

REVIEW

CYCLOSPORIASIS IN NEPAL

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Abstract. Nepal is one of the most highly endemic areas for cyclosporiasis in the world. Initial studies of the parasitosis in Nepal were among expatriates and tourists in the country. The present report, however, is a review of studies carried out since 1995 on the epidemiology of *Cyclospora cayetanensis* and infection in the Nepali populations. The parasitosis was found to occur mostly in children 2-11 years of age. Stools from several thousands of patients with diarrhea were examined, and approximately 6-30% were positive for oocysts of the parasite. The majority of patients were seen during the rainy season. Stool specimens from animals were examined, and oocysts were recovered from six chickens, two monkeys, three dogs, and five rats. PCR/RFLP analysis of oocysts from one monkey, one dog, and 2 chickens were positive for *Cyclospora* sp. Oocysts of *Cyclospora* were also found in sewage, a pond and two irrigation canals, and the washwater of cabbage, lettuce, mustard leaves, and basil. Basil may be an important source of infection since it is often eaten raw.

INTRODUCTION

Cyclospora cayetanensis is recognized as an important cause of diarrheal disease, and Nepal is considered one of the most highly endemic areas in the world. The disease was first recognized in Nepal among tourists and expatriate residents and reported at the time to be caused by cyanobacterium-like bodies (CLB) (Shlim *et al*, 1991). The epidemiology, clinical features, and treatment of the parasitosis were later studied in a similar type of patients (Hoge *et al*, 1995, 1996). Since little was known about cyclosporiasis in Nepali populations, studies were carried out from 1996 throughout 2004 on the transmission, distribution, and possible reservoir hosts of the parasite in Nepal.

METHODS

Populations throughout Nepal and patients seen at health care facilities were examined for infection with *C. cayetanensis*. Stool specimens from humans, animals, vegetable washwater, and various water bodies were examined for oocysts. The stool specimens were examined by direct

microscopy (400×); leaves from vegetables were washed with distilled water, the washings centrifuged, and the sediment examined. Water samples were centrifuged and the sediment examined. Positive specimens were placed into 2.5% potassium dichromate and the organism examined periodically for sporulation.

RESULTS

The initial study was carried out at the Kanti Children's Hospital in Kathmandu, and the stools from 180 children with diarrhea were examined by direct microscopy for oocysts. Fifteen children, 2 months to 11 years-of-age were found to be positive (Cross *et al*, 1997). During 1995-1998, over 2,000 stools from Nepali as well as few expatriates with diarrhea were examined; approximately 30% were positive for oocysts. Most positive stools were from children 2-11 years of age (Sherchand *et al*, 1991). Over 4,000 stools were examined during 1997-2000, and over 22% were found to be positive (Sherchand *et al*, 2001). A number of stools from various districts of Nepal were examined, and many were found to contain *C. cayetanensis*-like oocysts indicating the widespread distribution of the parasitosis throughout the country. Additional stools from rural populations were examined during 2002-2003, and approximately 6% were positive (Sherchand *et al*, 2004). More recently (2005-2006), 1,842 stool samples from different areas of Nepal and health facilities were examined,

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and approximately 8 % were found to be positive. Most of the infected people manifested diarrhea with the passage of 1-15 stools per day. The patients also reported abdominal discomforts, anorexia, fatigue, flatulence, and weight loss. Most patients responded to treatment with co-trimoxazole (Hoge *et al*, 1995). Although infections were detected throughout the year, the majority occurred during the rainy season, May to September.

Over 1,400 animal stools (chicken, pig, monkey, dog, cat, cow, buffalo, goat, rat, and pigeon) were examined, and cyclospora-like oocysts were recovered from six chickens, two monkeys, three dogs, and five rats (Sherchand *et al*, 2001, 2004). Oocysts from these animals were analyzed by PCR/RFLP. Cyclospora sp was demonstrated in monkey and dog samples, and *Eimeria* and *C. cayetanensis* were found in chicken samples (Chu *et al*, 2004).

During the rainy months, samples were collected from tap water, wells, ponds, irrigation canals, rivers, springs, as well as sewage. Cyclospora-like oocysts were recovered from sewage on 5 occasions, ponds once, and irrigation canals twice. Oocysts have also been recovered from chlorinated water during an epidemic of cyclosporiasis among a small British military detachment in Pokahara, Nepal (Rabold *et al*, 1994). Irrigation canal water is usually fecally contaminated, and these waters are used to irrigate crops and to keep vegetables fresh in the market. Over 300 samples of washwater from vegetables were examined for *C. cayetanensis* oocysts. The vegetables included cabbage, lettuce, cauliflower, spinach, green onion, radish, mustard leaves, and basil leaves. Oocysts were recovered from washwater of cabbage (5), lettuce (5), mustard leaves (3), spinach (3), and basil leaves (2). The oocysts recovered either were sporulated or were found to sporulate after several days in 2.5% potassium dichromate. Of interest is the finding of oocysts on basil leaves as basil is a holy plant in Nepal and considered an incarnation of God, and it helps purify eternal life. It is also considered a medicinal plant. The leaves of basil are usually eaten fresh without washing. Many of the people found infected with *C. cayetanensis* were vegetarians.

DISCUSSION

We have obtained a great deal of information on cyclosporiasis in Nepal; however, parasitosis remains an enigma. The disease is endemic in the Nepali population throughout the country, occurring mostly in the rainy season, May through September. Although most of the cases have been documented from a few medical facilities, it is not a reportable disease and therefore many more cases seen elsewhere have not been reported. The disease has been found primarily in children 2-11 years of age with relatively few reports in the older Nepali age groups.

