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Co-infection of *Giardia intestinalis* and *Cyclospora cayetanensis* in an Immunocompetent Patient with Prolonged Diarrhea: Case Report

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***Cyclospora cayetanensis* is an agent of emerging infectious disease, and a recognized cause of diarrhea in some patients. Also, the flagellated protozoan, *Giardia intestinalis,* induces a diarrheal illness of the small intestine. Cases of cyclosporiasis are frequently missed, primarily due to the fact that the parasite can be quite difficult to detect in human fecal samples, despite an increasing amount of data regarding this parasite. On the other hand, *G. intestinalis* can be readily recognized via the microscopic visualization of its trophozoite or cyst forms in stained preparations or unstained wet mounts. In this report, we describe an uncommon case of co-infection with *G. intestinalis* and *C. cayetanensis* in an immunocompetent patient with prolonged diarrhea, living in a non-tropical region of Turkey.**

Keywords: *Giardia intestinalis*, *Cyclospora cayetanensis,* diarrhea

Cyclospora cayetanensis is a coccidian protozoa which causes recurrent gastrointestinal infections in humans, and is spread by the consumption of food or drinking water contaminated with stool from an infected person (Ortega *et al.,* 1994). *C. cayetanensis* is distinct from many other organisms, as it is not infectious at the time it is passed via the stool of an infected person. In fact, the parasite requires days or weeks to become infectious after being passed in a bowel movement. Persons of all ages are at risk for infection. However, people who have traveled in tropical and developing countries may be at increased risk of acquiring the organism. *C. cayetanensis,* a worldwide intestinal pathogen, has been implicated in a number of sporadic cases and epidemic outbreaks of diarrheal illness in several endemic areas (Herwaldt, 2000). This intestinal parasite has been shown to be involved in a number of registered food/ water borne outbreaks in Europe and North America (Herwaldt *et al.,* 1997). *Giardia intestinalis* is the most frequent protozoan detected in the gastrointestinal tract. *G. intestinalis* can be spread via contaminated water, food, and human contact. Children tend to suffer more frequently from *G. intestinalis* than adults. In chronic cases, such as occur with immunodeficient subjects, patients frequently prove resistant to drug treatment. In some cases, giardiasis may effect a significant shortening of lifespan in immunodeficient patients (Farthing, 1996; Ortega *et al.*, 1997).

A 24-year-old woman from Ankara, Turkey was first admitted to the Gulhane Military Medical Hospital, Ankara, in August 2003, with an acute onset of abdominal cramps, low-grade fever, and nausea. The patient's diarrhea was described as pale yellow, bloody, and mucous. The patient complained of 8-10 episodes of this diarrhea per day. Stool parasitological analysis samples were collected in a sterile container, and soon after, were transported to the laboratory for examination. Each stool specimen was microscopically examined in fresh normal saline smear and Lugol's iodine preparations. All of the stool samples were initially examined for ova and parasites by the Parasitology Laboratory at Gulhane Military Medical Hospital. Modified acid-fast staining, containing 5% sulfuric acid, was employed for decolorization. This was utilized as a screening procedure for all coccidian parasites, including *C. cayetanensis*. Stool examinations via modified-acid fast staining revealed no *G. intestinalis* and coccidian parasites in the stool samples. The patient's *G. intestinalis* infection was

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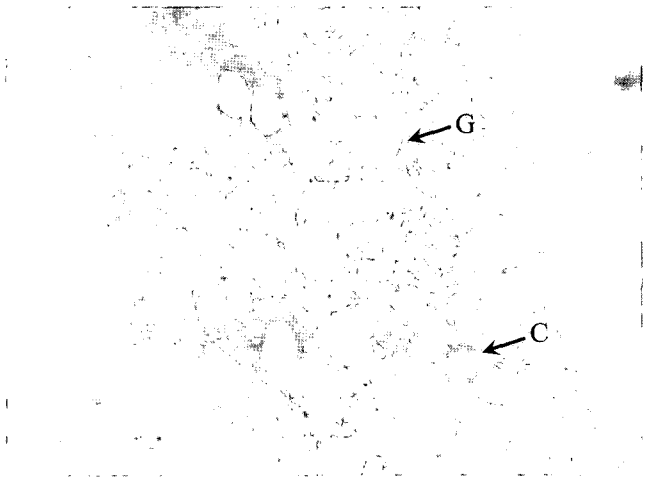

