Point Prevalence of Cryptosporidium, Cyclospora, and Isospora Infections in Patients Being Evaluated for Diarrhea

Julie A. Ribes, MD, PhD,1,2 John P. Seabolt, EdD, MT(ASCP)SM,3 and Sue B. Overman, MA, SM(AAM)2

Key Words: Cryptosporidium; Cyclospora; Diarrhea; Intestinal parasites; Intestinal coccidian

Abstract

From March to September 2001, 315 specimens from “nonrepeat” patients that were submitted for ova and parasite examination were stained using the Kinyoun modified acid-fast stain to detect the intestinal coccidians. Four patients (1.3%) were infected with coccidians, 2 with Cryptosporidium parvum and 2 with Cyclospora cayetanensis. No infections with Isospora belli were detected. In comparison, 15 patients (4.8%) had infections with one or more intestinal parasites detected by routine trichrome staining: 5 had Giardia lamblia; 2, Dientamoeba fragilis; 3, Strongyloides stercoralis; 1, Iodamoeba bütschlii; 3, Endolimax nana; 6, Blastocystis hominis; and 1, Entamoeba coli. Four patients were multiply infected. Coccidians made up 29% of the clinically significant parasitic infections. The coccidians were missed in all 4 cases because no special staining was ordered. Clinicians need to be reminded that additional tests should be ordered to fully evaluate patients with chronic diarrhea in which no diagnosis is found by routine testing.

The intestinal coccidians, Cryptosporidium parvum, Cyclospora cayetanensis, and Isospora belli, are associated with gastrointestinal disease in humans. Although the first outbreak of well water–acquired cryptosporidiosis in the United States was seen in Texas in 1983,1 the most widely recognized outbreak occurred a decade later in 1993 in association with contamination of a municipal water source with Cryptosporidium oocysts originating from cattle.2 An estimated 403,000 immunocompromised and immunocompetent people were infected with this organism. Since that time, outbreaks have been linked to contaminated drinking and recreational water.3-5 Disease transmission also has been described via consumption of unpasteurized apple cider6 and through person-to-person contact.7 C parvum produces a diarrheal syndrome with profuse, watery diarrhea and disease manifestations that tend to be most severe and protracted and more common in immunocompromised patients, in whom it can produce extreme weight loss and wasting.8,9

C cayetanensis was first described as causing prolonged, watery diarrhea in humans in 1977.10 Although the disease occurrence is predominantly tropical and subtropical, it is seen worldwide, usually associated with importation of contaminated food products or contaminated water sources.10 Outbreaks of diarrheal disease in humans have been linked to the consumption of Guatemalan raspberries,11-16 basil,17 a variety of green leafy vegetables,18,19 and Asian freshwater clams.20 Although most cases reported in the literature represent healthy hosts, illness with the organism also is seen in immunocompromised populations.21

I belli is an uncommon human pathogen, linked most commonly to the handling of contaminated cat feces.22 This organism can cause disease in immunocompetent and
immunocompromised hosts, often producing prolonged, profuse, watery diarrhea; anorexia; weight loss; and fever.\textsuperscript{23,24} It has emerged as a significant cause of protracted diarrhea in patients with AIDS in Haiti and, to a lesser extent, in the United States.\textsuperscript{25,26}

Every year, thousands of patients examined at the University of Kentucky, Lexington, are evaluated for diarrheal syndromes. Bacterial cultures remain the first line of diagnosis for acute illnesses, while parasitic infections generally are considered in patients with chronic symptoms and those with appropriate travel histories or other risk factors. Most intestinal parasites can be detected with the combination of a formalin-ethyl acetate concentrate and standard trichrome stain.\textsuperscript{27,28} Wet preparations and trichrome stains do not adequately detect the coccidian organisms, \textit{C. cayetanensis}, \textit{C. parvum}, and \textit{I. belli}. Identification of these organisms requires special staining techniques.\textsuperscript{22,29,31} The modified acid-fast stain may be used to detect the oocysts of all of these coccidian organisms.\textsuperscript{27,29,32} The size distribution of the modified acid-fast staining oocysts, together with their overall morphologic features, permits differentiation of these organisms.\textsuperscript{22,30,31} We used the Kinyoun modified acid-fast stain\textsuperscript{33} to perform a point prevalence assessment of these coccidian diseases in patients being evaluated for diarrhea with examination for ova and parasites (O&P) during a 7-month period in central Kentucky.

