Review Paper

Cyclospora cayetanensis: "Look and you will find"

John H Cross[1] and Jeevan Sherchand[2] [1]*Uniformed Services University of the Health Sciences, Bethesda, Maryland, USA;* [2]*Tribhuvan University Public Health Laboratory, Kathmandu, Nepal*

Abstract

Cyclosporiasis caused by the elusive coccidian parasite *Cyclospora cayetanensis* is reported from many parts of the world, but at the present time it is probably being under-diagnosed. The stools of patients presenting with diarrhoea should be examined for oocysts. Finding the oocysts by light microscopy at high magnification (400 X) is time consuming but it provides the definitive diagnosis. Scanning by ultraviolet fluorescence microscopy is helpful and acid-fast staining may provide a presumptive diagnosis. The life cycle of the parasite has not been completely elucidated and although water may be a vehicle of transmission of the organism other means of spread are suspected. However, many of the answers must wait until a natural host for the parasite is found or until the life cycle is established in a laboratory animal. The clinical manifestations of the disease are diarrhoea, fatigue, anorexia, weight loss, nausea, vomiting, abdominal cramping, myalgia flatulence, bloating and dyspepsia. Treatment is co-trimoxazole (TMP160/SMZ 800) twice daily for 7 days.

Key words: Cyclospora cayetanensis; laboratory diagnosis; treatment; clinical features

Introduction

A story that circulated among parasitologists a number of years ago regarding the teaching of protozoology at a university in the United States went something like this. Week after week a young female student complained to her professor thar she could not find protozoa under the microscope and each week the professor would say "look and you will find." At the last laboratory session of the course the young lady became quite excited and called to the professor and said "I found it." The professor impassively answered, "see, I told you, look and you will find." This can be said today with the recently described *Cyclospora cayetanensis;* it is small, illusive and easily missed when examining fecal specimens, but when carefully looked for it can be found.

Cyclospora species have been known from animals such as moles, rodents, insects worms and snakes (Soave, 1996) but the first recognition of what is now considered *C. cayetanensis* was by Ashford in 1973 (Ashford, 1973; Ashford *et al.*, 1993). He reported rhe finding of an undescribed coccidian in stools from two children and one adult with diarrhoea in Papua New Guinea. Soave *et al.* (1986) reported finding round bodies 8-9 μm in diameter with a well defined outer wall and granular material inside in stool specimens from healthy immunologically competent patients with diarrhoea after they had visited Haiti and Mexico. Similar organisms were described by Long *et al.* (1990), in diarrhoea stools of AIDS patients who had travelled to tropical countries in the Caribbean. The authors suggested the organisms were blue-green alga, cynobacterium or CLB. Additional reports of CLB organisms in stools from patients with diarrhoea followed: Long *et al.* (1991) reported the organism from patients in Southeast Asia and the United States and reports were also made by Shlim *et al.* (1991), and Hoge *et al.* (1993) from Nepal and Bendall *et al.* (1993), from England.

Ortega *et al.* (1993; 1994) described these organisms referred to as cynobacterium-like organism as a new protozoan pathogen of humans, *Cyclospora cayetanensis.* Mature oocysts of this parasite have two *sporocyst* each containing two sporozoites. In electron microscopic studies they found the sporozoites possess a membranebound nucleus and micronemes characteristic of coccidians of the phylum Apicomplexia. Further studies by Relman *et al.* (1996) using phylogenetic analysis confirmed that *C. cayetanensis* was a coccidian related to *Eimeria* species closely related to *Isospora.*

Geographic Distribution

Based upon present knowledge it appears that *C. cayetanensis* has a universal or world-wide distribution. It was first reported from Papua New Guinea and years later in travellers returning to the United States or Britain from Haiti, Mexico, Puerto Rico, Morocco, Cambodia, Pakistan, India and the Solomon Islands (Wurtz, 1994). More recent reports have been from Guatemala (Pratdesaba *et al.*, 1994), Italy (Caramello *et al.*, 1995), Brazil (Schubach *et al.*, 1997), Malaysia (Sinniah *et al.*, 1994), Thailand (Wanachiwanawin *et al.*, 1995), Indonesia (Fryaufff *et al.*, 1996) and China (Han *et al.*, 1996). The most highly endemic area, however, are in Peru (Ortega *et al.*, 1993) and Nepal (Hoge *et al.*, 1993). During 1996 and 1997 hundreds of cases of cyclosporiasis has been reported from many parts of North America (Herwaldt *et al.*, 1997).

Morphology

The oocysts of *C. cayetanensis* when observed in feces by light microscopy measures 8-10 µm in diameter. Under electron microscopy the oocyst is seen with a bilayered cell wall, 113 nm thick; outer layer 63 nm and rough inner layer 50 nm and smooth. A polar body and oocyst residuum are also present. Sporocysts are ovoidal 4.0 µm wide by 6.34 µm long with stieda and substieda bodies. The sporocyst residuum has large spherical globules. Sporozoites are 1.2 µm wide by 9.0 µm (Ortega *et al.*, 1993;1994).

