A Report Case of *Cyclospora* and *Cryptosporidium* Mixed Infection in a HIV-Negative Child in Thailand

KULKANYA CHOKEPHAIBULKIT, M.D.*,
KANOKPORN TOSASUK, M.D.*,
SANAY CHEARSKUL, M.D.*,
DARAWAN WANACHIWANAWIN, M.D.**,
NIRUN VANPRAPA, M.D.*,

Abstract

The first case of cyclosporosis in a non HIV-infected child in Thailand, co-infected with *Cryptosporidium*, was reported. The patient was a 3 year-old malnourished orphan who presented with fever, abdominal distension and relapsing diarrhea. There was no leukocyte in her stool, however, numerous *Cyclospora* and *Cryptosporidium* oocysts were identified by modified acid-fast staining. The illness was cured by co-trimoxazole and fluid therapy. More coccidial infections in Thailand may be detected if modified acid-fast staining is routinely performed.

Key word : *Cyclospora, Cryptosporidium*, Relapsing Diarrhea

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CASE REPORT

A three year-old girl with spastic diplegia and a mild degree of malnutrition from Pakred Orphanage presented with fever, lethargy and watery diarrhea for 2 days. On physical examination, she was moderately dehydrated with fever of 39.5°C, respiratory rate of 30 / minute, pulse rate of 130 / minute and blood pressure of 90/60 mmHg. The initial laboratory tests revealed hematocrit of 40 per cent, hemoglobin of 12.9 g / dL, white blood count of 14,600 / mm^3 (69% polymorphonuclear cell, 25% lymphocyte, and 6% monocyte) and platelet count of 376,000 / mm^3. Her serum electrolytes revealed sodium of 153 mmol / L, potassium of 6.3 mmol / L, chloride of 114 mmol / L, and bicarbonate of 9 mmol / L. Routine stool examinations, which were

* Department of Pediatrics,
** Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

taken twice, were negative for leukocytes, red blood cells or parasites. Her condition improved with fluid therapy and correction of electrolyte imbalance. Diarrhea and fever ceased. Three days later, she developed a high fever of 39°C with abdominal pain and distension. The roentgenogram and ultrasonogram of the abdomen demonstrated generalized ileus. The symptoms were slightly improved with ceftriaxone and metronidazole therapy for presumptive enterocolitis. Four days later she developed mucous watery diarrhea that occured 5-6 times per day. The stool examination revealed no white blood cells but numerous *Cyclospora* oocysts, appearing as spherical organisms, approximately 8-10 μm in size and containing refractile greenish tinge granules, were identified. They were deep red with a mottled appearance on modified acid-fast staining. Moreover, numerous *Cryptosporidium* oocysts were also identified in the same specimen. Both organisms were differentiated by size, staining pattern, and the presence of autofluorescence under ultraviolet light (UV) microscopy. Her symptoms improved with co-trimoxazole treatment at the dosage of 20 mg/kg/day of trimethoprim for 5 days. In the subsequent stool examinations after treatment and complete recovery from the illness, neither *Cyclospora* nor *Cryptosporidium* were found. Her anti-HIV serology was negative.

DISCUSSION

The genus *Cyclospora* is closely related to other pathogenic coccidia such as *Cryptosporidium*, *Isospora*, *Toxoplasma* and *Sarcocystis*. Cyclosporosis has been found worldwide with notable endemicity in Nepal, Haiti and Peru[1-3]. The infection may occur in both immunocompromised and immunocompetent individuals[2,4]. The illnesses caused by *Cyclospora* infection include watery diarrhea, which may be prolonged, relapsing or cyclical pattern, anorexia, vomiting, weight loss, abdominal cramps, profound fatigue, and may be preceded by flu-like symptoms[5]. However, infection in endemic areas may be less symptomatic[6]. HIV infection status is associated with a higher incidence of *Cyclospora* infection and HIV-infected patients harbor higher quantities of organisms than non HIV-infected patients[2,7]. *Cyclospora* is not commonly found in Thailand and nearby countries. The cases of cyclosporosis reported in Thailand were all HIV-infected[8-11]. Manatsathit et al[8] found *Cryptosporidium* in 9 (20%) but *Cyclospora* in only 1 (2.2%) of stool samples from 45 AIDS patients with chronic diarrhea. Another study in Thailand by Wanachiwanawin et al[9] found only one patient with cyclosporosis in 91 HIV-infected patients but none in 103 non HIV- infected patients with chronic diarrhea. The relative low incidence of *Cyclospora* is also seen in North America and Europe [5]. This is probably due to the widespread use of co-trimoxazole for *Pneumocystis carinii* pneumonia prophylaxis.

