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Case Report

Cyclospora infection in five immunocompetent patients in a Turkish university hospital

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Infection with *Cyclospora* has been increasingly reported worldwide in both immunocompetent and immunocompromised individuals. Here the cases of five patients infected with *Cyclospora cayetanensis*, who sought medical care at Hacettepe University in Turkey, are reported. Diarrhoea occurred from five to fifteen times a day in all of these patients, whose ages ranged from 27 to 67 years. All the patients were considered immunocompetent. Identification of *C. cayetanensis* was made by detection of the oocysts by using a modified acid-fast stain.

Introduction

Cyclospora cayetanensis is a newly recognized coccidian parasite. It has emerged as an important cause of epidemic and endemic diarrhoeal disease in humans called cyclosporiasis. It causes prolonged watery diarrhoea, fatigue, abdominal pain, weight loss and anorexia. The length of time between becoming infected and developing symptoms has not been well established. People usually experience *Cyclospora* symptoms about 1 week after they have been infected, but some people can be infected without symptoms (Chacin-Bonilla *et al.*, 2003; Eberhard *et al.*, 1999; Ortega *et al.*, 1993). If not treated, symptoms can last for a few days to a month or longer.

People infected with *C. cayetanensis* excrete oocysts that are not infectious. Oocysts become sporulated in one to several weeks and infect individuals who ingest them. As the parasite requires 1–2 weeks to sporulate, it is unlikely that it can pass directly from one person to another. The infection dose is presumed to be low (Jackson *et al.*, 1997). Outbreaks have been linked to contaminated water and various types of fresh produce.

Females and males are equally susceptible to *Cyclospora* infection. *C. cayetanensis* can cause illness that varies significantly with age and condition of the host. Infection occurs in people of all ages and in both immunocompetent and immunocompromised hosts (O'Mahony & Mannion, 1998; Ooi *et al.*, 1995). In AIDS patients the infection causes a more severe and chronic illness. Most AIDS patients develop prolonged, severe life-threating diarrhoea.

In recent years, several studies have shown that *C. cayetanensis* is distributed worldwide (Sterling & Ortega, 1999; Brown & Rotschafer, 1999), and its prevalence is considerably higher in developing countries than in Europe and North America. It has caused a number of sporadic cases and epidemic outbreaks of diarrhoeal illness. The majority of the isolates from humans have been from residents of developing countries or from travellers returning from Haiti, Mexico, Guatemala, Puerto Rico, Morocco, Pakistan and India (Clarke & McIntyre, 1996; Pollok *et al.*, 1992).

Since 1995, many foodborne outbreaks of cyclosporiasis have been documented in the USA and Canada (Huang *et al.*, 1995; Koumans *et al.*, 1998; Herwaldt & Ackers, 1997; Centers for Disease Control and Prevention, 1998). These outbreaks were mostly attributed to imported raspberries from Guatemala and other imported fruits and vegetables where contaminated water had been used (Herwaldt & Ackers, 1997; Centers for Disease Control and Prevention, 1996; Osterholm, 1997).

Here we report five cases of cyclosporiasis in immunocompetent patients who were not related and had no common activities.

Case reports

Case 1

In August 2004, a 67-year-old woman visited the department of gastroenterology. Her main complaint was watery diarrhoea. She had no history of recent travel. Physical examination revealed normal findings.

Case 2

In August 2004, a 28-year-old woman presented with watery diarrhoea for 3 days, 15 times a day, weight loss and anorexia. Physical examination was normal. She had travelled to the south coast of Turkey a week before her complaints.

Case 3

In August 2004, a 44-year-old man presented with diarrhoea accompanied by anorexia, weight loss and abdominal

cramps. Two weeks before his complaint, he had travelled to Pakistan for a week and to the south coast of Turkey for a couple of days.

Case 4

In August 2004, a 27-year-old woman visited the department of gastroenterology, complaining of a 5-day history of watery diarrhoea and malaise. She had no fever or abdominal cramp. Physical examination was found to be normal. She had no history of recent travel.

