Human Infection of *Cyclospora cayetanensis*: A Review on its Medico-biological and Epidemiological Pattern in Global Scenario

Ghimire TR[a] and Sherchan JB[b]

Abstract

Introduction Newly emerging *Cyclospora cayetanensis* is a coccidian protozoan parasite found in the chronic diarrhoeal disease patient and in immunocompetent and immunocompromised patients worldwide and has limited biological, epidemiological recognition of disease pattern and treatment information in academic and applied medico-biological sciences.

Objectives The main aim of this study is to highlight the medico-biological and epidemiological characters of *Cyclospora* with especial emphasis to *C. cayetanensis* Ortega, Gilman and Sterling, 1994.

Methods This retrospective description is based on the reliable literatures available all over the world. Different full texts, Medline and abstracts or summary of the journals, reports, books, reliable and related websites and thesis published or unpublished from 1870 AD to 2005 AD have been used during the explanation of the subject.

Results *Cyclospora* was detected in different animals. It was reported in humans in 1979 and it was classified, and then reclassified in Genus *Cyclospora* in 1994. Classification is not seemed to be complete due to its homology with *Eimeria* species. Transmission of *Cyclospora* is through food, water, faeces, soil, domestic animals and arthropod vectors. It affects small intestine of human body. Symptoms include acute and chronic watery diarrhoea or protracted diarrhoea, abdominal discomfort, myalgia, nausea and vomiting with the average 7 day-incubation period. Extra-intestinal cyclosporiasis have been observed. Treatment consists of TMP-SMZ after the parasites are detected in stool by different diagnostic tools and techniques.

Conclusion The establishment of a reliable pathology lab, training on outbreaks and stool processing and examination and molecular diagnostic tools and techniques in food, water and animal faecal and histological materials are necessary to prevent *Cyclospora*.

Keywords *Cyclospora,* Review, Medico-biology, Epidemiology

Introduction

Cyclospora cayetanensis is a coccidian protozoan parasite found in chronic diarrhoeal disease patient and in immunocompetent and immunocompromised patients worldwide. There are no complete literatures that include the biological and epidemiological characteristic features of this parasite. So, this study has been conducted retrospectively to explain these characters.

Methods

This description is based on the reliable literatures available all over the world. The literatures were collected from the library of Central Department of Zoology, Kirtipur, Central Library, Kirtipur, library of Nepal Health Research Council, Ramshah Path, Internet websites, and Web database. Different full texts, Medline and abstracts or summary of the journals, reports, books, reliable and related websites and thesis published or unpublished from 1870 AD to 2005 AD have been used during the explanation of the subject. The contents contain the complete retrospective secondary data.

Corresponding Authors: Mr. Tirth Raj Ghimire, **E-mail:** ghimiretr@hotmail.com, [a]Department of Biology, Bagmati Modern College, Naxal; [b]Tribhuvan University Teaching Hospital, Maharajgunj, Kathmandu, Nepal.

Results

Historical Prospectus

First of all, the presence of a parasite with cyclosporan morphology was reported in the intestine of the mole *Talpa europaea* in 1870, but no name was given at this time[1]. The genus was created for the parasite *Cyclospora glomericola* Schneider 1881 from the millipede *Glomeris* (Diplopoda)[2]. The asexual and sexual stages of life cycle were elaborated for the mole parasite, *Cyclospora caryolytica* Schaudinn 1902[3]. *Cyclospora* has previously been found in myriapods[2], insectivores[3-8], reptiles[9], one murine host[10] and African green monkey, colobus monkey and olive baboon[11].

The first published report of *Cyclospora cayetanensis* in humans appears to be by Richard Ashford, the British parasitologist working in 1979. He found scanty organisms or unidentified *Isospora*-like coccidia in the faeces of three individuals (only 2 of the 3 infected persons were ill) in Papua New Guinea[12]. Sporulation was so delayed that he had almost discarded the specimens and so he remarked them unsporulated oocysts[13]. Subsequent reports of infection caused by these organisms isolated from faeces suggested the presence of a new pathogen that might be an unsporulated, coccidian body or a fungal spore[14], unsporulated coccidian[15], a flagellate[16], *Cryptosporidium* muris like oocysts[16,17], a large cryptosporidium[17], a blue green algae or cyanobacterium-like body or CLB[17-20] or a coccidian-like body[17-19]. It was described under the genus *Cyclospora*[21], then details about this coccidian[22] and finally a complete morphologic description and its taxonomic status on the basis of in-vitro sporulation, mechanical excystation and transmission electron microscopy[22] from Peruvian isolates of human-associated *Cyclospora* was published to validate the name as *Cyclospora cayetanensis* Ortega, Gilman and Sterling, 1994[23]. The nomen triviale combines the name of the university where the principal studies on this parasite were conducted cayetan- (Cayetano Heredia University, Lima, Peru) and -ensis (L., belonging to)[23].

Cyclospora cayetanensis (or a morphologically similar species) has been reported in duck from Peru[24], in baboons and chimpanzees from Tanzania[25], in dogs from Brazil[26], in chicken[27] from Mexico and in chicken, dogs, rats, mice and monkey from Nepal[28-32]. It is not known whether this *Cyclospora* sp. represents *Cyclospora cayetanensis* Ortega, Gilman and Sterling, 1994[21] or other species. Nucleotide sequence variability in the first internal transcribed spacer regions within *Cyclospora cayetanensis* from different geographic origins has been observed and suggests the existence of multiple strains[33,34]. *Cyclospora cayetanensis* is more phylogenetically related to *Eimeria mitis* and *Eimeria tenella* than to *Cryptosporidium* spp[35,36]. There has been much debate as to where to place *Cyclospora cayetanensis* taxonomically due to its homology with *Eimeria* species[37]. The taxonomic status of *Cyclospora* on the basis of modern system[38] can be described as: Kingdom: Protozoa (Goldfuss, 1818) R. Owen, 1858, Subkingdom: Biciliata, Infrakingdom: Alveolata Cavalier-Smith, 1991, Phylum: Myzozoa Cavalier-Smith and Chao, 2004, Subphylum: Apicomplexa Levine, 1970, Class: Conoidasida Levine, 1988, Subclass: Coccidiasina Leuckart, 1879, Order: Eucoccidiorida Léger & Duboscq, 1910, Suborder: Eimeriorina Léger, 1911, Family: Eimeriidae Minchin, 1903, Genus: *Cyclospora* Schneider, 1881, Species: *Cyclospora cayetanensis* Ortega, Gilman and Sterling, 1994.

Morphology

Different parasitologists explained the asexual and sexual stages of the *Cyclospora cayetanensis* on the basis of morphologic and electron microscopic studies[39,40]. It is a polymorphic intracellular coccidian parasite and consists of the following morphological stages in its life cycle:

Oocyst: The infective stage of this coccidian is the sporulated 'oocyst,' which is the environmentally resistant cystic stage. Only unsporulated oocysts come out of the host during shedding of oocysts in the faeces. These oocysts need time (days or weeks) after being passed in bowel movements to develop into an infectious stage. The immature oocyst is uniformly spheroidal with a diameter of 8-10 µm (7.7-9.9µm).This oocyst has a 113 nm thick bilayered wall. The outer rough coat is 63 nm thick, while the inner smooth layer is 50 nm thick. A polar body and ooocyst residuum are present. The unsporulated oocysts may have a granular cytoplasm. They appear as non-refractile, round, hyaline structures containing an arrangement of refractile membrane with 6-9 bound globules. The mature oocysts have a fibrillar coat and cell wall similar to that of the unsporulated oocysts[23]. Ozone at appropriate concentration inactivates these oocysts in water[41]. They survive in the standard chlorination process. They are unlikely to survive the temperature achieved in anaerobic digestion and do not survive well under low moisture conditions[42]. The unsporulated oocysts are killed by the gamma irradiation of ^{137}Cs at 0.5 K Gy on the fruits and vegetables[43].

Sporocyst: Each oocyst contains two ovoidal sporocysts with 6.3 µm long and 4.0 µm broad. Each

sporocyst has a of 62 nm thick wall. Sporocyst has Stieda and substieda bodies. Sporocyst residuum has large spherical globules[23].

Sporozoite: Spore formula of this coccidian is 0.2.2 *Cyclospora* A.Schn[3]. It has an oocyst with 2 sporocysts, each with 2 sporozoites with a total of 4 sporozoites. The size of each sporozoite is 9.0 µm long and 1.2 µm wide[23]. They are slender and crescent-or spindle-shaped, motile and are infective.

Trophozoite: The trophozoite is intracellular and is quite incapable of any kind of movement. It is about 4 µm long and 2 µm wide in size.

Merozoites: There are two types of meronts: the first meront (meront I) having 8-12 merozoites and the second meront (meront II) having 4 merozoites. These meronts are about 3-4 µm long and 0.5µm wide[44]. The merozoites are banana-shaped[45].

Microgamonts: The 'meront II' forms male gamonts known as microgamonts or microgametocytes. Inside microgametocytes, numerous flagellated sperm-like motile microgametes are formed due to multiple fission.

Macrogamonts: Most of the 'meront II' forms female gamonts known as macrogamonts or macrogametocytes. They are larger in size than microgamonts.

