

Human cyclosporiosis in Turkey

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Abstract

Six patients infected with *Cyclospora cayetanensis* who sought medical care at three different hospitals in Turkey are herein presented. Four patients were male and the others were female and their ages ranged from 7 to 62 years. The first patient was HIV-positive and presented with watery diarrhea with a frequency of up to 18 times a day for more than ten months and diagnosed as cyclosporiosis in Kayseri, 1996. The second patient was also HIV positive and diagnosed as cyclosporiosis in Kayseri, 2000. The third patient was an acute myeloblastic leukemia (AML) patient and diagnosed in Istanbul, 2000. The fourth patient was idiopathic hepatic cirrhosis complaining of diarrhea and weakness and diagnosed in Kayseri, 2001. The fifth and sixth patients were immunocompetent patients complaining of diarrhea and diagnosed in Izmir and Kayseri, 2002. Diarrhea occurring from one to ten times a day continued for 7 to 70 d in the last 5 patients. Treatment with a trimethoprim/sulfamethoxazole compound was done for all patients. Both symptomatic and parasitologic improvements were quickly observed. In summary, *C. cayetanensis* infection is rare in Turkey and most patients infected with this pathogen tend to be immunosuppressive individuals at present.

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INTRODUCTION

The genus *Cyclospora* is in the subclass Coccidia, phylum Apicomplexa. This genus is taxonomically related to coccidian genera in humans. This organism is a unilocular parasite previously known as cyanobacterium-like or coccidian-like body (CLB) and, in recent years, a new coccidian pathogen, *Cyclospora*, has been putatively identified^[1,2]. The organisms are seen as nonrefractile spheres and are acid-fast variable with the modified Kinyoun's acid-fast stains, those are unstained appear as glassy, wrinkled spheres. Modified acid-fast stains stain the oocysts from light pink to deep red, some of which may contain granules or have a bubbly appearance. The oocysts autofluoresce (strong green or intense blue) under UV epifluorescence^[3]. The first known human cases of illness caused by *Cyclospora* infection (i.e., cyclosporiosis) were reported in the medical literature in 1979^[4]. Our aim in this study was to present cases of human cyclosporiosis in Turkey and to review the literature.

MATERIALS AND METHODS

We evaluated all of the human cases in our country

retrospectively which were belongs to Kayseri (three cases), Istanbul (one case) and Izmir (one case) regions between 1996 and 2002^[5-9]. Following these, we determined a new case with *C. cayetanensis* in December 2002. In this study, we discuss all of the six cases with cyclosporiosis in Turkey.

RESULTS

Case 1^[5]

A 50 year-old woman with acquired immune deficiency syndrome (AIDS) was admitted in December 1996 for chronic diarrhea, vomiting, and fever to the Erciyes University Medical Faculty Hospital. There was preceding history of episodic watery diarrhea, vomiting, and weight loss along with intermittent fever over a period of one year. She was cachectic, with mild abdominal tenderness and alert, thrush and palpable small cervical lymph nodes. She had anemia (Hb 8.4 g/L). Enzyme immunoassays for HIV antibodies were positive and the T4/T8 ratio was 0.6 in serum. *E. coli* and *Proteus* spp. were found at 10⁴ cfu/mL from urine specimens. Microscopical analysis of the stool revealed numerous spherical double walled microorganisms 8-9 µm in diameter, some with internal granulation. After Kinyoun's acid fast staining of the stool, the organisms appeared faint pink to red in colors, some cysts not taking up the stain and appearing as "ghosts". Empty cysts varied in shape but had generally collapsed into crescents. The organisms were identified as *Cyclospora* sp. The patient was treated with TMP/SMZ (160/800 mg) bid for three weeks. Following treatment, re-examination of a stool sample revealed no more organisms and diarrhea stopped. This case was the first report of *Cyclospora* infection in Turkey.

Case 2^[6]

A 40-year old man with AIDS was evaluated parasitologically for the etiologic agent of his persistent diarrhea for two months in Erciyes University Medical Faculty Hospital in 2000. Stool samples were examined by conventional coprological methods such as fresh preparation, iodine stain, and flotation. Suspicious organisms (8-10 µm in diameter) were seen in stool, which were stained with Kinyoun's acid-fast stain and identified as *C. cayetanensis*. He was treated with TMP/SMZ (160/800 mg) bid for two weeks. We could not find the organism in the stool samples after the treatment and diarrhea stopped.

