Original article

An epidemiological study of *Cyclospora cayetanensis* in Nepalese people

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Background: *Cyclospora cayetanensis is a* coccidian parasite that causes recurrent gastroenteritis among children living under poor sanitary condition and adults from industrialized countries who lived or traveled in endemic developing countries.

Materials and Methods: A total of 1842 stool specimens from gastroenteritis patients were examined between March 2005 to February 2006 and collected various types of faecal specimens from human, animals and samples of water and green leafy vegetables.

Results: Of 1842 stool specimens collected from different areas of Nepal, 146 (7.9 %) were found to be positive for *Cyclospora cayetanensis* with the majority were from children 2-9 years of age. The highest rate of infections was found in the month of June; (31.6%). *Cyclospora cayetanensis* were found to be contaminated in green vegetables including leaves of basil, mint leaves, water sources and feces of domestic animals and these findings were presented in the paper.

Conclusion: *Cyclospora*-like oocysts were isolated for the first time from the leaves of basil, a holy plant of Nepal and the study indicated that vegetarian people were also found highest rate (65.1%) of Cyclosporiasis. However, to obtain more evidence on the source of infection, specimens from rodents, birds, insects and domestic animals are need for an extensive study.

Key-word: Cyclospora cayetanensis, oocysts,

Introduction:

Cyclospora cayetanensis has emerged as an important cause of acute and chronic gastroenteritis worldwide [1, 2, 3]. The organism was first described by Ashford [4] in 1979, and recently it was categorized as an important gastrointestinal parasite. *Cyclospora* infection has been reported from Papua New Guinea, Indonesia, India, Pakistan, Nepal, the Middle East, North Africa, South Africa, the United Kingdom, the Caribbean, the United States of America, Central America, and South America [5,6,7,8,9,10,11].

Although *C. cayetanensis* is reported from all areas of the world, little is known about the biology of the organism and the means of transmission remains an enigma. Susceptible humans are suspected to be infected by ingesting sporulated oocysts. Water is probably an important vehicle, either drinking parasite contaminated water directly or indirectly when water is used to grow plant foods. Water has been implicated in outbreaks in the United States, [12] and in Nepal [11,13]. Food borne transmission is also suspected with reports of finding oocysts in washing of leafy vegetables in Peru [14] and Nepal [15]. Patients suffer from a chronic watery diarrhea, fatigue, nausea, vomiting, abdominal cramps, anorexia, weight loss and myalgia. However, it is not known what is responsible for the pathogenesis. Our study between 1995 and 2004 in Nepal, has identified that the possible sources of infection with *Cyclospora* is due to the consumption of undercooked, contaminated green leafy vegetables and exposure to contaminated water and some domestic animals. [17, 18].

To extent the further work, the present studies were carried out from March 2005 to February 2006 in order to determine the prevalence and distribution of the parasite from different topographical areas of Nepal. Further study on animals' feces, water, green leafy vegetables including basils and mint were collected and examined.

Materials and Methods

A total of 1842 stools samples were collected from different areas of Nepal and examined in Tribhuvan University Institute of Medicine, Health Research Laboratory and in Infectious and Tropical Diseases Research Centre, Nepal. The collected stool samples were examined soon after the passage by direct light microscopy at 400x and stools were also preserved in 2.5% potassium dichromate solution for further study. During the study period, the patients were asked to respond questionnaires to record their age, sex, educational status, occupation, clinical history including duration of illness, current diarrhoeal status, demographic and food habit of family either vegetarian or non vegetarian.

Samples of water and green leafy vegetable

During sampling collection in the different districts, samples from irrigation canal, pond water, public drinking water of different sources and green-leafy vegetables were collected to determine the contamination of *Cyclospora* oocyst and to investigate the possible sources of infection. The leaves were washed in distilled water, the washings and the samples of water were centrifuged and the sediments were examined microscopically. The excess amounts of sediments were resuspended in 2.5 % potassium dichromate solution, and the recovery of sporulation was noted.

***Cyclospora* study in various animals**

A study of 439 stools were examined for *Cyclospora* oocysts from chicken, pigs, monkeys, dogs, cats, goats, cows, buffaloes, rats and pigeons.

Microscopic examination and standard staining application

Direct microscopic examination after concentration with formalin-ether and sucrose solution, phase contrast microscopy was performed. All the positive samples from human and animals faeces as well as sewage and green leafy vegetables were further confirmed by following modified acid fast staining method: The samples were heat fixed and stained with carbolfuchsin (Sigma, St. Louis) for 30 minutes. Samples were decolorized for 1 minute with a 1 % solution of HCl in 75 % ethanol.

C. cayetanensis oocysts were identified with their characteristic size (8-10 um), round shape and red coloration. Other protozoa and helminthes eggs were also recorded.

