The Seasonal Outbreaks of *Cyclospora* and *Cryptosporidium* in Kathmandu, Nepal.

Ghimire TR[a], Mishra PN[b], Sherchand JB[c]

Abstract

Introduction *Cryptosporidium* and *Cyclospora* are obligate, intracellular, protozoan pathogens that cause diarrhea in children. From 2002 to 2004, we conducted study of people in two hospitals, one private clinic, one community and vegetables in 4 markets and water from different water sources in Kathmandu.

Objectives The study aim to determine the prevalence of and identify the seasonality of these two coccidian parasites.

Methods A total of good stool samples between Janwary 2002 to December 2004 from different health care centre and were examined using standard formalin ethyl acetate concentrated method as well as modified acid fast stain. Infection of Cryptosporidium distribution were analysed.

Results *Cyclospora* was identified in 8.2 percent and *Cryptosporidium* was in 11.3 percentof 9000 stool samples. In 2002, prevalence of *Cyclospora* in May, June, July, August, September and October was 9.3 percent, 17.5 percent, 18.6 percent, 6.2 percent, 2.0 percent, 1.6 percent and prevalence of *Cryptosporidium* was 17.5 percent, 7.8 percent, 7.1 percent, 9.0 percent, 9.2 percent and 4.2 percent In 2003, 0.6 percent, 1.8 percent, 35.2 percent, 9.7 percent, 7.6 percent, 7.5 percent, respectively for *Cyclospora* and this prevalence was 19.2 percent, 15.3 percent, 11.9 percent, 11.6 percent, 12.6 percent and 30.8 percent respectively for *Cryptosporidium*, and in 2004 2.3 percent, 20.6 percent, 14.3 percent, 9.1 percent, 0.5 percent and 0.1 percent respectively for *Cyclospora*, 8.4 percent, 7.9 percent, 9.8 percent, 15.7 percent, 13.3 percent and 13.0 percent respectively for *Cryptosporidium*.

Conclusion The detection of both parasites in water sources and in some vegetables proves that water and food are important vehicles for these coccidian transmissions. The molecular studies for these two parasites in waste water and different vegetables should be made in future for the confirmation of water and food borne transmission.

Key words *Cryptosporidium, Cyclospora, Nepal, Prevalence*

Introduction

Cryptosporidium parvum and *Cyclospora cayetanensis* are obligate, intracellular, coccidian protozoan pathogens that cause prolonged diarrhea during childhood[1,2]. The same modified acid–fast and hot-safranin staining techniques can be used to detect both organisms[3]. *Cryptosporidium* is zoonotic[4,5], while human isolates may not be capable of infecting non-human primates[6]. Water has been implicated in outbreaks of these both parasites[7,8,9,10,11]. In the majority of countries, including developing regions, the lack of surveillance, and limited availability of appropriate diagnostic tests have hindered public health efforts to prevent and control outbreaks caused by these two waterborne protozoan pathogens[12]. Both show a high rate of infection with a distinct seasonality in developing countries[9,13,14,15]. Both micro-organisms are attracting more attention as important public health hazards[2,12]. This study presents on seasonal outbreaks of *Cyclospora* and *Cryptosporidium* in vegetable markets, water resources and diarrheal an non-diarrheal

Corresponding Author: Tirth Raj Ghimire, **E-mail:** ghimiretr@hotmail.com/ghimiretr7@yahoo.com [a]Bagmati Modern College, Department of Biology, Bhagwatibahal, Naxal, Kathmandu, Nepal. [b]Central Department of Zoology (Parasitology), Tribhuvan University, Kathmandu, Nepal. [c]Department of Microbiology/Parasitology Health Research Laboratory, Institute of Medicine, Tribhuvan University,

people of Kathmandu valley and ensures the endemicity of the two pathogens.

Methods

Stool sampling

The study was conducted from January 2002 to December 2004 in Kathmandu valley, Nepal. A total of 9000 stools were collected from health care units and community: 5000 from Kanti Children's hospital, 2000 from Sukra Raj Tropical and Infectious Disease Hospital, 1000 from private clinics and 1000 from Kirtipur community. In each locality, we screened stool samples and recorded about age, sex and current diarrheal status (defined as 3 or more loose or watery stools in 24 hours) and other clinical symptoms.

Laboratory methods

Stool specimens were processed using a standard formalin-ethyl acetate concentration method and examined by two methods: direct light microscopy and stool smear stained with modified acid fast stain. Measurements of oocysts were done with ocular micrometer to distinguish the oocysts of *Cyclospora cayetanensis* from the oocysts of *Cryptosporidium parvum* and *Isospora belli*. All stool samples were preserved in 2.5 percent potassium dichromate solution. All the *Cyclospora positive* specimens were stored at an ambient temperature (approximately 23 degree centigrade) and were examined at regular intervals over a period of 2 weeks starting from the time of excretion[3]. We categorized the number of parasites in each specimen: "many" was equivalent to 10 or more oocysts, "moderate" to 3 to 9 oocysts and "few" to 2 or fewer oocysts per 10 oil immersion (1000 X) fields[16].

Samples of water and green leafy vegetables

From January 2002 to December 2004, samples of water and green-leafy vegetables were collected every month from various areas of Kathmandu valley to determine the possible sources of infection. The leaves of radishes, cauliflowers, green onions, cabbages, mustard leaves and carrots were washed in distilled water and the washings were kept in each test tube. We sampled 30 washings of each vegetable from four markets. Similarly, 100 water samples were taken in a test tube from each of sewage, pond, well, Bagmati and Bishnumati rivers, and municipal taps in Kirtipur community. Then the former washings and water of the latter sources were centrifuged and the sediments were examined microscopically. Then the recovery of oocysts was noted again by bisporulation assay from each microscopic positive sample of *Cyclospora cayetanensis*.