Life Cycle

It is a monoxenous parasite because life cycle is completed in only one host[39,40]. Life cycle in human *Cyclospora* is similar to that observed in animal species. It is completed in two phases as: exogenous and endogenous. The stage, which takes place outside the body of the human hosts, is called exogenous stage. Further development of sporocysts and sporozoites is called as sporogony or sporulation, which occurs only in the presence of appropriate atmospheric oxygen concentrations, temperature, humidity etc. It takes place from two weeks at 25^0C and up to four to six months at 4^0C[46]after oocysts are passed into stool.

The endogenous stage takes place inside human body. Human beings are infected when they ingest the sporulated oocysts by swallowing contaminated food or by drinking contaminated water. After ingestion of oocysts, the sporozoites are liberated from sporocysts and oocysts in the small intestine due to special stimuli such as an anaerobic environment in the presence of bile salts and enzymes. These sporozoites invade the enterocytes of jejunum and reside intracellular in the parasitophorous vacuole at the luminal end of the cells. The merogonic or schizogonic or asexual stage involves the maturation and development of meronts to reproduce and multiply in the infected cell or to infect enterocyte. This allows the infection to spread too many enterocyte, even if the host is not exposed repeatedly to the organisms. The sporozoites change into round shaped trophozoite and then increase in number by mitosis (schizogony or merogony) to form first generation meronts. These first generation meronts invade new host cells and again increase in number by mitosis forming second generation meronts. The gametogonic or sexual stage involves the maturation and development of sporozoites enclosed in cysts or spores. The meront II penetrates new enterocyte cells to form male and female gametocytes (stimuli unknown). Among the second generation meronts, some develop into microgamonts while most develop into macrogamonts which further give rise to macrogamete. Some microgametocytes undergo multiple fission to form numerous sperm-like 'microgametes'. Mature microgametes exit the microgametocyte and fertilize the macrogamete. The zygote then changes into an unsporulated fertile oocyst. As the infected enterocytes die, the oocysts or spores are sloughed into the gut lumen along with the host cells into the external environment with the faeces[39,40,44,45]. Certain abnormal cases of the persistence and further multiplication of the reduction nuclei of the female element (i.e., the nuclear portions given off during maturation); followed by multiple fertilization have been observed[3].

Geographical Distribution and Prevalence

Cyclospora cayetanensis has now been identified worldwide in the faeces of both immunocompetent and immunocompromised patients with diarrhoea[15-20,47-50]. The infection from international travel related countries are Australia, Belgium, Czech Republic, Germany, Greece, Ireland, Italy, Japan, The Netherlands, Spain, Switzerland, the United Kingdom, the United States where prevalence is about more or equal to 4 percent of the returning travelers with diarrhoea. The infected travellers were found to be return from Haiti, Indonesia, Mexico, Nepal, Puerto Rico, Morocco, Cambodia, Pakistan, India and the Solomon Islands[50-57]. The endemic areas are in Bangladesh, Brazil, Chile, China, Cuba, Dominican Republic, Egypt, Guatemala, Haiti, India, Indonesia, Jordan, Mexico, Morocco, Nepal, Nigeria, Pakistan, Peru, Puerto Rico, Romania, Saudi Arabia, Tanzania, Thailand, Turkey, Venezuela, Viet Nam, and Zimbabwe. In these areas, the prevalence varies significantly with season and year, 1-15 percent being

the highest in spring and early summer. Children accounts for 70-80 percent cases, asymptomatic rate in adults (>10-20 years) is higher than in children and infection rate in those with HIV is significantly higher than overall prevalence[50,52].

The prevalence of *Cyclospora* is different in different countries. It is 1.8 percent-85.7 percent[28-30,50, 58-66] in Nepal, 5.3 percent-9.8 percent[67,68] in Venezuela, 1.6 percent-18 percent[22,23,69] in Peru, 2.5 percent-22.5 percent[70-73] in Haiti, 0.9-3.8 percent[74-76] in Guatemala, 1 percent[77] in Tanzania, 2.2 percent[78] in Thailand, 6.8 percent[79] in Honduras, 3 percent[80] in Cuba, 1.3 percent-5.6 percent[81-83] in Egypt, 0.7 percent-3.3 percent[84,85] in India, 3.7 percent[86] in Ethiopia, 0.3 percent-50.0 percent[20, 87-89] in the United States, 0.07 percent in the United Kingdom[90], 6.4 percent in Indonesia[91], 0.99 percent[92] in Nigeria, 3 percent[93] in Mexico, 11 percent[94] in Saudi Arabia, 6 percent[95] in Jordan, 2.3 percent[96] in china, 1.1 percent[97] in Albania.

Clinical Features

Evidence favouring a role for this organism as a pathogen includes a significant association of oocysts with clinical illness (in absence of other known pathogens), clinical response to antimicrobial therapy, and clearance of organisms coincident with clinical resolution[19,59,72,98].

The time between becoming infected and shedding of oocysts through the faeces is about 7 days[50]. The incubation period is about 1-11 days[52] or 2 to 11 days (average 1 week[89,99]), but may be as short as 12 to 24 hours[50] or12 hours to 11 days[100].

Human cyclosporiasis is clinically indistinguishable, however, from cryptosporidiosis, isosporiosis, giardiasis and microsporidiosis because of the similar clinical features. Flu like syndrome with myalgias and arthralagias may precede the onset of diarrhoea[99]. Unusual fever but when present it is low grade. In the presence of moderate to severe dehydration, compensatory tachycardia, systolic blood pressure (SBP<90 mmHg) and decreased skin turgid may occur. But mild infection produces few or no clinical symptoms[28-30,50,62,63,98]. The frequency with which asymptomatic infection is identified depends on the sensitivity of the assay used to detect infection and on the immunologic status of the patients studied.

The symptoms are: relapsing[87,89,100,101-103], non blooded[87], watery[20] and self limited diarrhoea with duration of 4 weeks[17,23,99,104-106] to 18 months[19], 2 to 6 weeks[107], 1-5 weeks[20], 12 days to 8 weeks[87], 94 days[108], about 28±8 and 37±12 days[109], mean (±SD) 43 ±24 days[19], more than 3 weeks[89], 6-7 weeks or several months[52] , 6-8 weeks[100], 3 days and many extended periods[110], several months to a year[52], many extended periods[110], 6-60 days[111], 6 weeks to 3 months[112], 3-25 days[23,98] in immunocompetent patients, but 3 months[100] to more than 6 months[50] in AIDS patients and several days in protein energy malnourished children[82], e"1 month in Hepatitis B, cancer and tuberculosis patients[50]. Other symptoms include weight loss[50,89,101], fatigue[20,50,89], anorexia[20,50,89], flatulence[101], abdominal discomfort[50,101,103], nausea[50,101], fatigue[50,102], fever[50,103]. Acute diarrhoea[50,77,113] is usually sudden in onset and associated with frequent, watery, loose stool, flatulence, and abdominal pain[50,110]. Symptoms usually subside within 72 hours of onset. The onset of illness may be abrupt (68%) or gradual (32%). After a few days, acute symptoms subside and then may recur (61% of cases) in a waxing-waning pattern[89]. Chronic diarrhoea[50,86], on the other hand, involves frequent attacks over many extended periods[110] and is the symptom of immunodeficient patients[50,114-116]. Weight loss, anorexia, and chronic weakness often coincide[50]. Cyclosporiasis is not typically a fatal disease. The only potential for a fatal outcome is critical dehydration secondary to protracted diarrhoea, particularly in children and HIV/AIDS patients who have not had proper rehydration[50].

Pathogenesis

The infection caused by *Cyclospora cayetanensis* leads to watery diarrhoea with the absence of any leucocytes or blood, indicating that probably the diarrhoea may be due to some, as yet unidentified toxin. Enterocytes are believed to be invaded by sporozoites, causing release of cytokines from epithelial cells[117]. Cytokines, in turn, activate and recruit phagocytes from the blood. These phagocytes release factors such as histamine, prostaglandins, and platelet-aggregating factors that increase intestinal secretion of chloride and water and inhibit absorption[118,119]. Another mechanism of enterocyte damage, besides direct damage caused by the parasite, is inflammation; T cells, proteases, and oxidants secreted from mast cells are responsible for this process[117].Consequently, erythema of the distal duodenum has been observed during upper endoscopic procedures[120]. Ultimately, marked destruction of enterocytes causes nutrient malabsorption and increased secretion of fluids and electrolytes from the gut, resulting into secretory and osmotic diarrhoea[121].

Impaired D-xylose absorption has been observed. This implies proximal small intestinal

involvement[19,120,122]. Duodenal and jejunal biopsies have shown blunting and atrophy of villi with crypt hyperplasia. Villous crypt ratio becomes 0.6:1.5, while its normal value is 3:1 to 4:1. There is mild to moderate inflammation of lamina propria with increased number of plasma cells[120]. There are focal vacuolation, loss of brush border and change in epithelial cells from columnar to cubical[122]. The changes occur in last part of duodenum and jejunum and jejunal biopsy shows an altered mucosal architecture with shortening and widening of the intestinal villi due to diffuse oedema and infiltration by a mixed inflammatory cell infiltrate[44]. There is a reactive hyperaemia with vascular dilation and congestion of villous capillaries. Parasitophorous vacuoles, located at the luminal end of the jejunal epithelium, contain sexual and asexual forms of parasites resulting into a supranuclear location within enterocytes[40,44,101]. The inflammatory changes associated with *Cyclospora* infection may persist beyond parasite eradication[111]. Besides, there is mild diffuse gastritis[112] and increased excretion of faecal fat[19,120].

