Identification of *Cyclospora* and *Isospora* from Diarrheic Patients in the Philippines

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In recent years, *Cyclospora cayetanensis* and *Isospora belli* have been recognized as causative organisms in cases of chronic diarrhea. The aim of this study was undertaken to determine the prevalence of enteric protozoa among diarrhea patients in the Philippines. Stools were collected and from 3456 samples examined, only one sample each was found positive for oocysts of *Cyclospora cayetanensis* and *Isospora belli*. Identification was based on autofluorescence of the oocysts with a 365 nm ultraviolet excitation filter. Both samples were obtained from male patients (18 and 73 years old, respectively) living in Iloilo province in the western islands of Visayas, Philippines. Both patients obtained their drinking water from deep wells. The identification of these two emerging pathogens, which are easily overlooked by less-trained technical staff, highlights the increasing awareness and technical capability on detecting these parasites in the Philippines.

Key Words: *Cyclospora, Isospora*, enteric protozoa, diarrhea

INTRODUCTION

Both *Cyclospora* and *Isospora* belongs to family *Eimeridae*, subclass *Apicomplexa*, which are closely related to *Cryptosporidium*. Like the latter, both are implicated as etiologic agents of diarrhea in immunocompromised persons, particularly patients with acquired immune deficiency syndrome (AIDS). *Cyclospora*, first observed in humans in Papua New Guinea (Ashford 1979), has been isolated from humans worldwide since 1985 with increasing frequency (Marshall et al. 1997). *Isospora belli* was first described in 1915 (Marshall et al. 1997) and is the only species of *Isospora* known to infect humans. It has been reported in tropical areas of south America and southeast Asia (Wittner et al. 1993), and has also been associated with diarrhea outbreaks in mental wards and day care centers (Marshall et al. 1997).

Previous reports have documented the presence of *Isospora* and *Cyclospora* in the Philippines (Markell et al. 1947; Faust et al. 1961; Jueco et al. 1984). *Isospora belli* was detected in five out of 103 United States naval personnel and their families upon returning to the US from the Philippines after World War II (Markell et al. 1947). Faust et al. (1961) also reported that there were American soldiers who were diagnosed with isosporiasis in the Philippines during World War I. Jueco et al. (1984) reported for the first time the
detection of *Isospora* from diarrhea cases among locals in the Philippines.

A case of traveler’s diarrhea caused by *C. cayetenensis* acquired in the Philippines was reported by Ohnishi et al. (2002). The patient stayed in the country for five days in 2001, and had persistent diarrhea and weight loss upon his return to Japan.

A study to determine enteric protozoans from patients with diarrhea in the Philippines is currently conducted by our group. The specific protozoans included in the study are *Giardia, Cryptosporidium, Cyclospora* and *Isospora*. The collection sites include selected areas in Luzon, Visayas, and Mindanao.

This paper documents the presence of *Cyclospora* and *Isospora* in two patients from two towns in Iloilo province.

**MATERIALS AND METHODS**

**Patients**

A total of 3,456 patients with diarrhea was included in this study. These were 1,934 males, 1,520 females, and 2 that had no data to indicate sex. The age range of the patients was <1 to 95 years old. Patients with irritable bowel syndrome were excluded.

**Collection Sites**

Single stool sample was collected from diarrheic patients who were seen in several collaborating hospitals and health centers from May 2004 to May 2005. There were 31 collaborating institutions from 15 cities/provinces in Luzon, 39 from 12 cities/provinces in the Visayas, and 9 from 5 cities/provinces in Mindanao.

**Preparation of Fecal Samples**

One milliliter of each stool sample was placed in a 15-mL polypropylene tube containing 9 mL of 10% formalin. The preserved stool samples were stored at 4° C until transported to the Research and Biotechnology Division of St. Luke’s Medical Center in Quezon City, Philippines for concentration and microscopic examination. All samples were concentrated using the formalin-ethyl acetate method and were centrifuged at 1,000 rpm at 20° C

Concentrated specimens were prepared for the detection of *Giardia* and *Cryptosporidium* by immunofluorescence at 450 nm wavelength and *Cyclospora* and *Isospora* by autofluorescence using 365 nm ultraviolet excitation filter.