Statistical analyses

The results were analyzed by using the chi-square test (χ^2-test) at less than 0.05 and 0.001 significant level so that they were determined to have statistically significant at these level.

Results

Of the total 9000 stool samples examined ,from all age group of people , *Cyclospora were* dentified in 739(8.2%) and *Cryptosporidium* infection was in 1017(11.3%) samples. The stools collected were from a 10-day infant to 94-year old man. The lowest age of *Cyclospora infected* infant was of 12 days and the age of *Cryptosporidium* infected infant was of 2 months, while the highest age of *Cyclospora* infected person was of 87 years and that of *Cryptosporidium* was 64 of age.

Table 1 reveals that out of 6357 diarrheal patients, *Cyclospora* and *Cryptosporidium* were in 523(8.2%) and 964(15.2%) patients respectively. Similarly, out of total 2643 non-diarrheal patients former ratios were in 216(8.2%) and 53(2.00%) respectively. Both diarheal (16.0%, 193 out of 1205) and non-diarrheal (24.6%, 79 out of 321) children showed the highest prevalence rate of *Cyclospora* in 1 to 4 years age groups. Presence of *Cryptosporidium* was highest (20.6%, 517 out of 2511) in 5-9 years age grouped dearrheal children and (34.9%, 29 out of 83) children in less than 1 year aged non-dearrheal children.

Presence of *Cyclospora with* the age groups of diarrheal and non-dearrheal people was statistically significant (χ^2=62.7, $p<$ 0.001). Presence of *Cryptosporidium* with the age groups of diarrheal and non-dearrheal patients was statistically significant ($\chi2$= 502.4, p<0.001). Similarly, presence of *Cyclospora and Cryptosporidium* in these patients was significant (χ^2= 675.3, p< 0.001).

In this study, *Cyclospora* infection was found highest in the 1-4 years of age groups (17.8%, 272 out of 1526 children), where as in the age groups 10-15 years, *Cryptosporidium* infection was the highest (16.4%, 201 out of 1228 children).

Prevalence of *Cyclospora* and *Cryptosporidium* in acute diarrhea was 19.8percent (149 out of 753 people) and 80.2 percent (604 out of 753 people) respectively. And in chronic diarrhea, it was 51.0percent (374 out of 734 people) and 49.0 percent (360 out of 734 people) respectively. Presence of these both parasites in acute and chronic diarrheal patients was statistically significant (χ^2= 158.3, p< 0.001).

Presence of *Cyclospora* oocysts in the frequency of "many", "moderate", and "few" in the prevalence of 59.7 percent, 36.1 percent, and 4.2 percent in the diarrheal stools and 3.2 percent, 15.3 percent and 81.5 percent in the non-diarrheal stools were observed respectively. Frequency was significantly associated with presence of *Cyclospora in* diarrheal and non-diarrheal stools (χ^2=475.5, p< 0.001). Similarly, presence of *Cryptosporidium* oocysts in the former ratio of 78.3 percent, 15.6 percent and 6.1 percent in diarrheal stools and 3.8 percent, 3.8 percent and 92.5 percent in non-diarrheal stools were observed respectively. Frequency was significantly associated with presence of *Cryptosporidium* in diarrheal and non-diarrheal stools (χ^2=395.4, p< 0.001). Presence of the both *Cyclospora and Cryptosporidium* in diarrheal and non-diarrheal stools was significant with number of oocysts (χ^2=1076.59, p< 0.001).

In 2002, prevalence of *Cyclospora* in May, June, July, August, September and October was 9.3 percent, 17.5 percent, 18.6 percent, 6.2 percent, 2.0 percent, 1.6 percent and no positive sample was detected in February, March, April and this result was statistically significant(χ^2=532.6, p<0.001). Similarly, in 2002, the prevalence of *Cryptosporidium,* in these months, was 17.5 percent, 7.8 percent, 7.1 percent, 9.0 percent, 9.2 percent and 4.2 percent respectively and during this year, positive samples were detected more or less frequently with statistically significant (χ^2= 150.8, p< 0.001). Similarly, Month-wise prevalence of *Cyclospora* and *Cryptosporidium* was statistically significant in 2002(χ^2= 81.71, p< 0.001).

In 2003, the prevalence of *Cyclospora,* in the above months, was 0.6 percent, 1.8 percent, 35.2 percent, 9.7 percent, 7.6 percent, 7.5 percent, respectively with absence in January and it was statistically significant (χ^2=1144.0, p< 0.001). In this year, the prevalence of *Cryptosporidium* was 19.2 percent, 15.3 percent, 11.9 percent, 11.6 percent, 12.6 percent and 30.8 percent respectively with more or less frequent during all months of the year. It was statistically significant (χ^2= 223.7, p< 0.001). Month-wise prevalence of *Cyclospora* and *Cryptosporidium* infection was statistically significant in 2003 (χ^2=224.0, p< 0.001).

In 2004, the prevalence of *Cyclospora,* in the above months, was 2.3 percent, 20.6 percent, 14.3 percent, 9.1 percent, 0.5 percent and 0.1 percent respectively with the absence in February, March and April and it was statistically significant (χ^2=606.1, p<0.001). In this year, the prevalence of *Cryptosporidium* obtained was 8.4 percent, 7.9 percent, 9.8 percent, 15.7 percent, 13.3 percent and 13.0 percent respectively with the more or less prevalence during all months of the year. It was statistically significant (χ^2=289.5, p<0.001). Month-wise prevalence of *Cyclospora* and *Cryptosporidium* was statistically significant (χ^2=143.6, p<0.001).