The extra-intestinal complications of *Cyclospora*, such as, biliary disease (cholangitis)[108], Guillain-Barre syndrome or acute febrile polyneuritis[123], Reiter syndrome [Reactive arthritis syndrome (RAS)][124], acalculus cholecystitis[125], pulmonary infection[126] and low haemoglobin concentration in the absence of efficient immune system[64] have been recorded in some chronic cyclosporiasis patients.

Vector and Reservoir: There are no biologic vectors though arthropods and rodents can act as mechanical vectors or carriers of cyclosporiasis. Life cycle is completed only in human body[39,40]. Human beings are the only important reservoir hosts of *Cyclospora cayetanensis.* There are no domestic animals to be the reservoir host for *Cyclospora*[127,128]. The detection of *Cyclospora* in the previous studies[24-32] arises the question whether these animals (duck, chicken, rat, mice and monkey) may act as paratenic[24] or reservoir hosts. But we can't predict them as reservoir hosts because these studies are based on cross-sectional description using microscopic method that may lead to error and bias. Only one study[31] confirmed *Cyclospora* by molecular method, but it studied only stool not the histological materials. The positivity in these animals might be either due to cross-contamination of human and animal stool through rain water or sewage or the detected species might be other species such as *Hammondia heydorni* or *Neospora caninum* from dogs and *Eimeria mitis* or *Eimeria tenella* or other *Eimeria* species from chicken and ducks or they might be pseudoparasites.

The case may be mild, missed or severe. A case or carrier is infectious as long as the parasites appear in stool. Some untreated persons excrete oocysts for >1 month after symptoms resolve[19,129] or have symptoms for several weeks longer than oocyst excretion is documented[98] and some diarrhoeal children excrete for a mean of 22-23 days (range, 7-70)[22]. Among treated patients, excretion of oocysts usually stops during therapy or by several days to 1 week after therapy[59,91,130,131] . The carriers may be temporary in immunocompetent hosts or chronic in immunocompromised hosts[50].

Infective material: Faeces, food and water containing the sporulated oocysts of *Cyclospora cayetanensis* are infective[50,74,75]. However, immediate source of infection is the soil contaminated with infective oocysts[74,75].

Source of infection: The primary sources of infection are faeces of cases or carriers; the secondary sources are contaminated water, food, fingers, flies and milk mixed with contaminated water.

Period of Infectivity: It remains as long as the person harbours the parasites. The histological changes occurred in enterocytes may remain after parasite eradication[111].

Infective Dose: Virulence and characteristics of *Cyclospora* necessary to infect human hosts are unknown. Differences in virulence characteristics of *Cyclospora cayetanensis* isolates appear not to have been a major factor in failing to establish infection[132].

Host Factors

Age and Sex: Although all age groups can acquire the disease, the most vulnerable age group seems to be less than 1 year to 15 years of children[30,50,63,64] with the highest attack rates among children older than 18 months[59]. The respective lowest and highest *Cyclospora* infected patient was 12-day-infant and 87-year-old man[63]. No sexual predilection for cyclosporiasis appears to exist.

Race: No racial predilection for this infection appears to exist.

Socio-Economic Status: The low socio-economic groups of people are more susceptible to *Cyclospora* than the people of high socio-economic groups.

Occupation: People who work in various farming practiced soil, municipality sewage and river water

resources have a great risk of being infected by *Cyclospora*. People with the occupation of sex work, farming, kitchen activities, hospital works, and school children are the vulnerable groups for *Cyclospora*[50,63,64,76].

Immunology: Study of immune response to *Cyclospora* has been hampered by the absence of an animal model of this infection. Immunity may not occur after infection[108] however, some amount of immunity may be present in adults who are exposed to the infection as the infection is less prevalent in adults living in endemic areas[30,50,62-64]. High oocysts frequency case has been observed in the stool of diarrhoeal persons[63] and AIDS patients[50]. It suggests that the infection with *Cyclospora* with increased frequency and severity in diarrhoeal and AIDS patients shows that immune mechanisms effectively keep parasite numbers low in most normal persons[50,63]. Re-infection can occur[98,111,120], but appears to become less common after repeated exposure.

Environmental factors: Cyclosporiasis appears to be seasonal, with peak incident during the rainy seasons from April to June in Peru[22,98] and May to September in Nepal[28-30,60-63]. 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[98]. This shows the environmental factors guide *Cyclospora* appearance. As the unsporulated oocysts require some days for sporulation outside the human beings, the environment may trigger the development process in the oocysts. Naturally occurring *Cyclospora* oocysts may survive for extended periods in the environment, given the marked seasonality of infection in areas where the disease is endemic[133].

The most intriguing environmental issue is oocyst sporulation time. The oocysts subjected to -20°C for 24 hours and exposure to 60°C for 1 hour cannot be induced to sporulate. Oocyst storage at 4°C or 37°C for 14 days retards sporulation[134]. *Cyclospora* requires 1 to 2 weeks to completely sporulate and become infectious under ambient conditions of 25°C to 30°C[23]. So, direct person-to-person *Cyclospora* transmission is unlikely. The prolonged sporulation time would imply that oocysts favour a moist environment, ideally water.

Mode of Transmission: Faecal-oral route is the usual mode of transmission of *Cyclospora*[73,74]. Indirect transmission is the usual mode of transmission and it consists of 4F's-"Flies, Fingers, Fomites and Food". Epidemiologic data indicate that the human-associated *Cyclospora* is transmitted by water and by food [98,135,136].

Waterborne Transmission: A number of waterborne outbreaks of cyclosporiasis have been reported from different regions. The Chicago outbreak was apparently due to the drinking of water supplied from a roof top reservoir[129]. There are other reports such as in Chicago, where an 8-year-old child was found to be infected with *Cyclospora* after swimming in Lake Michigan[51] and in Utah, a man passed oocysts after cleaning his basement, which had been flooded by sewage backup following heavy rains[137]. The man's house was located near a dairy farm and much of the sewage backup was attributed to water runoff from this site. Besides, consumption of well water was implicated in the infection of *Cyclospora* in Massachusetts[87] and in Haiti[70].

In Nepal, in the first outbreak in summer 1992, expatriates, who were more likely to drink untreated water or milk reconstituted with water, became ill with diarrhoea and passed oocysts[98]. The second waterborne disease outbreak occurred in 1994, 12 of 14 British soldiers and dependents stationed in a small military base detachment in Pokhara, Nepal, despite chlorination of the water involved. In this outbreak, *Cyclospora* oocysts were demonstrated for the first time in drinking water, which consisted of a mixture of river and municipal water[58].

Though not directly connected with water-associated disease outbreaks, *Cyclospora* oocysts have been isolated and confirmed from the sewage and river[28,30,62,63] and municipal pipe water[63] in Nepal and from wastewater in sewage lagoons adjacent to Lima, Peru[138].

Foodborne Transmission: It is sometimes referred to as the "yuppie disease" due to outbreaks in the United States from faecally-contaminated imported raspberries. Foodborne transmission was suspected when consumption of raw or undercooked meat and poultry products was reported as part of case histories before the infectious organism was identified as *Cyclospora*[12,15]. Foodborne transmission was first suggested in 1995 when the illness of an airline pilot was associated with food prepared in a Haitian kitchen and brought on board the airplane[139]. Approximately 90 percent of cyclosporiasis cases in the United States are thought to be food borne, amounting to an estimated 14,638 cases each year[140].

Raspberries from Guatemala, blackberries from Guatemala or undetermined source, mesclun (young

salad greens, such as spring mix, field greens, baby greens, gourmet salad mix) from Peru or United States and basil from Mexico or United States are epidemiologically linked with *Cyclospora* outbreaks in Canada and US and lettuce imported from Southern France and Southern Italy in Germany and that of watercress in Mexico[52,141].

A number of food items, especially fresh fruits and vegetables[101] or dishes containing these items such as raspberries[142], strawberries, blackberries and blueberries, mesclun lettuce and basil[143], cabbage, lettuce and mustard leaves[28-30], radish, cauliflowers, mustard leaves[63] and 2 herbs (*Huacatany* and *Yerba buena*) and many vegetables[144] have been implicated in spread of *Cyclospora cayetanensis*. The vegetables and fruits are probably contaminated due to either use of sewage for irrigation or use of contaminated water for washing these items. Washing vegetables does not completely remove *Cyclospora* oocysts[63,144]. Experimental studies have shown that oocysts may be concentrated in fresh water clams[144].

From 2002 to 2004, there were 31 *Cyclospora* positive cases from a total of 10 outbreaks [3 school outbreaks, 4 school trips out breaks and 3 religious ceremony outbreaks] in Kathmandu valley[63]. But this study lacks epidemiologic surveillance study though it is based on positive laboratory results on the basis of direct wet mount, acid-fast staining, ocular micrometer and bisporulation assay and is far from pseudoinfection.