\section*{Materials and Methods}

\subsection*{Patient Recruitment}

Patients were included in the study if they submitted at least 1 stool specimen for examination for O&P during the study period, March 9 to September 30, 2001. Only 1 sample per patient was used so as to not overrepresent patients submitting multiple specimens as part of a routine workup for diarrheal illness. All specimens represented those submitted for diagnostic purposes, and no specimens were collected specifically for the purpose of the study. Limited demographic data (sex, age, and evaluating service) were obtained, and patient identifiers were dissociated to maintain patient confidentiality. The protocol was approved by the University of Kentucky Institutional Review Board for patient safety.

\subsection*{Specimen Testing}

Specimens were processed following routine laboratory methods for diagnostic purposes. This included evaluation of formalin-ethyl acetate concentrates and trichrome-stained polyvinyl alcohol slides.\textsuperscript{27} The results of these evaluations were reported in the laboratory MISYS computer system (Misys Healthcare Systems, Raleigh, NC). All patient specimens positive for O&P during this study period were identified by a computer search of the database (MISYS Epi Report, Misys Healthcare Systems). Repeated positive specimens from a single patient were excluded so as not to overrepresent the prevalence of parasitic disease in the patient population.

Smears were made from formalin-ethyl acetate–fixed stool specimens and stained with the modified acid-fast stain (Difco BBL, Becton Dickinson, Sparks, MD).\textsuperscript{33} Positive and negative control materials were processed with each batch of patient slides stained. Staining was performed within 1 month of specimen acquisition, with most slides being stained within 1 week.

All slides were reviewed by 1 observer (J.P.S.) who reviewed at least 200 fields using light microscopy at \(\times100\). All positive slides were confirmed by a second observer (J.A.R.). The coccidians were identified by their modified acid-fast staining, size, and overall morphologic features \textbf{Image 1}. No additional confirmatory testing, such as \textit{C. parvum} direct fluorescent antibody (DFA) staining or enzyme-linked immunoassays, was performed for the purposes of this study.

\section*{Results}

Of the 315 unique samples studied, 19 (6.0\%) were positive for one or more intestinal parasites. Of the specimens, 14 (4.4\%) had clinically significant pathogenic organisms, while 8 (2.5\%) specimens contained nonpathogenic organisms, alone or in combination with an intestinal pathogen \textbf{Table II}. Patients with noncoccidian parasites had a mean age of 40.4 years, with only 2 of the 15 younger than 21 years. Four of these patients had mixed parasitic infections. There was no association of infection with any of the parasites with sex, as roughly half of the infected patients were male and half were female. As expected, \textit{Giardia lamblia} was the most common pathogen identified, while infections with nonpathogenic levels of \textit{Blastocystis hominis} were seen in a similar frequency. The 3 cases of \textit{Strongyloides stercoralis} were not unexpected because several cases of infection with this pathogen are identified each year in our facility.

Coccidian organisms were identified in 4 (29\%) of the 14 specimens that contained clinically significant pathogens \textbf{Table II}. The 2 cases of cryptosporidiosis and the 2 cases of cyclosporiasis occurred as pure parasitic infections with no other intestinal parasites seen in any of the cases. Fewer than 10 organisms were seen in each of these 4 cases. In the present study, \textit{C. parvum} and \textit{C. cayetanensis} were the only coccidians detected. There were no cases of \textit{I. belli} identified. In comparison with the specimens containing noncoccidian organisms, patients with coccidian organisms tended to be young: 3 of 4 patients were younger than 17 years.
Physician ordering patterns were studied for the 6-month period July 1 through December 31, 2003. During this period, 520 routine examinations for O&P were ordered. Of these, 146 (28.1%) also were tested for Cryptosporidium and Giardia organisms by DFA. Evaluation for Cyclospora and Isospora organisms by modified acid-fast stain was ordered for only 40 specimens, representing only 7.7% of the specimens for which examination for O&P was ordered.