The year-wise prevalence of *Cyclospora* and *Cryptosporidium* was 8.2 percent (211 out of 2573) and 7.7 percent (199 out of 2573) in 2002, 9.8 percent (304 out of 3115) and 14.7 percent (458 out of 3115) in 2003 and 6.8 percent (224 out of 3312) and 10.9 percent (360 out of 3312) in 2004 respectively.

Only during the months of June and September of 2002, 2003 and 2004, the *Cyclospora* and *Cryptosporidium* were detected in 3.3percent (1 out of 30) and 16.7 percent (5 out of 30) in washings of radishes, 3.3 percent of (1 out of 30) and 0.0 percent (0 out of 30) in washings of cauliflower and 10.0 percent (3 out of 30) 13.3 percent (4 out of 30) in washings of cabbage, 6.7 percent (2 out of 30) and 3.3 percent (1 out of 30) in washings of mustard leaves respectively with the frequency of oocysts in "few" category. The presence of *Cyclospora* in different vegetables was not significant (χ^2=2.0, p>0.05) whereas, that of *Cryptosporidium* was significant (χ^2=11.0, p<0.05). The total prevalence of *Cyclospora* and *Cryptosporidium* was 5.3 percent (8 out of 150 vegetables) and 6.7 percent (10 out of 150 vegetables) respectively with no significant difference (χ^2=4.97, p> 0.05).

The prevalence of *Cyclospora* and *Cryptosporidium* was 4.0 percent (4 out of 100) and 13.0 percent (13 out of 100) in sewage water, 2.0 percent (2 out of 100) and 9.0 percent (9 out of 100) in river water, and 0 percent (0 out of 100) in pond water, 0 percent (0 out of 100) in well water and 1.0 percent (1 out of 100) and 0.0 percent (0 out of 100) in municipal water supplies respectively from May to September of 2002, 2003 and 2004 with statistically no significant (χ^2=1.8, p> 0.05).

Hence the total prevalence of *Cyclospora* and *Cryptosporidium* in different water sources was 1.4 percent (7 out of 500) and 4.4 percent (22 out of 500) respectively. There was statistically no significant of water sources (χ^2=2.0, p>0.05) with *Cyclospora but,* was statistically significant of water sources (χ^2=12.1, p< 0.05) with *Cryptosporidium.*

From January 2002 to December 2004, we followed 31 *Cyclospora* infected diarrheal cases and 17 *Cryptosporidium* infected diarrheal cases each months.

Out of 31 cases, there were ten nursery outbreaks reported; 3 school outbreaks (16 positive cases), 4 school trip outbreaks (8 positive cases) and 3 religious ceremony outbreaks (7 positive cases). Out of 17 cases, we reported eight nursery outbreaks; 2 school outbreaks

(6 positive cases), 1 school function outbreak (2 positive cases) and 5 religious ceremony outbreaks (9 positive cases). Both of these results were from May to September of study years and the pathogens were found solely with "many" category.

TABLE 1: Age-wise prevalence of *Cyclospora cayetanensis* and *Cryptosporidium* in nine thousand people, in Kathmandu valley, Nepal, from January 2002 to December 2004*.

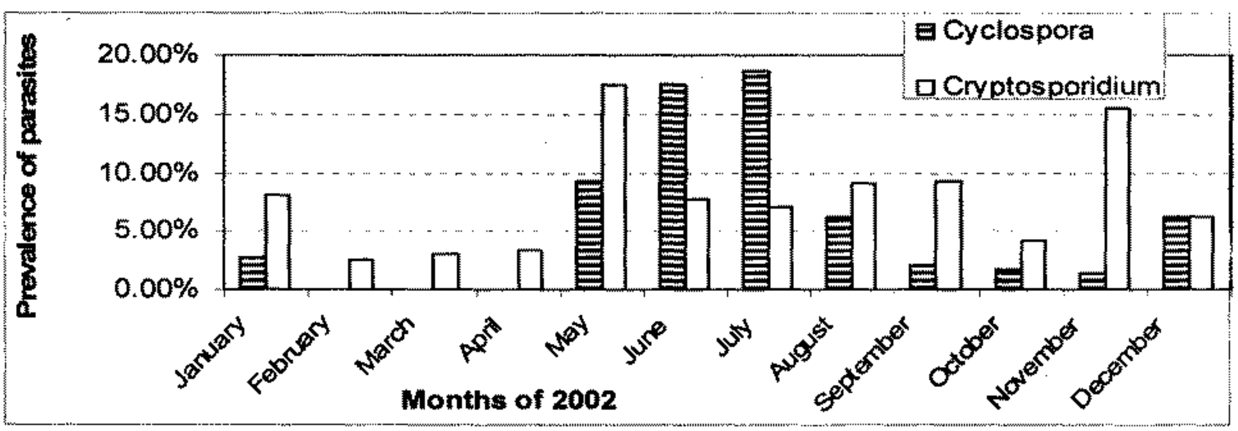
AGE (yrs.)	Cyclospora**				Cryptosporidium ***			
	With diarrhea		Without diarrhea		With diarrhea		Without diarrhea	
<1	4/221	1.8	7/83	8.4	17/221	7.7	29/83	34.9
1-4	193/1205	16.0	79/321	24.6	201/1205	16.7	10/321	3.1
5-9	279/2511	11.1	58/948	6.1	517/2511	20.6	6/948	0.6
10-15	35/1019	3.4	43/209	20.6	198/1019	19.4	3/209	1.4
16-29	3/875	0.3	15/754	2.0	2/875	0.2	1/754	0.1
30-39	4/156	2.6	4/228	1.8	1/156	1.8	1/228	0.4
40-49	1/161	0.6	3/36	8.3	3/161	1.9	1/36	2.8
>50	4/209	1.9	7/64	10.9	25/209	12.0	2/64	3.1
All Ages	523/6357	8.2	216/2643	8.2	964/6357	15.2	53/2643	2.0

*Values are the number positive/number tested (% positive).