Laboratory Diagnosis

Unstained Wet Mount Preparation: The oocysts are excreted in low numbers from an infected patient. So, identification of oocysts in concentrated stool specimens require 3 or more stool specimens collected 2 days apart. A single negative stool specimen does not rule out the diagnosis. *Cyclospora cayetanensis* can be detected by unstained (0.9% w/v) normal saline or 2.5 percent (w/v) potassium dichromate solution at X400 magnification. Measurement of oocysts is necessary to differentiate from the oocysts of *Cryptosporidium* (4-6 µm in diameter), cysts of *Entamoeba histolytica* (10-15 µm diameter), cysts of *Entamoeba hartmanni* (5-10 µm diameter), cysts of *Entamoeba histolytica* (10-30 µm diameter), cysts of *Giardia lamblia* (14 µm×7µm) and oocysts of *Isospora* (20-33 µm×10-19 µm in size), while *Cyclospora* oocysts are 8-10 µm in diameter[22,23]. The modified Ziehl Neelsen technique stains *Cyclospora* variably. In fresh faecal samples, most of the oocysts show good staining, while in older samples majority may be decolorized[146]. Recent assessment of different procedures that included routine trichrome[147] modified trichrome[148], Giemsa, chromotrope, Gram-chromotrope, Kinyoun acid-fast[149], auramine-rhodamine[150] and safranin stains indicated that heating of faecal smears prior to safranin-based staining yield a uniform, fast, reliable and easy to perform procedure that was superior to acid-fast staining[95,151]. Compared to Ziehl Neelsen technique, sensitivity of different procedures is: wet mount 75 percent, safranin 30 percent and auramine 23 percent[95]. Concentration methods may enhance detection of low numbers of oocysts. The methods used may be Sucrose Centrifugal Flotation Method, Formalin-ethyl acetate concentration [65,152]. The wet mount technique, stool staining and concentrations methods are usually followed by bisporulation assay and ocular micrometer for the confirm detection of *Cyclospora cayetanensis*[50,74,75,152].

Primary fluorescence of oocysts is a feature that appears to be unique to *Cyclospora* oocysts. It is more sensitive than Ziehl-Neelsen staining[150]. The method also allows the detection of oocysts even if they are covered with faecal debris[18,150]. The colour of the oocysts appear neon blue and green at the excitation wavelength of 330-380 nm and 450-490 nm fluorescent light respectively[153]. Although monoclonal antibodies (immunological reagents) are not yet commercially available[154], epifluorescence microscopy may still be used microscopy, however, is often a tedious and time-consuming procedure, potentially leading to analyst fatigue and false-negative results. The estimated sensitivity of acid-fast staining versus fluorescence microscopy is 78 percent[155].

Flow cytometry appears to be a useful alternative to microscopy for the screening of large numbers of stool specimens for *Cyclospora* oocysts, such as in an outbreak situation[156].

Distal duodenal biopsy and aspiration specimens reveal acute and chronic inflammation, crypt hyperplasia, epithelial disarray and partial villous atrophy[19,39,40,101,120,122]. The morphology of *Cyclospora* in the intestine is similar to that of *Isospora*, but differs from *Cryptosporidium*. The morphology of the oocysts of *Cyclospora* resembles that of Cryptosporidium, but differs from that of *Isospora*. Thus, a combined study of both stool and intestinal biopsy should readily distinguish *Cyclospora* from *Cryptosporidium* and *Isospora*[39].

Recent outbreaks[157], as well as the finding of *C. cayetanensis* on vegetables[158] have stimulated

interest in the development of molecular diagnostic methods to detect this organism when recovered from foods. Polymerase Chain Reaction (PCR) is the most sensitive method of detection[31,34,159-170]. PCR has the potential to be more sensitive than microscopy[166], but it cannot distinguish between sporulated and unsporulated oocysts. Besides, it is expensive and is rarely found in routine laboratory.

Treatment

The drug of choice is trimethoprim–sulfamethoxazole (TMP-SMZ, bactrim, bactrim DS, septra, septra DS, cotrim)[59] with respective half lives 11 hours and 10 hours. Combination of these two antibiotics inhibits two sequential steps in bacterial folate synthesis and has a wide spectrum of this drug. It is usually administered orally, but, can be administered intravenously if the patient can't tolerate per oral medications because of nausea, vomiting or underlying gastrointestinal problems. After oral administration, TMP peaks by 2 hours and SMZ by 4 hours. Children should receive TMP 5 mg/Kg plus SMZ 25 mg/Kg body weight twice a day for at least 7 days. The adult dose is 160/800 mg PO bid for 7 days while in immunocompromised patients with AIDS; the dose is 160/800 mg PO qid for 10 days. In children less than 2 months, it is not administered because side effects are anaemia, hypersensitivity and hepatic damage[171,172].

In immunocompromised patients, long-term secondary prophylaxis is necessary to prevent recurrence, which occurs in approximately 50 percent of patients[50,72,100,173]. And an effective sulfa-free alternative is needed for treatment and prevention of cyclosporiasis.

Conclusion

Cyclospora cayetanensis is endemic but enigmatic parasite in Nepal. There are four reasons why the prevalence in Nepal is too high: An untrained observer might confuse *Cyclospora* with *Entamoeba* and *Cryptosporidium,* an acid-fast staining, bisporulation assay and ocular micrometer are ignored during stool examination, trimethoprim-sulfamethoxazole prophylaxis is rarely used and *Cyclospora* have an underlying high prevalence in Nepalese people from the beginning years when a large numbers of cases were observed[20] and it is due to due to poor socio-economic status and human behavior[50,63].

In 1995 and 1996, Sherchand and Cross has extensively studied on *Cyclospora* among children and presented in XIV international congress for Tropical Medicine and Malaria[174]. The study was extended by Cross and Sherchand in different areas of Nepal[175]. From these times, this coccidian has been regularly reported in well documented cases in Nepal. Continuous diagnosis of *Cyclospora* in immnocompetent and immunocompromised patients and several green leafy vegetables and sewage, river and municipal water in Nepal shows that this is endemic country for *Cyclospora cayetanensis*.

The most importance gaining by *Cyclospora* in Nepal is due to the rapidly growing HIV/AIDS cases and a greatly high prevalence of this opportunistic parasite[50]. There are also reports from animal faeces on the basis of microscopic examination and PCR/ RFLP analysis but without tissue analysis. There is no evidence of zoonotic transmission of human and animal *Cyclospora*[127,128]. We can't easily explain its morphological, taxonomical and epidemiological features due to many encumbrances that have leaded them enigma. So, further study should be made by collecting the histological materials of cyclosporiasis animals from Kathmandu valley. Besides, there must be proof of epidemiological outbreaks of *Cyclospora* in Nepal during rainy season. Thus establishment of a reliable pathology lab, training on outbreaks and stool processing and examination and molecular diagnostic tools and techniques in food, water and animal faecal and histological materials are necessary in Nepal.

References

1. Eimer T. Ueber die ei-und Kugelförmigen sogenannten Psorospermien der Wirbelthiere, A Stuberr's Verlangshandlung, Würzburg, Germany, 1870.
2. Schneider A. Sur Les Psorospermies Oviformes Ou Coccidies: Especes Nouvelles Ou Peu Connues. *Arch Zool Exp* 1881; 1: 387-404.
3. Schaudinn F. Studien über krankheitserregende Protozoen I. *Cyclospora caryolitica* Shaud., der Erreger der perniciösen Enteritis des Maulwurfs. *Arb K Gesundheitsamte* 1902; 18: 378-416.
4. Pellerdy L, Tanyi J. *Cyclospora talpae* sp. n. (Protozoa: Sporozoa) From the Liver of *Talpa europaea*. *Folia Parasitol (Praha)* 1968; 15: 275-77.
5. Duszynski DW, Wattam AR. Coccidian Parasites (Apicomplexa: Eimeriidae) From Insectivores. IV. Four New Species in *Talpa europaea* From England. *J Protozool* 1988; 35: 58-62.