Life Cycle

The life cycle has not been determined. It is assumed that oocysts containing sporocysts or released sporozoites are ingested and that the sporozoites enter intestinal cells to go through schizogony and gametogony. Biopsies of intestinal tissue have been made (Connor *et al.*, 1993) but the parasite was not found. In other studies, however, asexual stage were seen by electron microscopic examinations of jejunal biopsies (Bendall *et al.*, 1993). On the other hand, in other studies, Sun *et al.* (1996) identified stages of sporozoites, trophozoites, schizonts and merozoites observed by light and electron microscopy, but no sexual stages were seen. Complete elucidation of the life cycle must wait further studies of intestinal biopsies or until a laboratory animal model is identified.

Disease

The incubation period for cyclosporiasis has not been definitively established, but one report indicates that the onset of illness can occur as early as 12-24 hours (Huang *et al.*, 1995). In another report the incubation period was 2-11 days. Watery diarrhoea, chronic and intermittent occur early in the infection at times alternating with constipation. The diarrhoea may last for 6 - 7 weeks with 6 or more stools per day. Characteristic of the disease is profound fatigue, anorexia, weight loss, nausea, vomiting, abdominal pain, flatulence, bloating, dyspepsia with D-xylose malabsorption (Connor *et al.*, 1993). Re-infection may rarely occur and immunity to infection is suspected because of the rare occurrence of infections in indigenous adult populations in endemic areas. No deaths have been associated with infections.

Histologic studies of duodenal and jejunal biopsies indicate that the parasite causes inflammatory changes, villous atrophy and crypt hyperplasia (Bendall *et al.*, 1993, Connor *et al.*, 1993).

Diagnosis

In freshly passed fecal specimens unsporulated occysts can be visualized under direct light microscopy. The organisms are usually in the morula stage and 8-10 µm in size. The specimen should be examined under 400x magnification. The organisms are auto fluorescent and under fluorescent microscopy will appear as blue or green circles depending on the filters, 365 or 450 to 490 DM. To improve the recovery of organisms concentration methods may be used, such as the formalin-ether or formalin-ethyl acetate concentration or the Sheather's sugar flotation methods. In a survey carried out in Nepal *C. cayetanensis* was found by both direct and concentration methods, often by one method and not the other or by both methods. Therefore multiple methods should be used in examining stools for this parasite (Cross *et al.*, 1997). The organism is acid-fast and can be seen in smears stained with Ziehl-Neilson stain or Kenyoun's stain. The staining of the parasite is variable, however, with some organisms being found unstained, pink of red. A method for staining *C. cayetanensis* with safranin and microwave heating has been reported to be superior to acid-fast staining (Visvesvara *et al.*, 1997). This will require further testing, however.

Treatment

Although the disease is self limiting, treatment with cotrimoxazole (TMP/SMZ 160/800), twice daily for 7 days is recommended. There is no alternative treatment for persons who are allergic to sulphamethoxazole. Eradication of the parasite from the stools was strongly correlated with clinical improvement (Hoge *et al.*, 1995b).

Epidemiology

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. It is more than likely a water-borne parasitosis and water has been implicated in outbreaks in the United States, (Huang *et al.*, 1995) and in Nepal (Rabold *et al.*, 1994). Person to person transmission is unlikely since the oocyst requires a number of days after passage in the faeces to sporulate and become infectious. Food-borne transmission is also suspected with reports of finding oocysts in washing of leafy vegetables. Epidemiological evidence reported by the U.S. Centers for Disease Control and Prevention incriminated raspberries imported into the United States from Guatemala. In 1996 a total of 1465 cases of cyclosporiasis were reported from 55 cluster events; one-half were laboratory confirmed. Studies were carried out to parasitologically confirm raspberries as the vehicle of transmission, but the parasite was never recovered from raspberries, strawberries or other berries. The epidemics occurred between May 3 to June 14, 1996. Additional epidemics occurred at about the same of the year time in 1997, and once again raspberries from Guatemala were considered to be the means of transmission and again this was never parasitologically confirmed. At least 126 people developed an intestinal illness caused by *C.*

cayetanensis that epidemiologically implicated basil-pesto pasta salad. There were several events in which the people had eaten basil-pesto salad or the sauce. In another outbreak in Florida, baby lettuce or mesclun lettuce was implicated epidemiologically but not parasitologically. In addition to these cases approximately 1430 cases of cyclosporiasis were reported in 1997. It is possible that these foods were irrigated or washed with contaminated water. In some instances contaminated water may have been used to mix with insecticides and pesticides which were subsequently applied to food crops. Although many animals have been examined and experimentally exposed to infection, none have been found to be infected. In Africa, however, baboons have been found to be passing *Cyclospora-like* oocysts in the faeces (Smith *et al.*, 1996), but preliminary generic evidence suggests the organisms may not be *C. cayetanensis*. It has been proposed that birds may be natural hosts for the parasite since these animals are known to harbor many different species of Eimerian coccidia. Osterholm (1997) has suggested that wild birds in Guatemala may be a source of contaminating raspberries.