**$p < 0.001$ (Chi-square test) for the comparison of prevalence of *Cyclospora cayetanensis* among those with and without diarrhea.

***$p < 0.001$ (Chi-square test) for the comparison of prevalence of *Cryptosporidium* among those with and without diarrhea.

Figure 1: Prevalence of *Cyclospora and *Cryptosporidium*** in 2573 people of Kathmandu valley, Nepal from January 2002 To December 2002.*****

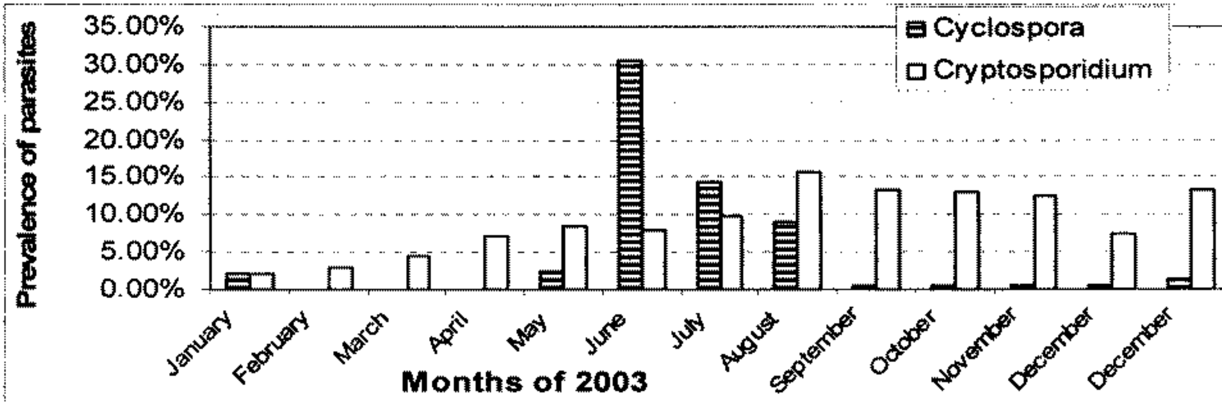


*This result was statistically significant (χ^2=532.6, p<0.001).

**This result was statistically significant (χ^2= 150.8, p< 0.001).

***Month-wise prevalence of *Cyclospora* and *Cryptosporidium* was statistically significant in 2002 (χ^2= 81.71, p< 0.001).

Figure 2: prevalence of *Cyclospora and *Cryptosporidium*** in 3115 people of Kathmandu valley, Nepal from 2003 January to December 2003.*****

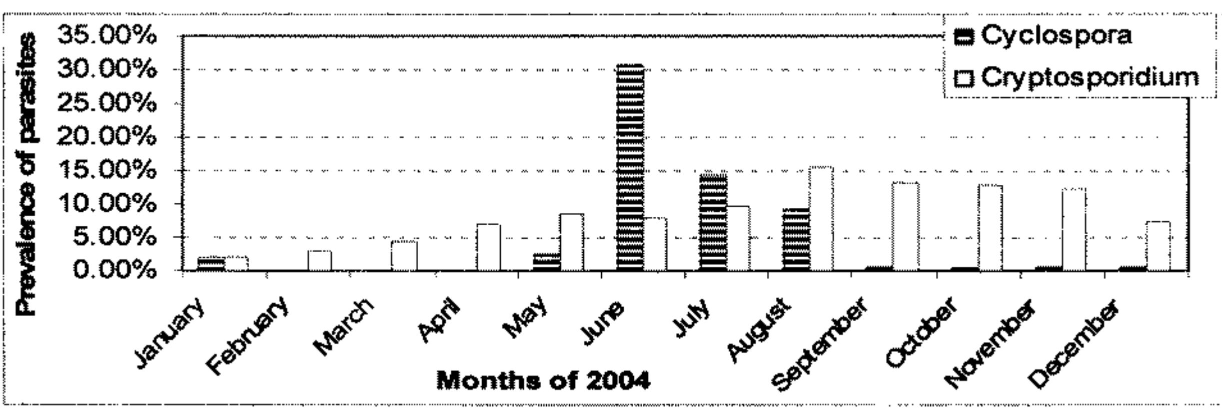


*It was statistically significant (χ^2=1144.0, p< 0.001)

**It was statistically significant (χ^2= 223.7, p< 0.001).

***Month-wise prevalence of *Cyclospora* and *Cryptosporidium* infection was statistically significant in 2003 (χ^2=224.0, p< 0.001).

Figure 3: Prevalence of *Cyclospora and *Cryptosporidium*** in 3312 people of Kathmandu valley, Nepal from January 2004 to December 2004***.**



Discussion

This is the first study to show the upgrade of epidemiologic relationship between *Cyclospora* and *Cryptosporidium* in Nepal though a similar study had been already conducted in Guatemala[16].

In the present study, the prevalence of *Cyclospora* 8.2 percent is lower than that reported in previous studies in Nepal[17,18] and approximately similar to the results of other studies[19-21]. The lower prevalence of *Cyclospora* in our study might be due to either we had not performed molecular study or some of the patients were from community during all months of the year.