6. Ford P, Duszynski. Coccidian Parasites from insectivores: VI six new species from the eastern mole, *Scalopus aquaticus*. *J Protozool* 1988; 35: 223-6.
7. Ford PL, Duszynski DW. Coccidian parasites (Apicomplexa: Eimeriidea) from insectivores. VII. Six new species from the hairy-tailed mole, *Parascalops breweri*. *J Parasitol* 1989; 75: 508-13.
8. Mohamed HA, Molyneux DH. Developmental stages of *Cyclospora talpae* in the liver and bile duct of the mole (*Talpa europaea*). *Parasitology* 1990; 101:345-50.
9. Pellerdy L. Coccidia and Coccidiosis. 2nd edition. Berlin, Germany: Paul Parey, 1974: 959.
10. Ford PL, Duszynski DW, McAllister CT. Coccidia (Apicomplexa) from heteromyid rodents in the southwestern United States, Baja California, and northern Mexico with three new species from *Chaetodipus hispidus*. *J Parasitol* 1990; 76: 325-31.
11. Eberhard ML, da Silva AJ, Liley BG, Pieniazek NJ. Morphologic and molecular characterization of new *Cyclospora* species from Ethiopian monkeys: *C. cercopitheci* sp. n., *C. colobi* sp. n., and *C. papionis* sp. n. *Emerg Infect Dis* 1999; 5: 651-8.
12. Ashford RW. Occurrence of an undescribed coccidian in man in Papua New Guinea. *Ann Trop Med Parasitol* 1979; 73: 497-500.
13. Ashford RW, Joshi AB. *Cyclospora cayetanensis*: is its emergence real or only apparent? *Health Renaissance* 1999; 1: 55-60.
14. Soave R, Dubey JP, Ramos LJ, Tummings M. A new intestinal pathogen? *Clin Res* 1986; 34:533A.
15. Hart AS, Ridinger MT, Soundarajan R, Peters CS, Swiatlo AL, Kocka FE. Novel organisms associated with chronic diarrhoea in AIDS. *Lancet* 1990; 335:169-70.
16. Naranjo J, Sterling CR, Gilman R. *Cryptosporidium muris*-like objects from faecal samples of Peruvians. presented at the 38th annual meeting of the American Society of Tropical Medicine and Hygiene, Honolulu, December 10-14, 1989.
17. Long EG, Ebrahimzadeh A, White EH, Swisher B, Callaway CS. Alga associated with diarrhoea in patients with acquired immunodeficiency syndrome and in travellers. *J Clin Microbiol* 1990; 28: 1101-4.
18. Long EG, White EH, Carmichael WW et al. Morphologic and staining Characteristics of a cyanobacterium-like organism associated with diarrhoea. *J Infect Dis* 1991; 164:199-202.
19. Shlim DR, Cohen MT, Eaton M, Rajah R, Long EG, Ungar BL. An alga-like organism associated with an outbreak of prolonged diarrhoea among foreigners in Nepal. *Am J Trop Med Hyg* 1991; 45:383-9.
20. Outbreaks of diarrhoeal illness associated with cyanobacteria (blue-green algae)-like bodies-Chicago and Nepal, 1989 and 1990. *MMWR Morb Mortal Wkly Rep* 1991; 40: 325-7.
21. Ortega YR et al. 1992. *Cyclospora cayetanensis*: A new protozoan pathogen of humans. Abstract 289. In: Proceedings of the 41st annual meeting of the American Society for Tropical Medicine and Hygiene. p. 210
22. 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: 1308-12.
23. Ortega YR, Gilman RH and Sterling CR. A new coccidian parasite (Apicomplexa: Eimeriidae) from humans. *J Parasitol* 1994; 80: 625-9.
24. Zerpa R, Uchima N and Huicho L. *Cyclospora cayetanensis* associated with watery diarrhoea in Peruvian patients. *J Trop Med Hyg* 1995; 85: 325-9.
25. Smith HV, Paton CA, Girdwood RW, Mitambo MM. *Cyclospora* in non-human primates in Gombe, Tanzania (letter). *Vet Rec* 1996; 138: 528.
26. Yai LE, Bauab AR, Hirshfeld MP, de Oliveira ML, Damaceno JY. The first two cases of *Cyclospora* in dogs, Sao Paulo, Brazil. *Rev Inst Med Trop Sao Paulo* 1997; 39: 177-9.
27. Garcia-Lopez HL, Rodriguez-Tovar LE, Medina-Dela Garza CE. Identification of *Cyclospora* in poultry. *Emerg Infect Dis* 1996; 2: 356-7.
28. Sherchand JB, Cross JH, Jimba M, Sherchand S, Shrestha MP. Study of *Cyclospora* cayetanensis in Health Care Facilities, Sewage Water and Green Leafy Vegetables in Nepal. *Southeast Asian J Trop Med Public Health* 1999; 30: 58-63.
29. Sherchand JB, Cross JH. Emerging pathogen *Cyclospora cayetanensis* infection in Nepal. *Southeast Asian J Trop Med Public Health* 2001; 32: 143–50.

30. Sherchand JB, Cross JH. *Cyclospora cayetanensis* in Nepal: A study of epidemiological and microbial aspects. *J Nepal Health Research Council* 2003; 3:1-8.

31. Chu D-M T, Sherchand JB, Cross JH, Orlandi PA. Detection of *Cyclospora cayetanensis* in animal faecal isolates from Nepal using an FTA filter-base polymerase chain reaction method. *Am J Trop Med Hyg* 2004; 71: 373-9.

32. Sherchand JB, Cross JH. Parasitic epidemiological studies of *Cyclospora cayetanensis* in Nepal. *Southeast Asian J Trop Med Pub Health* 2004; 35:1-8.

33. Okhuysen PC, Chappell CL, Crabb JH, Sterling CR, Dupont HL. Virulence of three distinct *Cryptosporidium parvum* isolates for healthy adults. *J Infect Dis* 1999; 180: 1275-81.

34. Olivier C, Van de Pas S, Lepp PW, Yoder K, Relman DA. Sequence variability in the first internal transcribed spacer region within and among *Cyclospora* species is consistent with polyparasitism. *Int J Parasitol* 2001; 31: 1475-87.

35. Relman DA, Schmidt TM, Gajadhar A, Sogin M, Cross JH, Yoder K et al. Molecular phylogenetic analysis of *Cyclospora*, the human intestinal pathogen , suggests that it is closely related to *Eimeria* species. *J Infect Dis* 1996; 173: 440-5.

36. Pieniazek NJ, Herwaldt BL. Re-evaluating the molecular taxonomy: is human-associated *Cyclospora* a mammalian *Eimeria* species? *Emerg Infect Dis* 1997; 3: 381-3.

37. Orlandi PA, Carter L, Brinker AM, daSilva AJ, Chu D-MT, Lampel KA et al.. Targeting single-nucleotide polymorphisms in the 18S rRNA gene to differentiate *Cyclospora* species from *Eimeria* species by multiplex PCR. *Appl Environ Microbiol* 2003; 69: 4806-13.

38. Adl SM, Simpson AGB, Farmer MA, Andersen RA, Anderson OR, Barta JR, et al. The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *J Eukaryot Microbiol* 2005; 52(5): 399-451.

39. Sun T, Ilardi CF, Asnis D, Bresciani AR, Goldenberg S, Robert B et al. Light and Electron Microscopic Identification of *Cyclospora* Species in The Small Intestine: Evidence of the presence of asexual life cycle in human hosts. *Clin Pathol* 1996; 105: 216-20.

40. Sadaka HAH, Zoheir MA. Experimental Studies on Cyclosporiasis. *J Egypt Soc Parasitol* 2001; 31: 65-77.

41. Khalifa AM, El-Temsahy MM, Abou El Naga IF. Effect of ozone on the viability of some protozoa in drinking water. *J Egypt Soc Parasitol* 2001; 31: 603-16.

42. Gerba CP, Pepper IL, Whitehead LF. A risk of assessment of emerging pathogens of concern in the land application of biosolids. *Water Science and Technology* 2002; 46: 225-30.

43. Dubey JP, Thayer DW, Speer CA, Shen SK. Effect of gamma radiation on unsporulated *Toxoplasma gondii* oocysts. *Int J Parasitol* 1998; 28: 369-75.

44. Ortega YR, Nagle R, Gilman RH, Watanabe J, Miyagui J, Quispe H et al. Pathological and clinical findings in patients with cyclosporiasis and a description of intracellular parasite life stages. *J Infect Dis* 1997; 176: 1584-9.

45. Nhieu JT, Nin F, Fleury-Feith J, Chaumette M-T, Schaeffer A, Bretagne S. identification of intracellular stages of *Cyclospora* species by light microscopy of thick sections using hematoxylin. *Hum Pathol* 1996; 27: 1107-9.

46. Taylor M. 10 CDC Report. Foodborne Disease Surveillance in England and Wales, 5th. November 1993; 3(12).

47. Taylor DN, Houston R, Shlim DR, Bhaibulaya M, Ungar BLP, Echevarria P. Aetiology of diarrhoea among travellers and foreign residents in Nepal. *JAMA* 1988; 260:1245-8.

48. Serpentini A, Dutoit E, Camus D. *Cyclospora cayetanensis*: review of an emerging intestinal pathogen. *Ann Biol Clin (Paris)* 1999; 57(6): 677-83.

49. Kansouzidou A, Charitidou C, Varnis T, Vavatsi N, Kamaria F. *Cyclospora cayetanensis* in a patient with travellers' diarrhoea: case report and review. *J Travel Med* 2004; 11(1): 61-3.

50. Ghimire TR. Cyclosporiasis in HIV and Non-HIV patients: A study in Kanti Children's Hospital, Maharajgunj and Sukra Raj Tropical and Infectious Disease Hospital, Teku, Kathmandu, Nepal. *Dissertation submitted in partial fulfilment of Master's Degree in Zoology (Parasitology), Central Department of Zoology, Tribhuvan University, Kirtipur, Kathmandu, Nepal,* 2004.

51. Wurtz R. *Cyclospora*: A Newly Identified Intestinal Pathogen Of Humans. *Clin Infect Dis* 1994: 18: 620-3.

52. William HS, Behrman AJ, Shepherd SM. *Cyclospora*. Websites: www.emedicine.com [Assessed date: December, 01, 2005].

53. Crowley B, Path C, Moloney C, Keane CT. *Cyclospora* species-a cause of diarrhoea among Irish travellers to ASIA. *Ir Med J* 1996; 89(3):110-2.

