

## Case Report

### Diarrhea due to *Cyclospora*-like organism in an immunocompetent patient\*

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#### Abstract

*Cyclospora cayetanensis* infection continues to be a major cause of diarrhea particularly in immunosuppressed patients. The emergence of new related parasite pathogens, is an issue of public health concern. We report here a case where a *Cyclospora*-like organism was the cause of diarrhea in an immunocompetent patient from Venezuela.

**Key Words:** *Cyclospora cayetanensis*, diarrhea, epidemiology, immunocompetent patients.

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#### Introduction

*Cyclospora cayetanensis* is a coccidian parasite that clinically manifests as a profuse watery diarrhea in both immunocompetent and, more frequently, in immunosuppressed patients [1,2]. Although there have been many advances in research, the parasite's life cycle and the pathogenic mechanisms are still poorly understood. Even more, the report that these new emerging parasite pathogens, including non-*cayetanensis* *Cyclospora* spp. as the *Cyclospora*-like organisms, are able to produce diarrhea is an issue of public health concern. We report here a case where a *Cyclospora*-like organism was the cause of diarrhea in an immunocompetent patient from Venezuela.

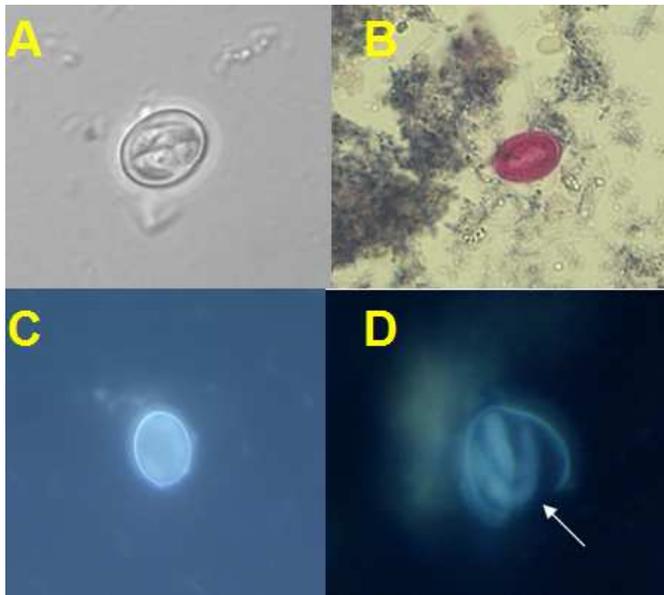
#### Case

A 27-year-old woman living in Cumaná, Sucre, Venezuela (a coastal zone located in the northeastern region of the country, with an annual temperature range of 26-32°C and a relative humidity of 89%, with significant poverty and unhealthy living conditions), was found with a 15-day history of sudden onset of watery diarrhea.

She was having more than 6 stools per day that were non-foul-smelling and with abundant mucus but without evidence of blood. The patient was afebrile the whole time. During this period she had noted the onset of epigastric pain that worsened with eating, but all the symptoms were self-limited and disappeared spontaneously two weeks after they occurred. She had been working in a fishing company for years and used to consume non-potable water and probably raw fish. Her family history was non-relevant for her current disease. Additionally in her house she had dogs, chickens and pigeons. On physical examination no significant alterations were found, neither in laboratory blood evaluations (except for 8% of eosinophilia). Her VDRL, HIV-1 and -2 –ELISA were negative. A complete evaluation of her stools sample was done. In physiological saline solutions was observed an ovoid oocyst with a non-retractile external wall, well-formed, with an undifferentiated cytoplasm that has 7 µm x 10 µm (Figure 1A). This form was very similar to the oocysts of *Cyclospora cayetanensis*. Kinyoun stain performed on a concentrated stool specimen demonstrated acid-fast oocysts having a uniform pink color without

dark granular inclusions (Figure 1B). Using an ultraviolet fluorescence microscope, the blue fluorescence phase was observed with 365 nm dichroic excitation filter similar to the *Cyclospora cayetanensis* oocysts (Figure 1C). After two months at environmental temperature the isolated organism did not make oocyst sporulation in a 2.5% potassium dichromate solution; however, using excystation solution we found one sporocyst enclosing two sporozoites, that have semilunar morphology with a pointed extreme and another round emitting interior fluorescence as well (Figure 1D).