The prevalence rate 11.3 percent of *Cryptosporidium* in the present study was similar to that of other studies from Cuba[22], Ethiopia[23] and developing countries in Asia, Africa, and Latin America excluding outbreak investigation[24,25,26]. This result is lower than that reported from Nepal[27] and India[28] which might be due to the methodological difference. This result is much higher than that reported from Jordan[29], Thailand[29] and Guatemala[16] It might be due to zoonotic infection[4,5,30] because Nepal is an agricultural country and the patients examined in this study might have a high contact with carriers of *C. parvum* such as cattle which is more common in this region (Kirtipur community).

Table1 shows that *Cryptosporidium*, in common with enteric pathogens such as rotavirus[31], affects children most in the first 2 years of life. *Cryptosporidium* is easily spread person-to-person among infants and young children in crowed home or day-care environments[32,33]. But direct person-to-person spread of *Cyclospora is* unlikely. Infants have lower risk of exposure to contaminated water and food than older children or they obtain passive immunity coming from breast milk. They are also less likely to eat fresh, raw produce that may be a source of sporulated *Cyclospora* oocysts[13]. These views support the presence of *Cyclospora* more frequently among somewhat–older children and that of *Cryptosporidium* in infants and children most in the first 2 years of life. However, the *Cyclospora* infection in a 12-day infant in this study might be explained on the basis of poor health, less infant care, low nutritional value and low socio-economic status of the Nepalese infants.

In the diarrheal stool examination, the frequency of "oocysts" of both *Cyclospora* and *Cryptosporidium* are in "many" category, whereas in the non-diarrheal stools, oocysts of the former parasites are in few category. It suggests that the infection with these both parasites with increased frequency and severity in diarhheal patients shows that immune mechanisms effectively keep parasite numbers low in most normal persons. Human studies indicate that intestinal antibodies can reduce parasite numbers[34,35,36,37,38]. The high prevalence of the *Cyclospora* and *Cryptosporidium* infections in the infants, young children and adults of >50 years of age groups of an endemic area[17,18] like Kathmandu valley suggests the possible presence of protective immunity in immunocompetent–persons[39].

In this study, a stronger association of *Cryptosporidium* with diarrheal illness was found as that of other studies[40,41]. However, chronicity of diarrhea is the symptom found in *Cyclospora infection* than that of *Cryptosporidium.* Presence of both organisms in non-diarrheal stools suggests that both of these commonly cause infection in the absence of diarrhea[6,40,42].

Cyclosporiasis appears to be seasonal with the peak incidence during the rainy seasons from April to June in Peru and May to September in Nepal[9,17,18,42,43]. The data so far suggest that the seasonality of *Cyclospora* in Kathmandu city is similar to that in Guatemala, at approximately the same altitude (1200-1500 meter) above the sea level[9,43]. Similar results of seasonality can be seen in the figure 1, 2 and 3 that show the aggregation of the both parasites during May to September, the rainy season of Nepal.

Cryptosporidiosis is more commonly seen during the warm rainy season, which probably reflects the increased oocysts contamination of surface and domestic water supplies and heavy seasonal rains[28,44] and high prevalence rates similar to our study during the wet seasons were 33-43 percent in the United States[45], 11 percent in Mexico City[46], 2-22 percent in England and Wales[47]. A few per cent of infection in cold climates might be explained on the basis of high levels of cryptosporidiosis, 33 percent, in the dry season in Lima, Peru[48], 22-27 percent, in the United States[45] and 8.3percent in Guatemala city[49] and spring and autumn peaks[50,51,52] which support the present study. This might be related to excretion patterns in animals such as cattle and sheep and possibly to farming practices such as sludge spreading as cryptosporidiosis is a zoonotic infection[4,5,30,53].

Though both parasites showed purely seasonal in the present study, a few percent of persons showed infection in the cold climates. This might be due to either irregular visit of some untreated chronic parasitosis patients suffered from protracted diarrhea to test the stool or usual contact with infected animals for Cryptosporidiosis.

In the present study, detection of *Cyclospora in* the sewage, river and municipal water proves that water is an important vehicle for *Cyclospora* transmission. The water and sewage contaminated with *Cyclospora have* previously been identified as risk factors for infection in multiple countries in both outbreak and non-outbreak situation[7-9,17,54-56]. One of the most important river and sewage pollution is from the contact of waste disposal deposits in Nepal[18]. The presence of in municipal water might be due to broken and unscientific water pipe. In Kathmandu, municipal water supply is contaminated through seepage of water from sewage water[17].

The epidemiology of cryptosporidiosis is complex, due to the existence of multiple transmission routes, including anthroponotic and zoonotic transmission, as well as waterborne and food borne transmission[57]. Outbreaks due to waterborne infection have been confirmed[58-61]. The presence of *Cryptosporidium* oocysts in the present study suggests that we can't rule out the waterborne infection of cryptosporidiosis in Nepal.