Fig. 1. Acid-fast staining revealed pink to reddish stained oval to round organisms 8-10 μm in diameter, suggestive of *Cyclospora cayetanensis* oocysts (C, arrow), and blue stained oval *Giardia intestinalis* trophozoites (G, arrow). Magnification x400.

treated with conventional doses of metronidazole; this resulted in a general improvement in her condition. In September 2003, the patient developed abdominal cramps, watery diarrhea, severe headache, and vomiting. She described a 10 kg weight loss over the course of a month, coupled with a considerable diminution of appetite. Laboratory studies on admission were notable, with an LDH of 342 and an AST of 72. HIV enzyme immunoassay test results were negative. Stool examinations by acid-fast stain again revealed *G. intestinalis* and *C. cayetanensis* oocysts (Fig. 1). Stool specimens were cultured on different and selective media for members of the Enterobacteriaceae, and no pathogenic bacteria were detected in the patient's stool specimens. She was treated with metronidazole 250 mg per day for 5 days and trimethoprim-sulfamethoxazole (160/800 mg) twice daily for 7 days. Gastrointestinal symptoms were completely resolved after only 48 hours of treatment. Fecal specimens were then collected on day 21; these confirmed that the patient's cyclosporiasis had been cured, resulting in complete clinical improvement.

C. cayetanensis is a recently described protozoan parasite which affects both immunosuppressed and HIV-infected patients, and also causes diarrheal illness worldwide. However, *C. cayetanensis* can be contracted via both water and food, and this manifests both sporadically and in outbreaks of diarrheal illness. Some reports have indicated that *C. cayetanensis* oocysts are resistant to current water treatment procedures, including chlorination (Rabold *et al.*, 1994). *Cryptosporidium parvum*, *G. intestinalis*, and *C. cayetanensis* are considered to be the major organisms contaminating drinking water supplies. More information regarding waterborne transmission can be found in recent research (Marshall *et al.*, 1997). The patient mentioned above was not found to suffer from immunosuppressive conditions, so it was theorized that her co-infection with *G. intestinalis* and *C. cayetanensis* was contracted via water and food, as in other sporadic cases. *C. cayetanensis* causes prolonged and relapsing watery diarrhea in immunosuppressed persons and AIDS patients. Other symptoms of infection can include loss of appetite, fatigue, anorexia, weight loss, bloating, increased gas, abdominal cramps, nausea, vomiting, myalgia, and low-grade fever (Hoge *et al.*, 1993; Berlin *et al.*, 1994). The parasites are harbored in the cells of the host's small intestine. At first, the parasite reproduces asexually, and the resultant merozoites infect more cells. Some of the merozoites generate gametes, which fuse and subsequently produce oocysts, and these oocysts are passed in the host's fecal material. After being passed in the feces, the oocysts become infective (sporulate) within one or two weeks. The next human is infected when he/she ingests food or water that has been contaminated by the oocysts. The practical diagnostic method consists of the identification of oocysts in stool specimens via light microscopy. *C. cayetanensis* stained orange with safranin are acid-fast variable, and under ultraviolet illumination, *C. cayetanensis* oocysts will autofluoresence, and appear as bluish-green circles (Eberhard *et al.*, 1997).

C. cayetanensis oocysts are not easily detected by direct microscopic observation. It should be noted that in cases in which the stool preparations are not carefully examined, oocysts might be misinterpreted due to the iodine staining of faecal compounds. Therefore, especially immunosuppressed patients with diarrhea and with prolonged and relapsing watery diarrhea should be examined thoroughly using acid-fast staining, in order not to overlook *C. cayetanensis* oocysts. Infectious disease physicians should be aware of this cause of diarrhea.

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