Case 5

In August 2004, a 31-year-old man presented with watery diarrhoea (five to six times a day) for 2 weeks. He complained of weight loss, cramping abdominal pain and malaise. He had no history of recent travel.

In all of the five cases, routine stool cultures for bacteria were negative. No leukocytes were seen on microscopic examination of the fecal samples. Although three stool samples collected 3 days consecutively examined for ova were found as negative, a modified acid-fast staining revealed pink to reddish stained oval to round organisms 8–10 μm in diameter, suggestive of *Cyclospora* spp. oocysts (Fig. 1). For modified acid-fast staining, 2 % sulfuric acid was used for decolorization. In none of the patients was immunodeficiency determined. All the patients were treated with trimethoprim (160 mg)/sulfamethoxazole (800 mg) b.i.d. for 7 days. After treatment, clinical symptoms of cyclosporiasis disappeared between 7 and 15 days.

Discussion

The first known human cases of illness caused by *Cyclospora* were reported in 1979 in Papua New Guinea (Ashford, 1979) but the organism was not named and classified until 1993 (Ortega *et al.*, 1993). Since the 1980s, more cases have been reported. The most probable reason for this is the previous underestimation of the presence of *Cyclospora* in examined stool specimens. The increased usage of modified acid-fast staining and experienced microbiologists made it possible to diagnose *Cyclospora* oocysts.

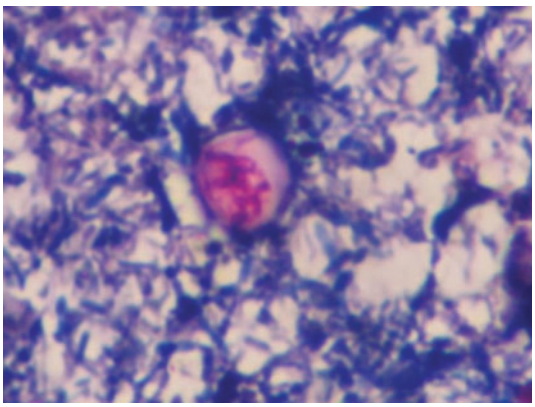

Fig. 1. Acid-fast oocyst of *Cyclospora* under oil immersion magnification (original magnification × 1000).

The prevalence of *Cyclospora* infection appears to vary depending on the population studied. The highest incidence has been described in underdeveloped countries, but infection is most common in tropical and subtropical areas. In the study of Wang *et al.* (2002) in Anhui, China, the infection rate was found to be 0·25 % in the normal population, 5·62 % in patients with diarrhoea and 9·32 % in immunocompromised patients. In a study by Chacin-Bonilla *et al.* (2003) in Venezuela, infection with *Cyclospora* was identified in 6·1 % of the subjects, and of the 13 subjects shedding oocysts, 11 (84·6 %) were asymptomatic. The incidence of the organism is very low in Europe and North America. In a laboratory-based study in the USA, *Cyclospora* was found in 0·2 % of stool specimens (Wurtz *et al.*, 1993). With the exception of some outbreaks, the overall prevalence of *Cyclospora* in North America appears to be far less than 1 %. It was also found in 0·1 % of 1333 stool specimens from healthy individuals in the UK (Soave, 1996). Most cases of *Cyclospora* infection reported from Europe have been sporadic, and almost all of them were detected in persons after travel to endemic areas (Pollok *et al.*, 1992; Gascon *et al.*, 1995; Petry *et al.*, 1997). Alakpa *et al.* (2002) collected 1109 stool samples between 1999 and 2000 from Nigeria, and only found 11 positive (0·99 %) samples of *C. cayetanensis* oocysts.