Human Prevalence

Most cases of cyclosporiasis have been reported from Haiti, Peru and Nepal until the outbreaks in 1996 and 1997 in the United States. There have, however, been few studies on the prevalence or incidence in population groups. In Haiti 450 HIV-Infected persons with chronic diarrhoea were examined and 11% were found infected with *C. cayetanensis* while 50 non-HIV infected adults with diarrhoea were not infected; 2000 infants with diarrhoea were also negative (Pape *et al.*, 1994). In Peru 18% of 26 children and 6% of 15 others 1-month to 2 years of age were positive. Nine of the 26 subjects and 2 of the 15 subjects had diarrhoea (Ortega *et al.*, 1993). In Nepal the organism was isolated in 10% of the diarrhoea cases seen in expatriates and tourists during the raining season and an incidence of 7% was found in 1992 in U.S. Embassy personnel and their dependents (Hoge *etal.*, 1993). In more recent reports 12 cases were diagnosed in U.S. Embassy staff in 1995 and 10 in 1996. In the same years the U.S. Embassy clinic labotatory detected 20 cases and 22 cases respectively in U.S. Peace Corps volunteers. Hoge *et al.* (1995a) also reporred 5% of Nepalese children under 5 yeats of age with diarrhoea positive for the organism while 2% of asymptomatic children over 18 months had the infection. None of 74 children over 18 months were infected. In a more recent study at the Kanti Children's Hospital in Kathmandu the stools of 180 children, 2 months to 13 years with diarrhoea were examined and 15 were found to have cyclosporiasis (Cross *et al.*, 1997). In continuing studies at this hospital and the Tribhuvan University Teaching Hospital children as well as adults were found to be infected. Prevalence data is not available for the United States but as reported above in 1996 a total of 1465 cases of cyclosporiasis were reported (Herwaldt *et al.*, 1997). The occurrence of the disease in 1997 is expected to equal or be greater than 1996. Few cases of cyclosporiasis have been reported out of Europe and those that have were usually in travelers to tropical lands. Few cases are reported out of the UK and according to a report in the ProMED healthnet in August 1997 only 53, 49 and 66 cases were reported in 1994, 1995 and 1996 respectively and 35 cases in the first 31 weeks in 1997.

Discussion

Although much is known about the clinical aspects of cyclosporiasis, little is known about the pathogenesis caused by the parasite. Patients suffer from a chronic watery diarrhoea, fatigue, nausea, vomiting, abdominal cramps, anorexia, weight loss, and myalgia. Howevet, it is not known what causes the symptoms. Studies of biopsied intestinal tissue have shown some but not all stages of the parasite as well as inflammatory changes, villous atrophy, crypt hyperplasia and surface epithelial disarray. More studies are tequired to establish the life cycle of the parasite in the intestines and the factors responsible for the pathogenesis.

The means of transmissions remains to be established. Water is probably an important vehicle, either drinking parasite contaminated water directly or indirectly when water is used to grow plant foods. *C. cayetanensis* has been recovered on several occasions from water and oocysts have been recovered from washing of lettuce and other leafy vegetables. Oocysts have also been found on Chinese parsley (Larry Ash, personal communication). No animal reservoir has been determined for the parasite but most investigators feel that there must be one other rhan humans. Although baboons and chimpanzees have been found infected with a *C. cayetanensis*-like parasite, no human infection have been found in the African locality where the monkeys came from. Furthermore preliminary genetic studies suggest the African organism to be similar but different than *C. cayetanensis*. Complete elucidation of the life cycle of this parasite must await the discovery of a suitable animal model.

The parasite can be visualized by direct microscopy and other methods of diagnosis but the present technology needs to be improved. Microscopic examination of stools requires high magnification, patience, perseverance and time. It is further recommended that several merhods of diagnosis be used to rule out the infection.

At a public meeting conducted by the U.S. Food and Drug Administration in July 1997 a review was presented on the current state of science relating to detection and control of *C. cayetanensis* on fresh produce. Much of the information presented in this paper were

discussed. One of the participants (Dr. Michael Osterholm from the Minnesota Department of Health) summarized the future research needs for cyclosporiasis as follows:

1. Methods of pasteurization of fresh food products must be developed to prevent infections with pathogenic agents.
2. Research on the propagation of the parasite.
3. Development of surveillance methods.
4. Development of better "trace-back" research.
5. Epidemiological investigations, case control studies.
6. Studies on natural history of the parasite; animal models, what happens in nature.
7. Determination of genetic sequences of recovered organisms, finger-printing, determine if outbreaks are related genetically.
8. Better methods of diagnosis and treatment.

The challenge is here and with diligence and dedication the problems will be solved. To go back to the old professor of protozology, we must "look and we will find" and someday we will know all that there is to know about cyclosporiasis and the elusive *C. cayetanensis*.

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