Case 3^[7]

In 2000, at the Istanbul University Medical Faculty Hospital there was another 7 year-old male patient with acute myeloblastic leukemia (AML). A sudden diarrhea developed while bone marrow transplantation was being planned. The patient's stool samples were examined with respect to pathogen bacteria and fungi and rotavirus. None of them was determined, isolated and/or seen. Two stool specimens, which were taken in one-week interval, also were examined with modified trichrome, acid fast and safranin staining methods. In microscopic examinations, *C. cayetanensis* oocysts were seen with all three staining methods. After four weeks of therapy with trimethoprim-sulphamethoxazole (15 mg/kg.d) diarrhea stopped and in the new stool specimens, *C. cayetanensis* oocysts were not encountered.

Case 4^[8]

A 52 year-old male patient with idiopathic hepatic cirrhosis

complaining of diarrhea and weakness was accepted to the gastroenterology clinic of Erciyes University Medical Faculty Hospital in 2001. In the patient's history, there were watery, bad smelling, bloodless episodes of diarrhea, fever, cold, sweating, and a 10 kg lost of weight which all began three weeks prior to hospitalization. The patient had never traveled to a foreign country. Physical examination did not reveal any abnormalities except subicteric conjunctivas and a hyperemic tongue. The patient was afebrile. In laboratory examination, blood values were found as Hb: 14.2 g/dL, white blood cells: 4 900/mm³, platelets: 69 000/mm³. Biochemical values were as follows: K: 2.9 mmol/L (↑), P: 1.7 mg/dL (↑), Ca: 7.6 mg/dL (↑), Mg: 0.9 mg/dL (↑), uric acid: 9.3 mg/dL (↑), total bilirubin: 4.2 mg/dL (↑), AST: 165 U/L (↑), ALT: 76 U/L (↑), CK-MB: 120 U/L (↑), albumin: 2.8 gr/dL (↑), acetone, protein and bilirubin were (+) in urine. Anti-HBs Ag and Anti-HAV were positive and other hepatitis markers were negative. Anti-HIV antibodies were found to be negative by ELISA test. In abdominal USG, liver with lobule contour was smaller than normal size, and its echo was increased. Widespread intraperitoneal exudate was seen.

In order to find out the causative etiologic agent of diarrhea, stool samples were examined by different methods and stained using modified Kinyoun's acid-fast stain. Following examination, acid-fast variable wrinkled spheres approximately 9 µm in diameter, were seen and diagnosed as *C. cayetanensis*. Confirmation of the diagnosis was established by fluorescent microscope (380 to 420 nm excitation filter), which showed bright green to intense blue autofluorescent oocysts.

Consequently, this organism was diagnosed as *C. cayetanensis*. The patient was treated with TMP/SMZ (160/800 mg) bid for 7 d. Following treatment, re-examination of a stool sample, however, did not reveal the presence of any organisms.

Case 5^{9]}

A 30 year-old female patient complaining of diarrhea and weakness was admitted to the gastroenterology clinic of Atatürk State Hospital in Izmir, 2002. In the patient's history, there were watery diarrhea, fever, nausea which all began one week prior to admission to hospital. Stool samples were examined by conventional coprological methods such as fresh preparation, iodine stain, flotation, modified Ritchie's method and modified Kinyoun's acid-fast stain. Acid-fast variable wrinkled spheres were seen and diagnosed as oocysts of *C. cayetanensis*. Confirmation of the diagnosis was established by fluorescent microscope. After one-week therapy with trimethoprim-sulphamethoxazole for 7 d, diarrhea stopped and in the new stool specimens, *C. cayetanensis* oocysts were not seen. This patient was different from the other four cases in Turkey because there were no abnormalities in immunologic tests of the patient. That is, she was an immunocompetent patient.

Case 6

A 62-year-old male patient complaining of diarrhea was admitted to the Erciyes University Medical Faculty Hospital in October 2002. This case was not reported anywhere. In the patient's history, there were watery, bad smelling diarrhea, fever, sweating, which all began one week before admission to hospital. Stool samples were examined by conventional coprological methods. Acid-fast variable oocysts were seen and diagnosed as oocysts of *C. cayetanensis* (Figure 1).

After one-week therapy with trimethoprim-sulphamethoxazole (160/800 mg) bid for 7 d diarrhea stopped and in the new stool specimens, *C. cayetanensis* oocysts were not encountered and all the symptoms disappeared. This patient was also immunocompetent similar to case 5. There were no abnormalities in immunologic tests of the patient.