Cryptosporidium is a prevalent coccidian parasite causing a broad spectrum of illnesses, ranging from asymptomatic to intractable diarrhea with significant weight loss, similar to cyclosporosis, in both normal and immunocompromised hosts[12-14]. Human immunodeficiency virus (HIV) infection is an important risk factor associated with both cryptosporidiosis and cyclosporosis[2,13,15]. *Cryptosporidium* is one of the most common causes of chronic diarrhea among patients with acquired immunodeficiency syndrome (AIDS) in Thailand [8,9].

To diagnose *Cyclospora* infection, examination of fresh stool by bright-field microscopy, UV microscopy, and modified acid-fast staining is required. The *Cyclospora* oocysts appear as 8-10 μm diameter nonrefractile spheres containing many refractile greenish globules inside. They are variably stained by modified acid-fast method and bright blue autofluorescence under UV fluorescence microscopy. Unlike *Cyclospora*, *Cryptosporidium* oocysts are smaller by one half and not autofluorescent. By electron microscopy, there are four naked sporozoites in each *Cryptosporidium* oocyst, different from *Cyclospora* oocyst which contains two sporocysts, in which there are two sporozoites[3].

The clinical symptoms of both cryptosporidiosis and cyclosporosis are similar and could be relapsing as manifested in this patient. Fever with abdominal pain and distension had misled to the diagnosis of enterocolitis. It is important to distinguish *Cyclospora* from *Cryptosporidium* by microscopic examination because the clinical manifestations are indistinguishable. The implication of distinguishing these two organisms is that infection by *Cyclospora* can be effectively treated while there is no effective therapy for the illness caused by *Cryptosporidium*[2,6,16]. The presenting patient may have been asymptomatically harboring *Cryptospridium* when she was suffering from cyclosporosis and therefore her diarrhea was resolved with

the clearance of *Cyclospora* by co-trimoxazole therapy. Co-trimoxazole is not effective in cryptosporidiosis. After treatment, however, the clearance of *Cryptosporidium* was probably due to the improvement of nutritional status after recovery from diarrhea.

Both *Cryptosporidium* and *Cyclospora* are water-borne pathogens therefore it is not surprising to find mixed infections from these two parasites. This patient is an orphan and might have been exposed to an unhygienic environment. Her malnutrition status was also another risk factor of these parasitic infections. To the best of our knowledge, this is the first report of *Cyclospora* infection in a non HIV-infected patient in Thailand. The incidence of coccidial infection in Thailand may be higher than previously expected if careful fecal examination with modified acid-fast staining is routinely performed.

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รายงานผู้ป่วยที่มีการติดเชื้อไซโครสปอรา ร่วมกับ คริปโตสปอริเดียม ในเด็กไทยรายหนึ่งที่ไม่ได้ติดเชื้อเอ็ชไอวี

กุลกัญญา โชคไพบูลย์กิจ, พ.บ., ดาราวรรณ วนะชิวนาวิน, พ.บ.**, กนกพร โตศะศุข, พ.บ.*, นิรันดร์ วรรณประภา, พ.บ.*, เสน่ห์ เจียสกุล, พ.บ.**

รายงานผู้ป่วยเด็กติดเชื้อ *Cyclospora* ซึ่งเป็นรายงานแรกในประเทศไทยในผู้ป่วยเด็กที่ไม่ติดเชื้อเอ็ชไอวี ซึ่งผู้ป่วยรายนี้มีการติดเชื้อ *Cryptosporidium* ร่วมด้วย ผู้ป่วยเป็นเด็กหญิงไทยกำพร้าอายุ 3 ปี และมีภาวะทุโภชนาการ มีอาการไข้ ท้องอืด และท้องเสียกลับซ้ำ จากการตรวจอุจจาระไม่พบเม็ดเลือดขาว แต่พบ oocyst ของเชื้อ *Cyclospora* และ *Cryptosporidium* จำนวนมากในอุจจาระโดยการตรวจวิธีย้อมสีแบบ modified acid-fast stain ผู้ป่วยได้รับการรักษาด้วยสารน้ำและยา co-trimoxazole ซึ่งทำให้หายจากอาการป่วย การติดเชื้อ *coccidial* อาจตรวจพบได้มากขึ้นหากได้ทำการตรวจอุจจาระของผู้ป่วยโดยการวิธีย้อมสีแบบ modified acid-fast stain เป็นประจำ

คำสำคัญ : *Cyclospora, Cryptosporidium,* ท้องเสียกลับซ้ำ

กุลกัญญา โชคไพบูลย์กิจ, ดาราวรรณ วนะชิวนาวิน, กนกพร โตศะศุข, นิรันดร์ วรรณประภา, เสน่ห์ เจียสกุล
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* ภาควิชากุมารเวชศาสตร์,
** ภาควิชาปรสิตวิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพ ฯ 10700