cyclospora* are shed unsporulated which can remain in environment in harsh conditions for a longer time maintaining infectivity[3].

5. Conclusion

The result obtained from this study implicates *Cyclospora* to be an important cause of childhood diarrhea, associated with consumption of untreated drinking water, fresh produce without proper washing and livestock presence at home. Provision for supply of safe drinking water and health education could be some of the measures of practical arbitration for controlling the infection of *Cyclospora* in the developing countries.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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References