Results:

***Cyclospora cayetanensis* in different areas:** Of 1842 stool specimens collected from 14 districts, 146 (7.9 %) were found to be positive for *Cyclospora cayetanensis.*

Gender and Age-wise distribution

Out of 1842 people, 846 were males and 996 were females ranging from 2 years to 70 years of age were examined. Of total 146 positive patients, 68 (46.6%) were male and 78 (53.4%) were female. The highest prevalence of *C. cayetanensis* infection 74 (50.7 %) was found between 2 and 5 years of age group where as the lowest prevalence 6 (4.1%) were above 28 years of age (Fig. 1).

Fig. 1: Age-wise distribution of Cyclospora infection

Occupation-wise distribution of *Cyclospora* infection:

The study samples were collected from different occupational groups: Farmer 658, Business people 106, Service holder 102, children/ students 913 and other such as unemployed people 67. The distributions of Cyclospora infection in these groups are shown in Figure 2. The highest infection rate was found among children/student (dependent group) (66%) and lowest (2%) among business people ($P < 0.05$).

Fig. 2: Occupation-wise distribution of Cyclospora infection

Education and *Cyclospora* infection:

The highest rate of *cyclospora* infection was found among children age group between 1-5 years of age (41.7%)

compared to other group of people ($P<0.05$) as shown in Fig. 3.

Fig. 3: Educational-wise distribution of *Cyclospora* infection

Characteristic of the patients with *Cyclospora* infection:

Most of the patients with *cyclospora* infection had diarrhea 83% (121/146). The duration of the diarrhoea was more than 3 days. About 44.4% cyclosporiasis patient had fever and 18.4% had vomiting. Headache, abdominal pain and anorexia were 43.2%, 99.4% and

92.8% respectively. On the basis of haemoglobin estimation, 94 (64.4%) cyclosporiasis patients were found anaemic with haemoglobin levels between 6-11gms/dl where as 52 (35.6%) patients were found with normal haemoglobin levels. 61% of the patients had leucocytosis and more than 50% had eosinophilia. Tuberculosis 7.5% (11/146) and HIV-seropositive patients 6.2 (9/ 146)had cyclosporiasis.

Distribution of one or more mixed parasites in Cyclosporiasis patients:

A total of 146 *Cyclospora* infected patients 34 (23.2) had one or more mixed intestinal parasites. The highest mixed parasitic infection was due to *Giardia lamblia* (24.7%) and the lowest was due to *Isospora belli* (1.2%). There was no difference in the distribution of co-infecting parasites between patients with diarrhea and non diarrhea (P= > 0.05).

More than 13% cyclosporiasis patients had mixed infection with other protozoa such as *Entamoeba coli, Iodamoeba butschilii* or *Endolimax nana*.

In *Cyclospora* negative samples of 1669 people, (31.6%) were positive with one or more intestinal parasites. Of the 528 positive samples, 39.1% *Giardia*, 8.1% *Trichuris trichura*, 7.8% *Ascaris*, 6.8% *hook worm*, 5%, 5.5% *Ent.Coli*, 3.0% *Oxyuris vermicularis*, 2.8% *Hymenolepis nana*, 2.8% *Entamoeba histolytica*, 2.1% *Blastocystic hominis*, *1.5% Cryptosporidium parvum* and 17.4% had more than two or more mixed infection of parasites including non pathogenic protozoal parasites.

Month-wise distribution of *Cyclospora cayetanensis* infection:

The higher distribution of *Cyclospora* infection in Nepal is during summer and rainy season- June and July as shown in Figure 4. The prevalence decreased during the winter, although there are few cases, which were identified, in early winter in October 2001.

Fig. 4: Month wise distribution of *Cyclospora cayeanensis* infection

Examination of Vegetables:

Green vegetables collected from 14 districts community and vegetable markets consisted of cabbage, lettuce, cauliflower, spinach, green onions, radishes, green leafy vegetables, mustard leaves and carrot, in which lettuce, spinach and mustard leaves were found to be contaminated with *Cyclospora* (Figure 5). ***Cyclospora*** were further confirmed by the development of 2 sporocysts, after 2 weeks incubation period in potassium dichromate solution. This is the first time that the basil leaves, a holy plant of Nepal were contaminated with oocyst of *Cyclospora*.

Fig. 5: Cyclospora study in green vegetables

Examination of water samples:

Water samples were collected from different areas and studied for *Cyclospora* as shown in Figure 6. *Cyclospora* contamination was found in June, July in irrigation canal and Pond water. Several other parasites: *Giardia lamblia, amoeba,* unidentified trophozoites, ova of *Ascaris*, larvae of helminthes and many small insects, worms were detected. In 2 sources of water samples (Pond, and irrigation canal) *Cryptosporidium parvum* oocysts were also identified.

Fig. 6: *Cyclospora* from different sources of water (June, July and August 2005)

Examination of stool samples from different animals

Of total 439 different animal samples examined, 1.8% animals were found to be positive for *Cyclospora* oocysts. Among these animals, one chicken (1.3%), monkeys (6.5%), dogs (3.8%) and rats (5.9%) were found positive as shown in figure 7.