One of the unknowns regarding cyclosporiasis is whether it is a zoonotic disease. *Cyclospora cayetanensis* oocysts have been reported elsewhere from the feces of ducks, chickens and dogs (Garcia-Lopez, 1996) and in chickens, monkeys, dogs and rats in Nepal (Sherchand *et al*, 2001, 2004). These infections, however, have been considered spurious as these animals are known to be coprophagic. In further studies, the PCR/RFLP analysis suggested the isolates from two dogs, one chicken, and one monkey from Nepal were *Cyclospora* sp (Chu *et al*, 2004). Experimental infections in animals and human volunteers have not been successful (Alfano-Sobsey *et al*, 2004). To understand fully the life cycle of *C. cayetanensis* and the pathology of the disease, it is imperative that an animal model be found.

Water from various sources has been suspected as a main source of infection with *C. cayetanensis* (Sterling *et al*, 1999; Cross *et al*, 2004). Untreated water has been implicated as a source of infection in Nepal, and oocysts were found in chlorinated water (Rabold *et al*, 1994). We have recovered oocysts from sewage, a pond, and irrigation canal waters (Sherchand *et al*, 2004). Fecally contaminated water is used in farm irrigation systems for vegetables and to keep vegetables looking fresh in markets. Other intestinal parasites such as *Ascaris lumbricoides* eggs, *Giardia lamblia* cysts, and *Cryptosporidium* sp oocysts have been recovered from pond and irrigation canal water. Furthermore, the drinking water supply is often contaminated with sewage water, especially in the Kathmandu valley. Many of the patients with cyclosporiasis report drinking untreated water.

Plants have been suspected as a source of infection (Sterling *et al*, 1999), but the organism is rarely isolated. Oocysts of the parasite have been found in the washwater of cabbage, lettuce, mustard leaves, and basil in Nepal (Sherchand and Cross, 2001), and fresh basil has been incriminated as a source of transmission of the parasite elsewhere (Lopez *et al*, 2001; Hoang *et al*, 2004). We have recovered *C. cayetanensis*-like oocysts from fresh basil washwater, and we consider this an important source of infection. In Nepal, basil is considered a "holy plant" by Hindu populations. It is also used in the treatment of respiratory disease. Basil is usually eaten raw. Although water is considered a source of infection, plants are probably contaminated with the organism during growth and irrigation with polluted water. The majority of the cases of cyclosporiasis among Nepali population have been found in vegetarians; therefore, the eating of raw vegetables is considered an important source of infection in the country.

REFERENCES

- Alfano-Sobsey EM, Eberhard ML, Seed JR, *et al*. Human challenge pilot study with *Cyclospora cayetanensis*. *Emerg Infect Dis* 2004;10:726-8.
- Chu DMT, Sherchand JB, Cross JH, Orlandi P. Detection of *Cyclospora cayetanensis* in animal fecal isolates from Nepal using an FTA filter-base polymerases chain reaction method. *Am J Trop Med Hyg* 2004;74:373-9.
- Cross JH, Sherchand JB. Cyclosporiasis. In: Cotruvo JA, Dufour A, Rees J, *et al*, eds. Waterborne zoonoses: classification, causes and conclusion. London: IWA, 2004:283-90.
- Cross JH, Sherchand J, Sharm P, Echeverria P. Cyclosporiasis at the Kanti Children's Hospital in Kathmandu, Nepal: a cursory survey. *J Trop Med Parasitology* 1997;20:30-2.
- Garcia-Lopez HL, Rodriquez-Tovar LE, Medina-de-la Garza CE. Identification of *Cyclospora* in poultry. *Emerg Infect Dis* 1996:256-7.
- Hoang LMN, Fyfe M, Ong C, Champagne S, Dixon B, Isaac-Renton, J. Outbreak of cyclosporiasis in British Columbia associated with imported Thai basil. *Epidemiol Infect* 2004;133:23-7.
- Hoge CW, Shlim DR, Ghimire M, *et al*. Placebo-controlled trial of co-trimoxazole for cyclospora infections among travelers and foreign residents in Nepal. *Lancet* 1995; 345:691-3.
- Hoge CW, Shlim DR, David R, *et al*. Epidemiology of diarrhea among expatriate residents living in a highly endemic environment. *JAMA* 1996;275:533-8.
- Lopez AS, Dodson DR, Arrowood MJ, *et al*. Outbreak of cyclosporiasis associated with basil in Missouri in 1999. *Clin Infect Dis* 2001;32:1011-7.
- Rabold JG, Hoge CW, Shlim DR, Kefford C, Rajah R, Escheverria P. Cyclospora outbreak associated with chlorinated water. *Lancet* 1994;344:1360-1.
- Sherchand JB, Cross JH, Jimba M, Sherchand S, Shrestha MP. Study on *Cyclospora cayetanensis* in healthcare facilities, sewage water and green leafy vegetables in Nepal. *Southeast Asian J Trop Med Public Health* 1991;30:58-63.
- Sherchand JB, Cross JH. Emerging pathogen *Cyclospora cayetanensis* infection in Nepal. *Southeast Asian J Trop Med Public Health* 2001;32 (suppl):143-50.
- Sherchand HB, Cross JH. Parasitic epidemiological studies on *Cyclospora cayetanensis* in Nepal. *Southeast Asian J Trop Med Public Health* 2004;35 (suppl):12-9.
- Shlim, DR, Cohen MT, Eaton M, Rajah R, Long EG, Unval LP. An alga-like organism associated with an outbreak of prolonged diarrhea among foreigners in Nepal. *Am J Trop Med Hyg* 1991;45:383-9.
- Sterling CR, Ortega YR. Cyclospora: an enigma worth unraveling. *Emerg Infect Dis* 1999; 5:48-53.