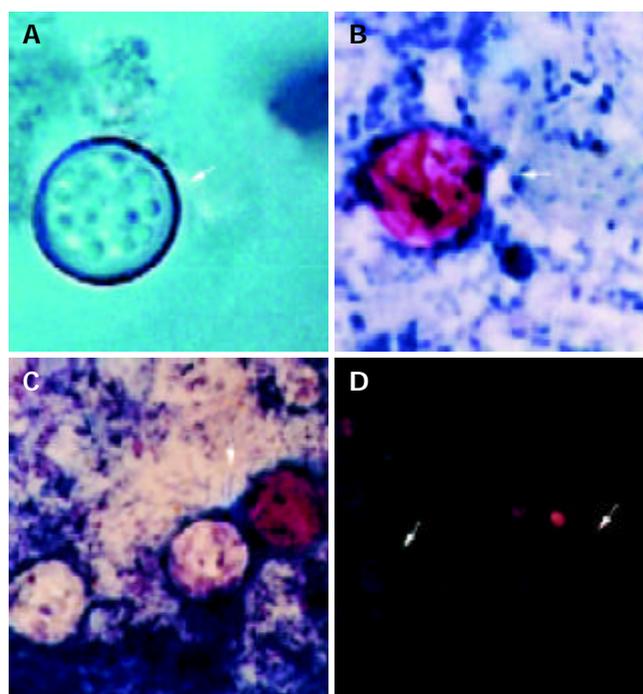


Figure 1 *Cyclospora* oocysts in stool smear preparations. A: *Cyclospora* oocysts in native preparation (original magnification. $\times 1\ 000$). B: Acid-fast stained *Cyclospora* oocysts (Modified Kinyoun's acid-fast stain: original magnification. $\times 1\ 000$). C: Acid-fast stained and unstained *Cyclospora* oocysts (Modified Kinyoun's acid-fast stain: original magnification. $\times 1\ 000$). D: *Cyclospora* oocysts' autofluorescence in wet preparation with fluorescent microscope of 380-420 nm wavelength. ($\times 400$).

DISCUSSION

Cyclospora organisms were first reported in the intestines of moles in 1870 by Eimer, and Schneider introduced the genus *Cyclospora* in 1881. The first report of human *Cyclospora* infection came from Papua New Guinea in 1979^[4].

Cyclospora oocysts in freshly excreted stool are non-infectious. The oocysts are thought to require days to weeks outside the host under favorable environmental conditions to sporulate and thus to become infectious. Direct person-to-person transmission by fecal exposure is unlikely, because excreted oocysts must sporulate to become infective^[10].

C. cayetanensis constitutes a significant cause of chronic and intermittent diarrhea in immunocompromised patients especially those with AIDS. A study in Haiti has documented the occurrence of chronic diarrhea in most patients with AIDS^[11]. *Cyclospora* infection has also been reported in patients with severe AIDS in other areas^[12,13]. Lontie *et al.*^[14] reported 2 cases of intestinal *Cyclospora* infection in immunocompetent Belgians. In Turkey, there were 6 cases between 1996 and 2002, 2 of them were AIDS patients^[5,6], 1 of them was an AML patient^[7], 1 of them was idiopathic hepatic cirrhosis^[8] and the last two patients were immunocompetent individuals^[9,10].

Both epidemiological and environmental data suggested that the organism be waterborne^[1]. The transmissibility of *Cyclospora* through water depends on the probability that the water source of interest could become contaminated and that the water treatment would kill or remove oocysts. *Cyclospora* oocysts, like *Cryptosporidium* oocysts, probably are highly chlorine resistant, but they could be more easily removed by conventional filtration because they are about twice as big as *Cryptosporidium* oocysts^[10].

In 1994, an outbreak of *Cyclospora* occurred among British soldiers and dependents stationed in a small military base detachment in Pokhara, Nepal. That outbreak was epidemiologically

Table 1 Similarities and differences between *Cryptosporidium parvum* and *Cyclospora cayetanensis*^[10]