**Microscopy**

Five µL of each concentrated specimen mixed with 5 µL of FITC-labeled monoclonal antibodies against *Cryptosporidium/Giardia* was examined by immunofluorescence microscopy at 450 nm wavelength. Using 365 nm wavelength, the same prepared slide was used for autofluorescence detection of *Cyclospora* and *Isospora*. The oocysts fluoresce blue with a 365 nm ultraviolet (UV) excitation filter (02UV, Zeiss), and are distinguished from each other based on their morphology. The same slide preparation was examined by phase-contrast microscopy using Zeiss Axiolab microscope. The oocysts of *Cyclospora* are round and measure 8–10 µm whereas those of *Isospora* are oval, measuring 20-33 µm by 10-19 µm and generally contain one or two immature sporoblasts.

Photomicrographs were taken using Nikon Coolpix 950.

**RESULTS**

The following enteric protozoa were detected in the 3,456 stool samples: *Giardia* (2 %), *Cryptosporidium* (1.94 %), *Cyclospora* (0.03 %), and *Isospora* (0.03 %).

Out of 3,456 diarrhea samples, only one was positive for *Cyclospora* (Figure 1) and also one was positive for *Isospora* (Figure 2). Both patients lived in two different towns in Iloilo province, located in western Visayan Islands (Figure 3). The possibility that contaminated water was the source of infection is high. In these towns, underground deep well water, which does not undergo any kind of treatment to ensure its safety, is used for drinking. *Cyclospora* was isolated from an 18-year-old male who had watery diarrhea. The stool sample was collected in Oct 2004 and was negative for other parasites. *Isospora* was isolated from a 73-year-old male who was passing soft stool. The stool was collected also in Oct 2004 and was negative for other parasites. *Isospora* was isolated from a 73-year-old male who was passing soft stool. The stool was collected also in Oct 2004 and was positive for eggs of two nematode species. One could either be eggs of *Ancylostoma duodenale* or of *Necator americanus*. The eggs of these two hookworms cannot be distinguished morphologically. The other nematode egg was identified as that of *Trichuris trichiura*. October falls during the rainy season, which is characterized by continuous heavy rains and floods in the Visayan Islands. Contamination of underground water becomes more pronounced.

There was no information on the travel history of the 2 patients who had the *Cyclospora* and the *Isospora*. The areas where the 2 patients live are in eastern Iloilo, about 35 km from the city. However, stool samples collected from diarrheic patients in 6 other towns nearby in Iloilo were negative for these protozoa.
Figure 1. *Cyclospora cayetanensis* oocyst from stool of an 18 year-old male with diarrhea: (A) fluorescence microscopy and (B) phase-contrast microscopy.

Figure 2. *Isospora belli* oocyst from stool of a 73 year-old male with diarrhea: (A) fluorescence microscopy and (B) phase-contrast microscopy.
DISCUSSION

This paper reports the detection of *Cyclospora* and *Isospora* in diarrhea stool samples from Iloilo. This is the second time that a local report is made on the occurrence of *Cyclospora* since the identification of *Cyclospora* from a Japanese traveler in the Philippines was previously reported (Ohnishi et al. 2002). *Isospora* has been locally reported only once (Jueco et al. 1984). As for the other enteric protozoa, results will be included in a forthcoming paper.

The identification of *C. cayetanensis* and *I. belli* in the Philippines highlights the development of increased capability and awareness of the technical staff.

*Cyclospora* and *Isospora*, which are autoflourescent, were detected by well-trained technicians. The use of UV epifluorescent illumination facilitates their detection. *Cyclospora* oocysts are not readily identified because these are excreted in low numbers of nondescript and unsporulated form. In the case of *Isospora belli*, microscopic identification is difficult because of the pale oocysts with transparent cyst walls (Marshall et al. 1997). Considering the enhanced technical capability and increased awareness for the need of a more thorough examination of diarrheic stool samples, more reports of these emerging enteric protozoa from the Philippines may be expected in the near future.

Figure 3. Map of the Philippines. The middle group of small islands is the Visayan islands. Arrow indicates the residence of patients positive for *Isospora* and *Cyclospora*. 
CONCLUSION

C. cayetanensis and I. belli are recognized as emerging protozoan pathogens of humans. Since these enteric protozoa are not included in routine stool examinations, it is possible that they are under reported. With improved detection methods, particularly the use of fluorescence microscopy, they can be easily identified due to their autofluorescence. With enhanced capability and awareness of laboratory staff, more accurate data on the prevalence of these pathogens are expected in the future.

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REFERENCES