Fig. 7: Cyclospora oocysts examination in different animals (2005)

Infection of *Cyclospora cayetanensis* among vegetarian and non-vegetarian

Out of 1842 gastroenteritis patients studied, 1106 (60.04%) were vegetarian and 736 (39.96%) non-vegetarian. The present results on 146 cases of cyclosporiasis, 95 (65.1%) were vegetarian group compared to non-vegetarian 51 (34.9%). Hence, the study indicated that vegetarian people of Nepal likely to be infected with highest rate of cyclosporiasis.

Discussion

The current study presented to explain upgrade information on *Cyclospora cayetanensis* a coccidian parasite that infect humans and causes prolonged diarrhoea in both immunocompetent and immunocompromised hosts. The mechanisms of pathogenesis and virulence factors of *Cyclospora cayetanensis* are yet to be defined, but tissue damage and jujunitis have been reported [14]. From the present study advances our understanding both of the epidemiology of *Cyclospora cayetanensis* in Nepal where there are repeated outbreaks of the emerging diseases associated with gastroenteritis. Although *Cyclospora* infection has been reported from all parts of the world, most of the epidemiological information comes from studies in Nepal, Haiti, Peru and United States, where it is endemic [11,13] 18,19,20,21. Cyclosporiasis appears to be seasonal, with peak incidence during the rainy seasons from April to June in Peru and May to September in Nepal [1,3,16,18]. Although all age groups can acquire the disease, the highest attack rates occur among children older than 18 months [19,22], where as in our study the highest attack rates was found among children age between 2 to 5 years. There is no apparent immunity to infection, and reinfection can occur at all ages [23, 24].

In the United States, the outbreak of diarrheal disease associated with *Cyclospora* in 21 medical residents in 1990 was epidemiologically linked to a contaminated water supply[11,12,13]. Subsequently, more than 1000 confirmed cases in the US and Canada were reported [26]. In this study oocysts of *Cyclospora* were found in irrigation canal and pond water in June, July during the high transmission period. *Cyclospora* infection occurs most commonly via contaminated water [12, 25] and the oocysts are resistant to chlorination and not readily detected by methods that are currently used to assure the safety of drinking water. Though *Cyclospora* has been detected in water samples, however, the available method for detection in water has very low sensitivity[25]. Contaminated food has long been proposed as a possible route for transmission of *Cyclospora* [23]. Vegetables in particular are suspicious since they are often ingested raw or undercooked. Vegetables are easily contaminated and provide organisms with an optimal environment for survival prior to host ingestion. It is believed that *Cyclospora*, must sporulate for at least 7-10 days in the environment to be infectious. Fertilization of plants with human waste or indirectly via contaminated water used for crop irrigation and to freshen produce could lead to contamination of vegetables with *Cyclospora.* In this study, 65.1% of the vegetarian people were found infected with Cyclosporiasis and the findings on vegetable studies have shown that lettuce, spinach, mustard and basil leaves were contaminated with *Cyclospora* which confirmed that food-borne transmission is feasible. It still remains to be determined if recovered oocysts are infectious. The source of vegetable contamination with oocysts is still unknown, but it may be due to fecally contaminated water used on the vegetables from the irrigation water or directly from contaminated hands of food handlers. Moreover, in Nepal, vegetables coming in the markets are dipped and rinsed into highly contaminated water of small ponds or rivers in order to wash and clean it, but actually it becomes too contaminated once again. There are thousands of such instances of how food is rendered unsafe due to unhygienic conditions, handling and practices and poor environment. In cities of Nepal, the water supply is contaminated through seepage into water pipes from sewage. In rural areas, the source of water itself (wells, ponds, rivers etc) is polluted from the contact of waste disposal deposits. One of the common food contamination problems is again from insects and rodents and as a result food becomes unfit for human consumption.

Although more studies are needed to clarify the direct link between *Cyclospora* infection and these sources, the results suggests that green leafy vegetables, water from sewage and irrigation canal are possible sources of infection in Nepal [18, 20].

To obtain more evidence on the source of infection, specimens from rodents, birds, insects and domestic animals are need for extensive study. Wider dissemination of skilled laboratory diagnosis is a prerequisite for a better understanding of the epidemiology of this infection and its association with disease. The frequency of malabsorption in clinical illness needs to be established. Detail histopathological and electron microscopical studies on biopsy material should be done to understand the life cycle and pathogenesis of the *Cyclospora.* In vitro cultivation system for drug screening and controlled trials of drug therapy are needed. Better knowledge of the behavior of *Cyclospora* in AIDS patients along with other coccidia, *Cryptosporidium*, *Isospora* and *Toxoplasma* need to be studied in the context of Nepal.

In addition, we need to identify alternative efficacious agents for therapy as well as prophylaxis and to develop inexpensive, reliable molecular and diagnostic tools.

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