Similarities	<i>C. cayetanensis</i>	<i>C. parvum</i>
Acid-fast staining of oocysts	Variable acid-fast	Acid-fast
Number of infective units (sporozoites) per sporulated oocyst	4	4
Completion of life cycle within humans	Yes, except for sporulation	Yes
Multiplication outside the host (e.g. in water or food)	No	No
Differences		
Size of oocysts	8-10 µm in diameter (intermediate in size between <i>Cryptosporidium parvum</i> and <i>Isoospora belli</i>)	Average width of 4.5 µm and average length of 5 µm
Number of organisms in stools of symptomatic nonimmune hosts	Typically excreted in low to moderate number	Often excreted in somewhat higher numbers
Autofluorescence of oocyst wall	Yes	No
Internal morphology of sporulated oocysts	Each oocyst has 2 internal sporocysts, each contains 2 sporozoites	The 4 sporozoites are naked within the oocyst
Infectivity of oocysts in freshly excreted stool	Must sporulate outside host to become infectious	Fully sporulated and infectious when excreted (sporozoites can be visualised when oocysts are excreted)
Zoonotic potential	Host range unknown	Infects virtually all commonly known wild and domestic mammals
Location in enterocytes of small bowel	Intracytoplasmic within a parasitophorous vacuole in apical supranuclear region	Intracellular, extracytoplasmic, within a parasitophorous vacuole at luminal surface of enterocyte
Susceptibility to antimicrobial agents	Treatment with TMP-SMZ leads to both clinical and parasitologic cure	Some antimicrobial agents (e.g. paromomycin) may cause clinical improvement, but no agents has been consistently demonstrated to provide parasitologic cure

linked to drinking water, because the organism was identified in the water source^[15].

In the 1990s, at least 11 definite and probable food born outbreaks of cyclosporiasis, affecting at least about 3 600 people, were documented, all of which occurred in North America^[10]. The outbreak that brought cyclosporiasis to prominence in North America and definitively established that *Cyclospora* was transmissible through food, occurred in 1996 in the United States and Canada and was linked to a third country, Guatemala, which was the source of implicated fresh raspberries^[16].

The symptoms presenting in one patient (watery diarrhea, nausea, weight loss, and abdominal pain) were similar to those classically *Cyclospora* infection^[11,13,17]. Since the oocysts of *Cyclospora* are acid-fast like those of *Cryptosporidium*, we recommend that all laboratories screening for the latter parasite include precise measurements of oocysts. *Cyclospora* oocysts are 8-10 µm in diameter (intermediate size between *Cryptosporidium parvum* and *Isoospora belli*). *Cryptosporidium parvum* oocysts have an average width and length of 4.5 µm 5 µm, respectively. It is possible that many cases of diarrhea reported to be due to *Cryptosporidium* might actually be due to *Cyclospora* because size discriminations are not often made. *Cyclospora* organisms have now been isolated in chronic diarrhea and this infection should be carefully distinguished from cryptosporidiosis^[11,11,18]. A list of some of the similarities and differences between these two organisms is shown in Table 1.

The treatment for *Cyclospora* infections cotrimoxazole (TMP-SMZ) was given for 7-10 d (longer, if symptoms persist)^[19]. The adult dosage was 160 mg TMP plus 800 mg SMZ orally twice daily. In a double blind, placebo controlled trial among Peruvian children, a three-day course of TMP (5 mg/kg.d) plus SMZ (25 mg/kg.d) decreased the duration of oocyst

excretion, but few symptomatic children were treated to address the effect on duration of diarrhea^[20]. Alternative treatments have not yet been identified. Limited data suggest that the following drugs are ineffective: albendazole, azithromycin, nalidixic acid, norfloxacin, tinidazole, metronidazole, quinacrine, tetracycline, and diloxanide furoate. Approaches to alternative treatment of patients who could not tolerate TMP-SMZ therapy include observation and symptomatic treatment^[21-25]. In a small, randomized, controlled-trial comparing oral TMP-SMZ and ciprofloxacin for treatment of and secondary prophylaxis for *Cyclospora* infection in HIV infected Haitians, ciprofloxacin (500 mg twice daily for 7 d as therapy and thrice weekly for 10 wk as secondary prophylaxis) was moderately effective, though it was less active than TMP-SMZ^[26]. These results suggest that ciprofloxacin might be an alternative for patients who cannot tolerate TMP-SMZ. However, these results should be confirmed in a large number of patients as well as in non-HIV population.

Sporadic cases of infection may be part of widespread outbreaks and should in any case be reported to public health officials. Public health personals and clinicians should also be aware that stool examination for *Cyclospora* should be specifically required in case of clinical suspicion of *Cyclospora* infection (protracted or relapsing diarrheal episode)^[10]. In conclusion, this organism should be considered in the differential diagnosis of unexplained diarrhea in both immunosuppressive and immunocompetent patients. However, further studies are needed to confirm the causative association with other diseases and to determine the incidence and epidemiological features of this organism.

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