Contaminated food has long been proposed as a possible route for transmissions of *Cyclospora*[13,62]. Vegetables are suspicious since they are often ingested raw or undercooked. They are easily contaminated and provide organism with an optimal environment for survival prior to host ingestion. In Nepal, one can see the fecal disposing people, especially by the children, near the road side or along river side at night time. Similarly people release cattle freely in these areas. These may contaminate the river water and sewage. In rainy season, the seepage of water from these sources may contaminate vegetables either when they are freely kept in soil for selling or when they are in the fields before just before harvesting. Besides, Fertilization of plants with human waste or indirectly via indirectly via contaminated water used for crop irrigation and to freshen produce could lead to contamination of vegetables with *Cyclospora* and *Cryptosporidium*. Vegetables in the markets are dipped and rinsed into highly contaminated water of small ponds or rivers in order to wash and clean it but washing vegetables does not completely remove *Cryptosporidium* and *Cyclospora* oocysts[13]. A common source of infection appears to be contaminated water[63] and transmission rates seem to peak between April and September[63,64]. That is why waterborne and foodborne transmission may cause outbreaks of *Cyclospora* and *Cryptosporidium* mostly in rainy season in Nepal.

The constant presence of *Cyclospora* and *Cryptosporidium* in Nepal might be explained on the basis of the following points: a) It is likely that these are under-diagnosed because clinicians fail to consider the diagnosis in patients with diarrheal disease (particularly immunocompetent adults and children). As result clinicians do not request stool analysis for these parasites, a test not normally included in routine stool analysis[65]. b) Acid-fast staining and ocular-micrometer are not used to diagnose these parasites. c) People do not normally use medicines for both of these parasites. d) Both of these are fecalborne, waterborne, foodborne, soilborne and *Cryptosporidium* alone is zoonotic and these means are easily created in Nepalese environments.

Detailed histopathological and electron microscopical studies on biopsy materials should be done to understand the life cycle of these both parasites. Similarly, molecular studies should be introduced to confirm these coccidia in waste water and market vegetables so that the food borne or waterborne and seasonal outbreaks of cyclosporiasis and cryptosporidiosis can be easily confirmed in endemic area like Nepal.

Acknowledgements

We acknowledge Professor Steve J. Upton, Division of Biology, Kansas State University, Manhattan, KS for his support during manuscript preparation, Professor Dr. Tej Kumar Shrestha, Central Department of Zoology, Tribhuvan University, Nepal, for providing necessary laboratory facilities and Associate Professor Dr. Ananda Ballav Joshi, Tribhuvan University, Teaching Hospital, Epidemiology Department for constant guiding during stool, water and vegetable sampling.

References

1. Griffiths JK. Human cryptosporidiosis: epidemiology, transmission, clinical disease, treatment, and diagnosis. *Adv Parasitol* 1998; 40:37-85.
2. Looney WJ. *Cyclospora* species as a Cause of Diarrhoea in Humans. *Br J Biomed Sci* 1998; 55: 157-161.
3. Eberhard, ML, Pieniazek NJ, Arrowood MJ, Laboratory diagnosis of *Cyclospora* infections. *Arch Pathol Lab Med* 1997; 121: 792-797.
4. Sallon S, Deckelbaum RJ, Schmid I, Harlaps, Baras M and Spira DT. *Cryptosporidium*, Malnutrition and Chronic Diarrhea in Children. *Am J Dis Child* 1988; 142: 312-315.
5. Tzipori S, and Griffiths JK. Natural History and Biology of *Cryptosporidium parvum*. *Adv Parasitol* 1998; 40:5-36

6. Eberhard ML, Nace EK and Freeman AR. *Cyclospora cayetanensis* in Domestic Animals in an Endemic Area in Haiti. *J Parasitol* 1999; 85: 562-563.

7. Rabold JG, Hoge CW and Shlim DR. *Cyclospora* Outbreak Associated with Chlorinated Drinking Water [letter]. *Lancet* 1994; 344: 1360-1.

8. Bern C, B Hernandez, MB Lopez, MJ Arrowood, MA de Mejia, AM de Merida, AW Hightower, L Venczel, BL Herwaldt, and RE Klein. Epidemiologic studies of *Cyclospora cayetanensis* in Guatemala. *Emerging Infectious Diseases* 1999; 5: 766-774.

9. Hoge CW, Shlim DR, Rajah R, Triplett J, Shear M, Rabold JG and Escheverria P. Epidemiology of Diarrheal Illness Associated with Coccidian-like Organism among Travelers and Foreign Residents in Nepal. *Lancet* 1993; 341: 1175-1179.

10. Goldstein ST, Juranek DD, Ravenholt O, Hightower AW, Martin DG, Mesnik JL, Griffiths SD, Bryant AJ, Reich RR, Herwaldt BL, Cryptosporidiosis: An Outbreak Associated with Drinking Water Despite State-of-the-art Water Treatment. *Ann Intern Med* 1996; 124: 459-468.

11. Mac Kenzie WT, Hosie NJ, Proctor ME, Gradus MS, Blair KA, Peterson DE, Kazmierczak JJ, Addiss DG, Fox KR, Rose JB, Davis JP. A massive outbreak in Milwaukee of *Cryptosporidium* infection transmitted through the public water supply. *N Engl J Med* 1994; 331:161-167.

12. Marshall MM, Naumovitz D, Ortega Y, Sterling CR. Waterborne Protozoan Pathogens. *Clin Microb Rev* 1997; 10: 67-85.

13. Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C, Sterling CR. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from Vegetables Collected in Markets of an Endemic Region in Peru. *Am J Trop Med Hyg* 1997; 57(6): 683-6.

14. Tangermann RH, Gordon S, Wiesner P, Kreckman L. An Outbreak of Cryptosporidiosis in a Day-care Centre in Georgia. *Am J Epidemiol* 1991; 133: 471-6.

15. Medico G, Mcdonald J Gilman RH, Cabrera L, Sterling CR. Epidemiology and Treatment of *Cyclospora cayetanesniss* Infection in Peruvian children. *Clin Infect Dis* 1997; 24: 977-981.