54. Lontie M, Degroote K, Michiels J, Bellers J, Mangelschots E, Vandepitte J. *Cyclospora* sp.: a coccidian that causes diarrhoea in travellers. *Acta Clin Belg* 1995; 50(5): 288-90.

55. Kansouzidou A, Charitidou C, Varnis T, Vavatsi N, Kamaria F. *Cyclospora cayetanensis* in a patient with travellers' diarrhoea: case report and review. *J Travel Med* 2004; 11(1): 61-3.

56. Lammers HA, van Gool T, Eeftinck Schattenkerk JK. Two patients with diarrhoea caused by *Cyclospora cayetanensis* following a trip to the tropics. *Ned Tijdschr Geneeskd* 1996; 140(16):890-2.

57. Dekker E, Kager PA. Prolonged diarrhoea and weight loss after a biking trip from Tibet to Nepal: infection with *Cyclospora*. *Ned Tijdschr Geneeskd* 2002; 146(32):1502-4.

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

59. Hoge CW, Shlim DR, Ghimire M, Rabold JG, Pandey P, Walch A et al. Placebo-controlled trial of co-trimoxazole for *Cyclospora* infections among travellers and foreign residents in Nepal. *Lancet (North American Edition)* 1995; 345: 691-3.

60. Sherchand JB, Ohara H, Sherchand S, Cross JH, Shrestha MP. Intestinal parasitic infections in rural areas of southern Nepal. *J Inst Med* 1997; 19: 115-21.

61. Sherchand JB, 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.

62. Sherchand JB and Sharma DR. A Study of *Cyclospora cayetanensis* and the possible contamination of vegetables and river water in Kathmandu, Nepal. *J Nepal Ass Med Lab Sc* 2003; 5: 13-7.

63. Ghimire TR, Mishra PN, Sherchand JB. The seasonal outbreaks of *Cyclospora* and *Cryptosporidium* in Kathmandu, Nepal. *Journal of Nepal Health Research Council* 2005; 3(1): 39-48.

64. Ghimire TR, Mishra PN. Intestinal parasites and haemoglobin concentration in the people of two different areas of Nepal. *Journal of Nepal Health Research Council* 2005; 3(2): 1-7.

65. Kimura K, Rai SK, Takemasa K, Ishibashi Y, Kawabata M, Belosevic M et al. Comparison of three microscopic techniques for diagnosis of *Cyclospora cayetanensis*. *FEMS Microbiol Lett* 2004; 238 (1): 263-6.

66. Kimura K, Rai SK, Rai G, Insisiengmay S, Kawabata M, Karanis P, Uga S. Study on *Cyclospora cayetanensis* associated with diarrhoeal disease in Nepal and Loa PDR. *Southeast Asian J Trop Med Public Health* 2005; 36(6):1371-6.

67. Chacin- Bonilla L, Estevez J, Monsalve F, Quijada L. *Cyclospora cayetanensis* infections among diarrhoeal patients from Venezuela. *Am J Trop Med Hyg* 2001; 65: 351-4.

68. Chacin-Bonilla L, de Young MM, Estevez J. Prevalence and Pathogenic Role of *Cyclospora cayetanensis* in a Venezuelan community. *Am Trop Med Hyg* 2003; 68: 304-6.

69. Madico C, McDondald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. *Clin Infect Dis* 1997; 24: 977-81.

70. Lopez AS, Bendik JM, Alliance JY, Robert JM, da Silva AJ, Moura LNS et al. Epidemiology of *Cyclospora cayetanensis* and other identical parasites in a community in Haiti. *J Clin Microbiol* 2003; 41: 2047-54.

71. Eberhard ML, Nace EK, Freeman AR, Streit TG, da Silva AJ, Lammie PJ. *Cyclospora cayetanensis* infections in Haiti: a common occurrence in the absence of watery diarrhoea. *Am J Trop Med Hyg* 1999; 60: 584-6.

72. Pape JW, Verdier R-I, Boncy M, Boncy J, Johnson WD Jr. *Cyclospora* infection in adults infected with HIV: clinical manifestations, treatments and prophylaxis. *Ann Int Med* 1994; 121: 654-7.

73. Rose I-V, Fitzgerald DW, Warren D, Johnson Jr, Pape JW. Trimethoprim-Sulfamethoxazole compared with ciprofloxacin for treatment and

prophylaxis of *Isospora Belli* and *Cyclospora cayetanensis* infection in HIV-infected patients: a randomized, controlled trial. *Ann Intern Med* 2000; 132: 885-8.

74. Bern C, Hernandez B, Lopez MB, Arrowood MJ, de Mejia MA, de Merida AM et al. Epidemiologic studies of *Cyclospora cayetanensis* in Guatemala. *Emerg Infect Dis* 1999; 5: 766-74.

75. Bern C, Hernandez B, Lopez MB, Arrowood MJ, Merida AM, Klein RE. The contrasting epidemiology of *Cyclospora* and *Cryptosporidium* among ou tpatients Guatemala. *Am J Trop Med Hyg* 2000; 63: 231-5.

76. Pratdesaba RA, Gonzalez M, Piedrasanta E, Merida C, Contreras K, Vela C et al. *Cyclospora cayetanensis* in three populations at risk in Guatemala. *J Clin. Microbiol* 2001; 39: 2951-3.

77. Cegielski JP, Ortega YR, McKee S Madden JF, Gardo L, Schwartz DA et al. *Cryptosporidium*, *Enterocytozoon* and *Cyclospora* infections in paediatric and adult patients with diarrhoea in Tanzania. *Clin Infect Dis* 1999; 28: 314-21.

78. Manatsathit S, Tunsupasawasdikul S, Wanachiwanawin D, Setawarin S, Suwanagool P, Prakasvejakit S, Leela-Kusolwong S et al. Causes of chronic diarrhoea in patients with AIDS in Thailand: a prospective clinical and microscopical study. *J Gastroenterology* 1996; 31: 533-7.

79. Girard de Kaminsky R. *Cyclospora cayetanensis*: Nuevo Apicomplexa intestinal, con observaciones en el hospital Escuela. *Rev Med Honduras* 1997; 65: 68-72.

80. Escobedo AA, Nunez FA. Prevalence of intestinal parasites in Cuban Acquired Immunodeficiency Syndrome (AIDS) patients. *Acta Trop* 1999; 72: 125-30.

81. Abou el Naga IF. Studies on a newly emerging protozoal pathogen: *Cyclospora cayetanensis*. *J Egypt Soc Parasitol* 1999; 29: 575-86.

82. Osman GA, Karun MM, Hanan M El-S, Doreya MM, Sahar SAA, Herba HS. Coccidian parasites as case of watery diarrhoea among protein energy malnourished and other immunocompromised Egyptian Children. *J Egypt Soc Parasitol* 1999; 29: 653-68.

83. Rizk H, Soliman M. Coccidiosis among malnourished children in Mansoura, Dakahlia Governorate, Egypt. *J Egypt Soc Parasitol* 2001; 31: 877-86.

84. Kumar SS, Ananthan S, Saravanan P. Role of coccidian parasites in causation of diarrhoea in HIV infected patients in Chennai. *Ind J of Med Research* 2002; 116: 85-9.

85. Mohandas K, Sehgal R, Sud A, Malla N. Prevalence of intestinal pathogens in HIV-seropositive individuals in Northern India. *Jpn J Infect Dis* 2002; 55: 83-4.

86. Mohammed A, Gebre-Selassie S, Kassa T, Kibru G. Prevalence of intestinal parasites in HIV- infected adult patients in South-western Ethiopia. *Ethiop J Health Dev* 2003; 17: 71-8.

87. Ooi WW, Zimmerman SK, Needham CA. *Cyclospora* species as a gastrointestinal pathogen in immunocompetent hosts. *J Clin Microbiol* 1995; 33: 1267-9.

88. Amin OM. Seasonal prevalence and host relationship of *Cyclospora cayetanensis* in North America during 1996. *Parasitol Int* 1998; 47: 53-8.

89. Fleming CA, Caron D, Gunn JE, Barry MA. A foodborne outbreak of *Cyclospora cayetanensis* at a weeding clinical features and risk factors for illness. *Archives Int Med* 1998; 158: 1121-5.

90. Clarke SC, McIntyre M. The incidence of *Cyclospora cayetanensis* in the stool samples submitted to a district hospital. *Epidemiol Infect* 1996; 117:189-93.

91. Fryauff DJ, Krippner R, Purnomo, Ewald C, 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.

92. Alakpa GE, Fagbenro-Beyioku AF, Clarke SC. *Cyclospora cayetanensis* in stool submitted to hospitals in Lagos, Nigeria. *Inter J Infect Dis* 2002; 6: 314-7.

93. Diaz E, Mondragon J, Enrique R, Bernal R. Epidemiology and control of intestinal parasites with nitaxoxanide in children in Mexico. *Am J Trop Med and Hyg* 2003; 68: 384-5.

94. Al Braiken FA, Amin A, Beeching NJ, Hommmel M, Hart CA. Detection of *Cryptosporidium* amongst diarrheic and asymptomatic children in Jeddah, Saudi Arabia. *Ann Trop Med and Parasitol* 2003; 97: 505-10.