**Discussion**

The coccidians are important emerging pathogens. They are capable of producing disease in immunocompetent and immunocompromised hosts. It is in this second category of patients that the coccidians take on the greatest significance. Many physicians remain unaware of their clinical importance, however. It is noteworthy that for all 4 cases in which *C. parvum* and *C. cayetanensis* were identified, no special testing had been ordered, and the cases represented missed diagnoses.

There are more efficient methods than the modified acid-fast stain for detecting *Cryptosporidium* oocysts in diarrheal stools. These include the highly sensitive and specific DFA stain (Meridian Biosciences, Cincinnati, OH), which is used as the primary test in our clinical laboratory for patient testing, or any of a number of commercially available enzyme immunoassays (EIAs). The DFA and EIAs have been shown to be more sensitive than the modified acid-fast stain, particularly when the organism burden is low.34,35 They are more efficient and less labor-intensive procedures for detecting *C. parvum* that require less technical skill for interpretation.34 False-positive results have, however, been identified using EIAs to detect *Cryptosporidium* infections.36,37 In addition, EIAs for *Cryptosporidium* species tend to be slightly less...
sensitive than the DFA.\textsuperscript{35,38} DFA and EIAs have the disadvantage of increased reagent cost, and they are organism-specific, detecting the oocysts only of Cryptosporidium species and not of the other coccidians.

Although a number of staining procedures have been reported to detect Cyclospora species,\textsuperscript{39} the modified acid-fast stain is the only stain capable of reliably detecting all 3 of the coccidians in clinical specimens.\textsuperscript{40} For this reason, it was selected for use in the present study. Owing to cost constraints of this study, no confirmatory testing or routine evaluation of the specimens was performed using the Cryptosporidium DFA. Had such a survey been performed, additional cases of cryptosporidiosis might have been detected.

The modified acid-fast stain requires significant time and expertise for interpretation. The stain is quite variable, with coccidian oocysts varying in staining intensity from clear (no stain uptake) to bright pink (Image 1).\textsuperscript{22,30-32,39,40} This variation may be seen within the organisms in a single sample. Staining intensity also has been reported to diminish with increasing time from slide preparation, which might pose a problem, particularly with control slides that are used to demonstrate appropriate staining for each batch of patient slides evaluated. This effect is seen particularly with control slides for C cayetanensis and to a lesser extent with C parvum. Difficulty detecting these oocysts in specimens can be compounded by their occurrence in small numbers, as in the present study. Adequate evaluation of each specimen needs to include review of at least 200 fields under oil before judging a specimen negative.

Aside from the present study, large numbers of patients have not been evaluated for these organisms in our laboratory. C parvum testing is ordered for only about one third of the roughly 85 patients tested each month for O&P. I belli and C cayetanensis examinations are ordered by our physician population in fewer than 10\% of the cases for which routine stool examinations for O&P have been requested. During the past 4 years for which data are available, only 11 patients have been identified at the University of Kentucky with C parvum infections, including 1 patient for whom the clinician ordered the assay only at the laboratory's recommendation and 2 who were identified during the present study. A full 27.3\% of positive results were identified in patients for whom testing was not ordered initially. Only 3 patients have been identified with cyclosporiasis, and 2 (67\%) of these were identified during the study and represent missed diagnoses. The remaining case was identified only because the laboratory recommended that a modified acid-fast stain be performed.

These data demonstrate that coccidian infections exist in the central Kentucky patient population being evaluated for diarrhea by routine examination for O&P. Clinicians need to be alerted to the fact that unusual organisms such as coccidians and microsporidians will not be detected unless specific diagnostic testing is ordered. Despite these interventions, clinicians might believe that these organisms do not occur in significant numbers in the community, might not be aware of the significance of these organisms in causing human disease, and might miss the opportunity to make the diagnosis in their patients. Reporting the results of a point prevalence study to the appropriate medical services might help heighten awareness of the relative frequency with which these pathogens are seen in patients with prolonged diarrheal illness.

From the Departments of 1Pathology and Laboratory Medicine and 2Department of Biology, University of Kentucky, and 3Clinical Microbiology Laboratories, University of Kentucky Medical Center, Lexington.

Acknowledgment: We thank Stephen Welch for expert assistance in preparing the image.

References