Anyone can become infected with *Cyclospora* but the prevalence in children tends to be much higher. *Cyclospora* is commonly associated with paediatric gastroenteritis in Guatemala, Peru, Nepal and other developing countries (Hoge *et al.*, 1995; Ortega *et al.*, 1993; Madico *et al.*, 1997). In the study of Lopez *et al.* (2003), the prevalence of *Cyclospora* infection was higher in children $\leqslant$10 years of age than in older people. Studies from Guatemala and Peru have similar results (Madico *et al.*, 1997; Bern *et al.*, 1999; Bern *et al.*, 2000). Ortega *et al.* (1993) found the parasite in 6–18 % of Peruvian children. In the study of Hoge *et al.* (1995), the prevalence of *Cyclospora* species among children at least 18 months of age was found to be 12 % in Nepal. Among asymptomatic children, the prevalence was 2 %.

Cyclospora causes prolonged and watery diarrhoea in both immunocompetent patients and immunocompromised (O'Mahony & Mannion, 1998; Ooi *et al.*, 1995; Wurtz *et al.*, 1993) patients such as those with AIDS. Here we report the cases of five immunocompetent patients who had watery diarrhoea associated with *Cyclospora*. Only six cases of *C. cayetanensis* infection have been reported recently in Turkey (Yazar *et al.*, 2004). Of these six cases, only two were in immunocompetent patients.

That all the patients were living in Ankara, the capital city of Turkey, and were diagnosed in August 2004 suggested that the organism could be waterborne or foodborne. However,

all the patients used bottled water for drinking and none of them consumed the same food. Two of the patients travelled to the same town on the south coast before developing watery diarrhoea. It is possible that these two could have been infected by the same source. However, it should be kept in mind that one of these patients could have acquired *C. cayetanensis* from his travel to Pakistan also. Therefore, as a result we could not find any source according to the histories of the patients.

Generally throughout the world, especially in developing countries, there are some difficulties in the identification of this parasite in stools. In most of the laboratories, stool specimens examined for ova and parasites are not usually examined for *Cyclospora* unless such testing is requested. The identification of this parasite requires special laboratory tests that are not routinely done. The stain mostly used to identify the oocysts of *Cyclospora* and *Cryptosporidium* is a modified acid-fast stain (Yazar *et al.*, 2004; Baxby *et al.*, 1984; Eberhard *et al.*, 1997). The oocysts show variability in staining with this modified acid-fast stain, ranging from no staining to staining pink or deep purple. This variability may result in the misidentification of the parasite. Also, *Cyclospora* oocysts can be secreted intermittently and shed in relatively low numbers. Therefore, one negative stool specimen does not rule out infection with *Cyclospora*. At least three stool samples should be tested before a negative result is reported.

Cyclospora and *Cryptosporidium* are obligate, intracellular coccidian protozoan parasites that infect the gastrointestinal tract of humans, causing severe diarrhoea. Both of them produce oocysts, which are excreted in the faeces. Since the oocysts of *Cyclospora* are stained acid-fast like those of *Cryptosporidium*, there is close similarity between these two parasites. Therefore, more attention should be given to differentiation between the two. The *C. cayetanensis* oocyst diameter (8–10 μm) is larger than that of *Cryptosporidium* (about 5 μm).

Patients infected with *Cyclospora* have non-specific symptoms such as watery diarrhoea, abdominal distention, abdominal pain and anorexia, which can easily be confused with those of other intestinal diseases. Therefore, if the patient has prolonged diarrhoea, a history of travel or is immunocompromised or if there is a waterborne/foodborne-related diarrhoea outbreak, one should always remember that it can be caused by *C. cayetanensis*. If several stool samples are found to be negative for routine bacteriological, virological and parasitological tests and the patient still has gastrointestinal symptoms, examination for *C. cayetanensis* with modified acid-fast staining must be done.

Cyclospora has been isolated in chronic diarrhoea of immunocompetent patients, therefore this infection must be taken into consideration, especially in patients with prolonged diarrhoea. More coccidial infections could be detected if modified acid-fast staining is routinely performed.

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