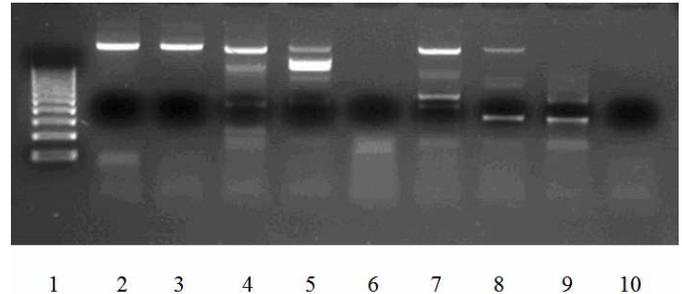
**Figure 1.** *Cyclospora*-like organism isolated from the patient's feces. Immature oocyst observed by phase contrast microscopy (1000X) (fecal sample) (A), stained with Kinyoun (1000X) (fecal sample) (B), at UV fluorescence microscopy with 365 nm dichroic excitation filter (1000X) (C), and excystation induced by sporulation solution showing sporozoites with typical morphology (arrow) at UV fluorescence microscopy (1000X) (D).



From the feces sample where the organism was isolated, using universal primers, the DNA was amplified and compared by PCR against *C. cayetanensis*, *Cryptosporidium parvum*, *Eimeria tenella*, *Eimeria sp.*, *Isospora belli*, and *Isospora sp.* (Figure 2) with the end result of confirming the organism to be *Cyclospora*-like. Additionally, her family members were all investigated, and showed positive for *Ascaris lumbricoides*, *Trichuris trichiura*, *Entamoeba coli*, *E. nana*. The same

negative results were found in her animals. The patient refused treatment and after 5 days her clinical symptoms disappeared and remained negative in further evaluations.

**Figure 2.** *Cyclospora*-like organism, comparison of its DNA amplified by PCR against other related organisms.



Lanes, 1: Molecular weight marker (100 bp ladder); 2: *Cyclospora cayetanensis*; 3: *Cryptosporidium parvum*; 4: *Eimeria tenella*; 5: *Eimeria sp.*; 6: *Isospora sp.*; 7: *Isospora belli*; 8: *Cyclospora*-like and *Cyclospora cayetanensis*; 9: *Cyclospora*-like (from patient's feces sample); 10: Negative control.

### Discussion

*Cyclospora cayetanensis* is a recently recognized protozoan parasite. It has been implicated as the etiologic agent of prolonged watery diarrhea, fatigue, and anorexia in humans. The mechanism of transmission is presumed to be ingestion of contaminated water. Cyclosporan organisms were first described in moles in 1870 and are believed to infect myriapods, vipers, and rodents [3,4]. Humans are the most recently confirmed host species [4,5] in which the parasites produce a syndrome bearing remarkable similarities to those caused by *Cryptosporidium spp* [6].

Human infection with organisms sharing morphologic and morphometric features with those of the *Cyclospora* genus was first described in 1979 [3,4], but still few reports of *Cyclospora*-like are found in literature. Our findings suggest that *Cyclospora*-like organism could be considered as a possible etiological agent for community-acquired diarrhea in the immunocompetent host, as we seen in this case. To date, few reports have described cases of *Cyclospora* diarrhea acquired in the community from exposure to contaminated sewage that had backed up into the patient's basement [6,7]. Many questions remain about the epidemiology of this newly described organism, and this is the first report from Latin America; therefore, we are performing field epidemiology studies to determinate the prevalence of this organism. Comparing the acute clinical

presentation of cryptosporidial enteritis and *Cyclospora* diarrhea, both are similar: frequent (often explosive), watery, nonbloody stools accompanied by crampy abdominal pain, nausea, and fatigue [6,8], most of them observed in our case. Fever is usually absent. It is important, therefore, for the clinical laboratory to differentiate between these two organisms. In the case of the *Cyclospora*-like organism identified in this report, it is also important to consider the sporulation of the cyclosporan oocyst, which took about 2 weeks for *Cyclospora cayetanensis* [6]. Although other reports have indicated formation of this sporocyst within 5 to 7 days [5,9], after 2 months we had not observed it. In the geographical area where this organism was isolated, other field studies have demonstrated a significant prevalence of other diarrhoeal and non-diarrhoeal parasites, specifically *Blastocystis hominis*, *Giardia duodenalis*, *Entamoeba histolytica*, and *Plasmodium vivax*, among others.

While there are many similarities between genera *Cryptosporidium*, *Eimeria* and *Cyclospora*, this last has been shown to diverge from predicted phylogenetic and environmental cycle paths [10]. In our PCR analysis we confirmed that the isolated organism was *Cyclospora*-like and unlike other protozoan parasites. Understanding these phylogenetic, clinical and epidemiological differences is not only important to expand our understanding of this organism, but to also aid in the study of other emerging pathogenic parasitic protozoa in both immunosuppressed and immunocompetent hosts [11].

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**Conflict of interests:** The authors declare that they have no conflict of interests.