95. Nimri LF. *Cyclospora cayetanensis* and other intestinal parasites associated with diarrhoea

in a rural area of Jordan. *Int Microbiol* 2003; 6: 131-5.

96. Wang KX, Li CP, Wang J, Tian Y. *Cyclospora cayetanensis* in Anhui, China. *World J Gastroenterol* 2002; 8(6): 1144-8.

97. Jelinke T, Lotze M, Eichenlaun M, Loscher T. Prevalence of infection with *Cryptosporidium parvum* and *Cyclospora cayetanenesis*. *Gut* 1997; 41: 801-4.

98. Hoge CW, Shlim DR, Rajah R, Triplett J, Shear M, Rabold JG et al. Epidemiology of diarrhoeal illness associated with coccidian-like organism among travellers and foreign residents in Nepal. *Lancet* 1993; 341: 1175-9.

99. Soave R. *Cyclospora*: An Overview. *Clin Infect Dis* 1996; 23: 429- 37.

100. Looney WJ. *Cyclospora* spp. as a cause of diarrhoea in humans. *Brit J Biomed Sc* 1998; 55: 157-61.

101. Ortega YR, Sterling CR and Gilman RH. *Cyclospora cayetanensis*. *Adv Parasitol* 1998; 40: 400-18.

102. Mosimann M, Nguyen XM, Furrer HJ. Excessive watery diarrhoea and pronounce fatigue due to *Cyclospora cayetanensis* infection in an HIV-infected traveller returning from the tropics. *Schweizerishe Medizinische Wuchenschrift* 1999; 21: 1158-61.

103. Chokephaibulkit K, Wanachiwanawin D, Tosasuk K, Vanprapa N, Chearskul S. A report case of *Cyclospora* and *Cryptosporidium* mixed infection in a HIV-negative child in Thailand. *J Medical Ass of Thailand* 2001; 84: 589-92.

104. Bendall RP, Lucas S, Moody A, Tovey G, Chiodini PL. Diarrhoea associated with cyanobacterium-like bodies: a new coccidian enteritis of man. *Lancet* 1993; 341: 590-2.

105. Wurtz RM, Kocka FE, Peters CS, Weldon-Linne CM, Kuritza A, Yungbluth P. Clinical characteristics of seven cases of diarrhoea associated with a novel acid-fast organism in the stool. *Clin Infect Dis* 1993; 16: 136-8.

106. Berlin OG, Novak SM, Porschen RK, Long EG, Stelma GN, Schaeffer FW. Recovery of *Cyclospora* organisms from patents with prolonged diarrhoea. *Clin Infect Dis* 1994; 18: 606-9.

107. Salvatella1 DR, Balleste R, Puime A, Rodriguez G, Eirale LC, Calegari L. *Ciclospora* cayetanensis en Uruguay. Agente de diarrea del viajero, adquirida en el exterior. *Rev Méd Urug Montevideo* 2002; 18 (2): 175-9.

108. Sifuentes-Osornio J, Porrascortes G, Bendall RP, Morales-Villarreal F et al. *Cyclospora cayetnensis* infection in patients with and without AIDS: Biliary disease as another clinical manifestation. *Clin Dis* 1995; 21: 1092-7.

109. Nassef NE, Saedia A, Sayed El-Ahl, Omaima K, El-Shafee and Nawar M. *Cyclospora*: a newly identified protozoan pathogen of man. *Soc Parasitol* 1998; 28: 213-9.

110. Longe RL, DiPiro JT. Diarrhoea and constipation. In: DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, Posey LM, eds. Pharmacotherapy: a pathophysiologic approach, 3rd ed. Stamford, CT: Appleton & Lange, 1997:767-74.

111. Connor BA, Reidy R, Soave R. Cyclosporiasis: clinical and histopathologic correlates. *Clin Infect Dis* 1999; 28: 1216-22.

112. Pinge-Suttor V, Douglas C, Wettstein A. *Cyclospora* infection masquerading as coeliac disease. *Med J Aust* 2004; 180(6): 295-6.

113. Popovici I, Dahorea C, Rugina A, Coman G. Acute diarrhoea associated with *Cyclospora cayetanensis*. *Rev Med Chir Soc Med Nat Iasi* 2003; 107(4):877-80.

114. Velasquez JN, Carnevale S, Cabrera M, Kuo L, Chertcoff A, Mariano M et al. *Cyclospora cayetanensis* in patients with AIDS and chronic diarrhoea. *Acta Gastroenterol Latinoam* 2004; 34(3):133-7.

115. Santana Ane M, Nunez Fernandez FA, Perez Avila J, Barrero Bringuez M, Velazquez Viamontes B. Emergence of a new pathogen: *Cyclospora cayetanensis* in patients infected with human immunodeficiency virus. *Rev Cubana Med Trop* 2000; 52(1): 6-9.

116. Scaglia MS, Bassi GP, Viale PL, Novati S, Ranieri S. Intestinal Co-Infection by *Cyclospora* sp. and *Cryptosporidium parvum*. First report in an AIDS patient. *Parasite* 1995; 1: 87-390.

117. Powell D. Approach to the patient with diarrhoea. In: Yamada T, ed. Textbook of gastroenterology, 2nd ed. Philadelphia: JB Lippincott, 1995:820-4.

118. Hinterleitner T, Powell D. Immune system control of intestinal ion transport. *Proc Soc Exp Biol Med* 1991; 197:249-60.

119. Ciancio M, Chang E. Epithelial secretory response to inflammation. *Ann N Y Acad Sci* 1992; 664: 210-21.

120. Connor BA, Shlim DR, Scholes JV, Rayburn JL, Reidy J, Rajah R. Pathogenic changes in the small bowel in nine patients with diarrhoea associated with a coccidia-like body. *Ann intern med* 1993; 119: 377-82.

121. Goodgame RW: Understanding intestinal spore-forming protozoa: *Cryptosporidia*, *Microsporidia*, *Isospora*, and *Cyclospora*. *Ann Intern Med* 1996; 124(4): 429-41.

122. Connor BA. *Cyclospora* infection: a review. *Ann Academ of Med Singapore* 1997; 26: 632-6.

123. Richardson RF Jr, Remler BF, Katirji B, Murad MH. Guillain-Barre Syndrome after *Cyclospora* infection. *Muscle and Nerve* 1998; 21: 669-71.

124. Connor BA, Johnson EJ, Soave R: Reiter syndrome following protracted symptoms of *Cyclospora* infection. *Emerg Infect Dis* 2001; 7(3): 453-4.

125. Zar FA, El-Bayoumi E, Yungbluth MM. Histological proof of acalculous cholecystitis due to *Cyclospora cayetanensis*. *Clin Infect Dis* 2001; 33: 140-1.

126. Di Gliullo AB, Cribari MS, Bava AJ, Cicconetti JS, Collazos R. *Cyclospora cayetanensis* in sputum and stool samples. *Rev Inst Med Trop S Paulo* 2000; 42:115-7.

127. Eberhard ML, Nace EK, Freeman AR. Survey for *Cyclospora cayetanensis* in domestic animals in an endemic area in Haiti. *J Parasitol* 1999; 85: 562-3.

128. Eberhard ML, Ortega YR, Hanes DE, Nace EK, Do RQ, Robl MG et al. Attempts to establish experimental *Cyclospora cayetanensis* infection in laboratory animals. *J Parasitol* 2000; 86: 577–82.

129. Huang P, Weber JT, Sosin DM, Griffin PM, Long EG, Murphy JJ et al. The first reported outbreak of diarrhoeal illness associated with *Cyclospora* in the United States. *Ann Intern Med* 1995; 123:409-14.

130. Madico G, McDonald J, Gilman RH, Cabrera L, Sterling CR. Epidemiology and treatment of *Cyclospora cayetanensis* infection in Peruvian children. *Clin Infect Dis* 1997; 24: 977-81.

131. Verdier R-I, Fitzgerald DW, Johnson WD, Pape JW. Trimethoprim-sulfamethoxazole compared with ciprofloxacin for treatment and prophylaxis of *Isospora belli* and *Cyclospora cayetanensis* infection in HIV-infected patients. *Ann Intern Med* 2000; 132: 885-8.

132. Alfano-Sobsey EM, Eberhard ML, Seed JR, Weber DJ, Won KY, Nace EK et al. Human Challenge Pilot Study with *Cyclospora cayetanensis*. *Emerg Infect Dis* 2004; 10(4): 726-8.

133. Lopez AS, Douglas R, Dodson, Arrowood MJ, Orlandi PA Jr, da Silva AJ et al. Outbreaks Of Cyclosporiasis Associated With Basil In Missouri In 1999. *Clin Infect Dis* 2001; 32: 1010-7.

134. Smith HV, Paton CA, Mtambo MMA, Girdwood RWA. Sporulation of *Cyclospora* sp oocysts. *Appl Envir Microbiol* 1997; 63:1631-2.

135. Pieniazek NJ et al. PCR confirmation of infection with *Cyclospora cayetanensis*. *Emerg Inf Dis* 1996; 2: 357-9.

136. Centres for Disease Control and Prevention (CDC). Update: Outbreaks of *Cyclospora cayetanensis* infection in the United States and Canada. *MMWR Morb Mortal Wkly Rep* 1996; 45: 611-2.