16. Bern C, B Hernandez, MB Lopez, MJ Arrowood, AM De Merida, and RE Klein. The Contrasting Epidemiology of *Cyclospora* and *Cryptosporidium* among Outpatients in Guatemala. *Am J Trop Med Hyg* 2000; 63: 231-5.

17. Sherchand JB, JH Cross, M Jimba, S Sherchand, and MP Shrestha. Study of *Cyclospora cayetanensis* in health care facilities, sewage water and green Leafy Vegetables in Nepal. *Southeast Asian Journal of Tropical Medicine Public Health* 1999; 30: 58-63.

18. Sherchand JB and Cross JH. Emerging Pathogen *Cyclospora cayetanensis* in Nepal. *Southeast Asian J Trop Med Public Health* 2001; 32: 143-150.

19. Fryauff DJ, Krippner R, Purnomo, Ewald C and Escheverria P. Short Report: Case Report of *Cyclospora* Infection Acquired In Indonesia and Treated With Co-Trimoxazole. *Am J Trop Med Hyg* 1996; 55: 584-5

20. Nimri LF. *Cyclospora cayetanensis* and Other Intestinal Parasites Associated with Diarrhea in a Rural Area of Jordan. *Int Microbiol* 2003; 6: 131-135.

21. Chacin-Bonilla L, de Young MM and Estevez J. Prevalence and Pathogenic Role of *Cyclospora cayetanensis* in a Venezuelan Community. *Am Trop Med Hyg* 2003; 68: 304-306.

22. Escobedo AA and FA Nunez. Prevalence of intestinal parasites in Cuban Acquired Immunodeficiency Syndrome (AIDS) patients. *Acta Tropica* 1999; 72: 125-130.

23. Awole M, Gebre-Selassie S, Kassa T and Kibru G. Prevalence of Intestinal Parasites in HIV-Infected adult patients in Southwestern Ethiopia. *Ethiop J Health Dev* 2003; 17(1): 71 – 78.

24. Fayer R, Ungar BLP. *Cryptosporidium* spp and cryptosporidiosis. *Microbiol Rev* 1986; 50: 458-484.

25. Current WL, and Gracia LS. Cryptosporidiosis . *Clin Microbiol Rev* 1991; 4: 325-358.

26. Navin TR, Juranek DD. Cryptosporidiosis: clinical, epidemiologic, and parasitologic review. *Rev Infect Dis* 1984; 6: 313-327.

27. Sherchand JB, Shrestha MP, Larsoon S, Hirai K, Abe N, Nakanishi M and Shrestha S. *Cryptosporidium* Infection and Diarrhea in Chidren from Jomsome, Chitwan and Kathamndu Valley of Nepal. *Zoon Bull* 1994; 2: 17-22.

28. Kaur R, Rawat D, Kakkar M, Uppal B, and Sharma VK. Intestinal Parasites in Children with Diarrhea in Delhi, India. *Southeast Asian J Trop Med Public Health* 2002; 33(4):725-28.

29. Janoff EN, Mead PS, Mead JR, et al. Endemic *Cryptosporidium* and *Giardia lamblia* infections in a Thai orphanage. *Am J Trop Med Hyg* 1990; 43: 248-56.

30. Current WL, Reese NC, Ernst JV, bailey WS, Heyman MB, Weinstein WM. Human Cryptosporidiosis in Immunocompetent and Immunodeficient Persons. *New Engl J Med* 1983; 308: 1252-7.

31. Black RE, Merson MH, Rahman AS, Yunus M, Alim AR, Huq I, Yolken RH, Curlin GT, 1980. A two-year study of bacterial, viral and parasitic agents associated with diarrhea in rural Bangladesh. *J Infect Dis* 142: 660-664.

32. Heijbel H, Slaine K, Seigel B, Wall P, McNabb SJ, Gibbons W, Istre GR, 1987. Outbreak of diarrhea in a day care center with spread to household members: the role of *Cryptosporidium. Pediatr Infect Dis J* 6: 532-535.

33. Newman RD, Zu SX, Wuhib T, Lima AA, Guerrant RL, Sears CL. Household epidemiology of *Cryptosporidium parvum* infection in an urban community in northeast Brazil. *Ann Intern Med* 1994;120: 500-505.

34. Tzipori S, Roberton D, Chapman C. Remission of diarrhea due to cryptosporidiosis in an immunodeficient child treated with hyperimmune bovine colostrum. *Br Med J [Clin Res]* 1986; 293: 1276-1277.

35. Lasser KH, lewin KJ, Ryning FW. Cryptosporidial Enteritis in a Patient With Congential Hypogammaglo- bulinemia. *Hum Pathol* 1979; 10: 234-40.

36. Sloper KS, Dourmashkin RR, Bird RB, Slavin G, Webster AD. Chronic Malabsorption due to Cryptosporidiosis in a Child with Immunoglobulin Deficiency. *Gut* 1982; 23: 80-2.

37. Weisburger WR, Hutcheon DF, Yardley JH, Roche JC, Hillis WD, P. Charache. Cryptosporidiosis in an Immunosuppressed Renal-transplant Recipient with IgA Deficiency. *AM J Clin Pathol* 1979; 72: 473-8.

38. Jacyna MR, Parkin J, Goldin R, Baron JH. Protracted Enteric Cryptosporidial Infection in Selective Immunoglobulin A and Saccharomyces Opsonin Deficiencies. *Gut* 1990; 31:714-6.

