Human cyclosporiasis 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 10 months and was diagnosed as cyclosporiasis in Kayseri, 1996. The second patient was also HIV positive and diagnosed as cyclosporiasis 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.

INTRODUCTION
The genus Cyclospora is in the subclass Coccidia, phylum Apicomplexa. This genus is taxonomically related to coccidians genera in humans. This organism is a unicellular parasite previously known as cyanobacterium-like or coccidians-like body (CLB) and, in recent years, a new coccidians 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 oocytes from light pink to deep red, some of which may contain granules or have a bubbly appearance. The oocytes autofluoresce (strong green or intense blue) under UV epifluorescence[3]. The first known human cases of illness caused by Cyclospora infection (i.e., cyclosporiasis) were reported in the medical literature in 1979[4]. Our aim in this study was to present cases of human cyclosporiasis 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 cyclosporiasis in Turkey.

RESULTS
Case 1[6]
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 10^4 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 oocytes 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 oocytes 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 conjunctivias and a hyperemic tongue. The patient was apyretic. 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 g/dL (↑), acitone, 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
A 30 year-old female patient complaining of diarrhea and weakness was admitted to the gastroenterology clinic of Ataturk 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.

DISCUSSION
Cyclospora organisms were first reported in the intestines of mole 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], 1 of them was an AML patient[6], 1 of them was idiopathic hepatic cirrhosis[7] and the last two patients were immunocompetent individuals[8,9].

Both epidemiological and environmental data suggested that the organism be waterborne[11]. 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
linked to drinking water, because the organism was identified in the water source\[18\].

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\[19\].

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 Isospora belli). Cryptosporidium parvum oocysts have an average width and length of 4.5 µm and 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\[11,13,18\]. A list of some of the similarities and differences between these two organisms is shown in Table 1.

The treatment for Cyclospora infections cotrimoxasole (TMP-SMZ) was given for 7-10 d (longer, if symptoms persist)\[10\]. 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. Pubic 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)\[19\]. 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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Yazar S et al. Human cyclosporiasis

13

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