137. Hale D, Aldeen W, Carroll K. Diarrhoea associated with cyanobacteria-like bodies in an immunocompetent host. An unusual epidemiological source. *JAMA* 1994; 271: 144-5.

138. Sturbaum GD, Ortega YR, Gilman RH, Sterling CR, Cabrera L, Klein DA. Detection of *Cyclospora cayetanensis* in wastewater. *Appl Environ Microbiol* 1998; 64: 2284-6.

139. Connor BA, Shlim DR. Foodborne transmission of *Cyclospora*. *Lancet* 1995; 346:1634.

140. Mead PS, Slutsker L, Dietz V, McCaig LF, Bresee JS, Shapiro C et al. Food-related illness and death in the United States. *Emerg Infect Dis* 1999; 5: 607-25.

141. Herwaldt BL Ackers M-L and the *Cyclospora* working group. An outbreak in 1996 of cyclosporiasis associated with imported raspberries. *N Engl J Med* 1997; 336:1548-58.

142. Centers for Disease Control and Prevention (CDC). Update: outbreaks of cyclosporiasis in United States and Canada, 1997. *MMWR Morb Mortal Wkly Rep* 1997; 46: 521-2.

143. Pritchett R, Gossman C, Radke V, Moore J, Busenlehner, Fischer K et al. Outbreak of

cyclosporiasis. Northern Virginia-Washington, DC.-Baltimore, Maryland, Metropolitan Area, 1997. *MMWR Morb Mortal Wkly Rep* 1997; 46:689-91.

144. Ortega YR, Roxas CR, Gilman RH, Miller NJ, Cabrera L, Taquiri C et al. Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanensis* from vegetables collected in market of endemic region in Peru. *Am J Trop Med and Hyg* 1997; 57: 683-6.

145. Graczyk TK, Ortega YR, Conn DB. Recovery of waterborne oocysts of *Cyclospora cayetanensis* by Asian freshwater clams (*Corbicula fluminea*). *Am J Trop Med Hyg* 1998; 59: 928-32.

146. Clarke SC, McIntyre M. Modified Detergent Ziehl-Neelsen Technique for the staining of *Cyclospora cayetanensis*. *J Clin Pathol (London)* 1996; 49: 511-512.

147. Ash LR, Orihel TC. Collection and preservation of faeces. Parasites: a guide to laboratory procedures and identification. Chicago: ASCP Press; 1991. p. 3-53.

148. Weber R, Bryan RT, Owen RL, Wilcox CM, Gorelkin L, Visvesvara GS. Improved light microscopical detection of *Microsporidia* spores in stool and duodenal samples. *N Engl J Med* 1992; 326:161-6.

149. Ash LR, Orihel TC. Atlas of human parasitology. 4th ed. Chicago: ASCP Press; 1997.

150. Berlin OGW. Mycobacteria. In: Baron EJ, Finegold SM, editors. Diagnostic microbiology. 8th ed. St. Louis: CV Mosby; 1990. p. 597-640.

151. Visvesvara GS, Moura H, Kovacsnace E, Wallace S, Eberhard ML.. Uniform staining of *Cyclospora* oocysts in faecal smears by a modified safranin technique with microwave heating. *J Clin Microbiol* 1997; 35:730-3.

152. Eberhard ML, Pieniazek NJ, Arrowood MJ. Laboratory diagnosis of *Cyclospora* infections. *Arch Pathol Lab Med* 1997; 121: 792-7.

153. Garcia SL, Bruckner AD. Intestinal protozoa: Coccidia and *Microsporidia* in Diagnostic medical parasitology. Washington, D.C: *American Society Microbiol* 1997: 54-89

154. Orlandi PA, Chu DT, Bier JW, Jackson GJ. Parasites and the Food Supply. *Food Technology* April 2002, 72-81.

155. Lopez B, de Merida AM, Arrowood MJ et al. Comparison of two microscopic diagnostic methods for *Cyclospora cayetanensis* [abstract P-2.3]. In: Program and abstracts of the International Conference on Emerging Infectious Diseases (Atlanta), 8-11 March, 1998:80.

156. Dixon BR, Bussey JM , Parrington LJ, Parenteau M. Detection of *Cyclospora cayetanensis* oocysts in human faecal specimens by Flow Cytometry. *J Clin Microbiol* 2005; 43(5): 2375–9.

157. Chambers J, Somerfeldt S, Mackey L, Nichols S, Ball R, Roberts D et al. Outbreaks of *Cyclospora cayetanensis* infection-United States, 1996. *MMWR* 1996; 45: 549-51.

158. Roxas C, Miller N, Cabrera L, Ortega Y, Gilman R, Sterling D. Vegetables as a potential transmission route for *Cyclospora* and *Cryptosporidium*. Abstracts of the Annual Meeting of the American Society for Microbiology, 1996, C-102, p.19.

159. Steele M, Unger S, Odumeru J. Sensitivity of PCR detection of *Cyclospora cayetanensis* in raspberries, basil, and mesclun lettuce. *J Microbiol Methods* 2003; 54(2): 277-80.

160. Verweij JJ, Laeijendecker D, Brienen EAT, Van Lieshout L, Polderman AM . Detection of *Cyclospora cayetanensis* in travellers returning from tropics and subtropics using microscopy and Real-Time PCR. *IJMM International Med Microbiol* 2003; 293: 199-202.

161. Orlandi PA, Frazar C, Carter L, Chu D-M T. Detection of *Cyclospora* and *Cryptosporidium* from fresh produce: isolation and identification by Polymerase Chain Reaction (PCR) and microscopic analysis. *Bacteriological Analytical Manual Online*. October 2004. Chapter 19 A.

162. Yoder KE, Sethabutr O, Relman DA. "PCR-based detection of the intestinal pathogen *Cyclospora*" in PCR protocols for emerging infectious diseases, a supplement to diagnostic molecular microbiology: principles and applications. D.H. Persing (ed.), ASM Press, Washington, DC, 1996 pp.169-76.

163. Jinneman KC, Wetherington JH, Hill WE, Adams AM, Johnson JM, Tenge BJ. Template preparation for PCR and RFLP of amplification products for the detection and identification of *Cyclospora* sp. and *Eimeria* sp. oocysts directly from raspberries. *J Food Prot* 1998; 61:1497-503.

164. Shields JM, Olson BH: PCR-restriction fragment length polymorphism method for

detection of *Cyclospora cayetanensis* in environmental waters without microscopic confirmation. *Appl Environ Microbiol* 2003; 69(8): 4662-9.

165. Johnson DW, Pieniazek NJ, Griffin DW, Misener L, Rose JB. Development of a PCR protocol for the sensitive detection of *Cryptosporidium* oocysts in water samples. *Appl Environ Microbiol* 1995; 61:3849-55.

166. Orlandi PA, Lampel KA. Extraction-free, filter-based template preparation for rapid and sensitive PCR detection of pathogenic parasitic protozoa. *J Clin Microbiol* 2000; 38: 2271-7.

167. Jinneman KC, Wetherington JH, Adams AM, Johnson JM, Tenge BJ, Dang N-L et al. Differentiation of *Cyclospora* sp. and *Eimeria* spp. by using the polymerase chain reaction amplification products and restriction fragment length polymorphisms. Laboratory Information Bulletin No. 4044. U.S. Washington, D.C: Food and Drug Administration; 1996.

168. Varma M, Hester JD, Schaefer FW, Ware MW, Lindquist HDA. Detection of *Cyclospora cayetanensis* using a quantitative real-time PCR assay. *J Microbiol Methods* 2003; 53:27-36.

169. Wu DY, Ugozzoli L, Pal BK, Wallace RB. Allele-specific enzymatic amplification of ß-globin genomic DNA for diagnosis of sickle cell anaemia. *Proc Natl Acad Sci USA* 1989; 86: 2757-60.

170. Jinneman KC, Wetherington JH, Hill WE, Omiescinski CJ, Adams AM, Johnson JM. An oligonucleotide-ligation assay for the differentiation between *Cyclospora* and *Eimeria* sp. polymerase chain reaction amplification products. *J Food Prot* 1999; 62:682-5.

171. Bayard PJ, Berger TG, Jacobson MA. Drug hypersensitivity reactions and Human Immunodeficiency Virus disease. *J Acquir Immune Defic Syndr* 1992; 5: 1237-57.

172. De Hovitz JA, Johnson WD Jr, Pape JW. Cutaneous reactions to trimethoprim-sulfamethoxazole in Haitian AIDS. *Ann Intern Med* 1985: 103: 479-80.

173. Pape JW, Verdier RI, Johnson WD Jr. Treatment and Prophylaxis of *Isospora belli* infection in patients with the Acquired Immune Deficiency Syndrome. *N Engl J Med* 1989; 320: 1044-7.

174. Sherchand JB, Cross JH. *Cyclospora* diarrhoea at the Kanti Children's Hospital Nepal. Paper presented abstract in XIV International Congress of Tropical Medicine and Malaria. "New Goal for the 21st century", Nagasaki, Japan, November 17-22, 1996; 296.

175. Cross JH, Sherchand JB. *Cyclospora cayetanensis*: "Look and you will find". *International Medical Research Journal* 1997; 1(2): 7-11.