39. Hoge, CW, DR Shlim, M Ghimire, JG Rabold, P Pandey, A Walch, R Rajah, P Gaudio, and P Escheverria. Placebo-controlled Trial of Co-trimoxazole for *Cyclospora* Infections Among Travelers and Foreign Residents in Nepal. *Lancet (North American Edition)* 1995; 345(8951): 691-693.

40. Newman RD, Sears CL, Moore S.R., Natoro JP, Wuhib T, Agnew DA, Guerrant RL and Lima AA. Longitudinal Study of *Cryptosporidium* Infection in Children in Northeastern Brazil. *J Infect Dis* 1999; 180: 167-175.

41. Molbak K, Wested N, Hojlyng N, Scheutz F, Gottschau A, Aaby P, da Silva AP. The etiology of early childhood diarrhea: a community study from Guinea-Bissau. *J Infect Dis* 1994; 169: 581-587.

42. Ortega YR, Sterling CR, Gilman RH, Cama V and Diaz F. *Cyclospora* species: A New Protozoan Pathogen of Humans. *N Engl J Med* 1993; 328: 1308-1312.

43. Sherchand JB and Cross JH. Studies on *Cyclospora cayetanensis* Infection in Nepal. The 10th International Congress of Parasitology-ICOPA 10, Vancouver (Canada), August 4-9, 2002; 71-88.

44. Meinhardt PL, Casemore DP, Miller KB. Epidemiologic Aspects of Human Cryptosporidiosis and the Role of Waterborne Transmission. *Epidemiol Rev* 1996; 18:118-136.

45. Amin OM. Seasonal Prevalence of Intestinal Parasites in the United States during 2000. *Am J Trop Med Hyg* 2002; 66:799-803.

46. Enriquez FJ, Avila CR, Tanaka-Kido JI, et al. *Cryptosporidium* Infections in Mexican Children: Clinical, Nutritional, Enteropathogenic and Diagnostic Evaluations. *Am J Trop Med Hyg* 1997; 56:254-7.

47. Smerdon WJ, Nichols T, Chalmers RM, et al. Foot and Mouth Disease in Livestock and Reduced Cryptosporidiosis in Humans,

England and Wales. *Emerg Inf Dis* 2003; 9:22-8.

48. Bern C, Ortega Y, Checkley W, et al. Epidemiologic Differences between Cyclosporiasis and Cryptosporidiosis in Peruvian Children. *Emerg Inf Dis* 2002; 8:581-585.

49. Cruz JR, Cano F, Caceres P, et al. Infection and diarrhea caused by *Cryptosporidium* sp. among Guatemalan infants. *J Clin Micro* 1988; 26: 88-91.

50. Casemore DP. Cryptosporidiosis. PHLS. *Microbiology Digest* 1987; 4:1-5.

51. Baxby D, Hart CA. The Incidence of Cryptosporidiosis : A Two Year Prospective Survey in a Children's Hospital. *Journal of Hygiene* 1986; 96:107-11.

52. Thomson MA, Benson JWT, and PA Wright. Two Year Study of *Cryptosporidium* Infection. *Arch Dis Child* 1987; 62:559-63.

53. Riberio CD, SR Palmer. Family Outbreak of Cryptosporidiosis. *Br Med J* 1986; 292: 377.

54. Hale D, W Aldeen, and K Carroll. Diarrhea Associated with Cyanobacteria-like Bodies in an Immunocompetent host: An Unusual Epidemiological Source. *JAMA* 1994; 271:144-145.

55. Ooi WW, Zimmerman SK and Needham CA. *Cyclospora* species as a Gastrointestinal Pathogen in Immunocompetent Hosts. *J Clin Microbiol* 1995; 33: 1267-1269.

56. Wurtz R. Cyclospora: A Newly Identified Intestinal Pathogen Of Humans. *Clin Infect Dis* 1994; 18: 620-623.

57. Monis, PT, Thompson, RC. *Cryptosporidium* and *Giardia*-zoonoses: Fact or Fiction? *Infect Genet Evol* 2003; 3: 233-244.

58. Issac-Renton JL, Foged D, Stibbs HH, Ongerth JE. Giardia and *Cryptosporidium* in Drinking Water. *Lancet* 1987; i: 973-4.

59. D'Antonio RG, Winn RE, Taylor JP, et al. A waterborne outbreak of cryptosporidiosis in normal hosts. *Ann Intern Med* 1985; 103: 886-8.

60. Hayes EB, Malte TD, O'brian TR, et al. Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. *New Engl J Med* 1989; 320: 1372-6.

61. Smith HV, Girdwood RWA, Patterson WJ, et al. Waterborne Outbreak of cryptosporidiosis. *Lancet* 1988; ii: 1484.

62. Connor BA, Shlim DR, Scholes JV, Rayburn JL, Reidy J. and Rajah R. Pathogenic Changes in The Small Bowel in Nine Patients with Diarrhea Associated with a Coccidia-Like Body. *Ann Intern Med* 1993; 119: 377-82.

63. Herwaldt B L, ML Ackers, and the *Cyclospora* Working Group. An outbreak in 1996 of Cyclosporiasis Associated with Imported Raspberries. *N Engl J Med* 1997; 336:1548-1556

64. Jinneman, K C, J H Wetherington, AM Adams, J M Johnson, BJ Tenge, NL Dang, and WE Hill. In Differentiation of *Cyclospora* spp. and *Eimeria* spp. by Using the Polymerase Chain Reaction Amplification Products and Restriction Fragment Length Polymorphisms. Laboratory Information Bulletin No. 4044. U.S. Food and Drug Administration, Washington, D.C., 1996.

65. Clark DP. New insights into human cryptosporidiosis. *Clin Microbiol Rev* 1999; 12: 554-63.