RE-EVALUATION OF COMMERCIAL AVAILABLE ENZYME-LINKED IMMUNOSORBENT ASSAY FOR THE DETECTION OF GIARDIA LAMBLIA AND CRYPTOSPORIDIUM SPP FROM STOOL SPECIMENS

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Abstract. This study aimed to detect Giardia lamblia and Cryptosporidium spp infection from stool specimens. A total of 345 stool specimens were examined by microscopy (both direct smear and formalin concentration) and EIA techniques (ProSpecT Microplate Assay) for G. lamblia and Cryptosporidium spp. Of 73 tests positive for G. lamblia, 41 (56.2%) were positive by microscopy, and 71 (97.3%) were positive by EIA. Of 16 tests positive for Cryptosporidium spp, 5 (31.3%) were positive by microscopy, and 16 (100%) were positive by EIA technique. The results demonstrate that this EIA method is quick, simple, and more sensitive than the microscopy method and should be used for the detection of G. lamblia and Cryptosporidium spp where the prevalence of these protozoan parasites is a public health problem.

INTRODUCTION

Diarrheal disease is a major cause of childhood illness in rural areas world-wide. Giardia lamblia and Cryptosporidium are enteric protozoan parasites that take up residence in the small intestine, causing giardiasis and cryptosporidiosis, respectively. Giardiasis occurs world-wide, but is more prevalent in warmer climates. It is also more common in children than in adults. After ingestion, the cysts excyst in the duodenum thereby releasing trophozoites that rapidly multiply and colonize the microvillous surface of the jejunum. This process can lead to endothelial dysfunction, malabsorption of essential nutrients, nausea, diarrhea, abdominal distension, cramps, weight loss, flatulence, anemia and general weakness lasting from a few weeks to several months. In the young this can lead to morbidity and even death. Chronic infection may also be asymptomatic (Black et al., 1977; Wolfe, 1979; Garcia, 2001). Cryptosporidiosis is also recognized as a significant human disease in worldwide distribution. Transmission of Cryptosporidium is direct, by either the fecal-oral route or ingestion of water contaminated with infective oocysts. Illness is characterized by acute, severe diarrheal illness, and often leads to persistent infection or death in immunocompromized patients. Asymptomatic infection may also occur (Dubey et al., 1990; McDonald, 1996).

The examination of stool specimens for detection of intestinal parasites in our laboratory involves six different techniques of microscopic analysis: (1) direct saline and (2) iodine wet mounts of fresh stool, a (3) DMSO (Dimethylsulfoxide)-mAFB stained smear of fresh stool, an (4) iodine wet mount and (5) DMSO-mAFB stained smear of a sample concentrated by the formalin-ethylacetate sedimentation concentration technique from a 10% buffered formalin preserved stool, and a (6) trichrome stained smear from a Zinc sulfate polyvinyl alcohol (Zinc PVA) preserved stool (Bronsdon et al., 1984; NCCLS, 1997; Kellogg and Elder, 1999; Garcia, 2001). In patients with either giardiasis or cryptosporidiosis, the use of such routine diagnostic methods may be insufficient to detect these organisms. Additionally, diagnosis of these infections by microscopic examination can be time-consuming and expensive. Recent studies have found enzyme-linked immunosorbent assays (ELISA) to be sensitive, cost-effective, simple and a rapid method for the detection of G. lamblia and Cryptosporidium in stool specimens (Nash et al., 1987; Addiss et al., 1991; Garcia and Shimizu, 1997; Aldeen et al., 1998). The assays employed in this evaluation are manufactured by Alexon-Trend (now Remel) and are currently available (Remel, Inc, 12076 Santa Fe Drive, Lenexa, KS 66215). ProSpecT Giardia and Cryptosporidium Microplate assays are solid phase immunoassays for the detection of Giardia (GSA 65) and Cryptosporidium (CSA) Specific Antigens in stool specimens.
RE-EVALUATION OF ELISA FOR DETECTION OF G. LAMBLIA AND CRYPTOSPORIDIUM

These antigens are produced by the organisms within the host intestinal tract and can be detected in stool specimens with or without the visible presence of cysts, trophozoites or oocysts. In this study the above assays were used and re-evaluated to determine the true prevalence of G. lamblia and Cryptosporidium spp in the study area.

MATERIALS AND METHODS

After informed consents were obtained, stool specimens were collected from children 3 months to 5 years of age from the Sangkhla Buri district in the west of Thailand along the Thai-Myanmar border. All stool specimens were processed by preparing a 1:3 suspension of stool and preservative, namely 10% buffered formalin and Zinc PVA (Kellogg and Elder, 1999). Saline and iodine wet mounts of fresh stools were also prepared. The slides of fresh stool smears were fixed in absolute methanol for 10 minutes and air dried before performing DMSO-mAFB staining (Bronsdon et al, 1984) for detecting Cryptosporidium and Cyclospora oocysts. An iodine wet mount and DMSO-mAFB smear were prepared from the formalin preserved specimens after performing a formalin-ethylacetate sedimentation concentration. Trichrome staining was performed on the stool specimens preserved in Zinc PVA (Bronsdon et al, 1984; NCCLS, 1997; Kellogg and Elder, 1999; Garcia, 2001).

Part of each specimen was kept in a cryogenic vial at -20°C for weekly shipment to AFRIMS in a box on dry ice. There it was tested for G. lamblia and Cryptosporidium spp antigens by enzyme immunoassay (EIA: ProSpecT Microplate Assay, Alexon-Trend 14000 Unity St, NW, Ramsey, MN 55303). Positive ProSpecT Giardia/Cryptosporidium Microplate Assay results were followed-up by performing individual assays of ProSpecT Giardia Microplate Assay and ProSpecT Cryptosporidium Microplate Assay. EIA results were read both visually, according to the manufacturer’s guidelines (0 to 4+), and spectrophotometrically via optical density (OD) readings.

RESULTS

From October 2001 through October 2002, a total of 345 stool specimens were examined by microscopy as described above and ProSpecT Giardia/Cryptosporidium Microplate Assay. Of 84 positive results by ProSpecT Giardia/Cryptosporidium Microplate Assay, subsequently, 71 tested positive by ProSpecT Giardia Microplate Assay and 16 were positive by ProSpecT Cryptosporidium Microplate Assay. Upon further analysis of the original 84 positives, 9 stool samples were positive by both ProSpecT Giardia and Cryptosporidium Microplate Assays and 6 were negative when tested by the individual assays (Table 1).

Of 73 tests positive for G. lamblia, 41 (56.2%) were microscopy positive, and 71 (97.3%) were positive by EIA. Of 16 tests positive for Cryptosporidium spp, 5 (31.3%) were microscopy positive, and 16 (100%) were positive by EIA technique. The sensitivity of EIA ProSpecT Giardia/Cryptosporidium, Giardia, and Cryptosporidium Microplate Assays were 95.5, 94.9, and 100%, respectively (Table 2).

Visual readings and OD results showed good correlation when comparing positive results. They yielded the same results, except one specimen, in which the EIA Giardia Assay was negative by visual reading (0), but the OD yielded a positive result (OD = 0.081). All negative specimens had OD readings equal to or lower than 0.05 (Table 3). Microscopic examination also revealed a number of co-infections: 13 (3.8%) Ascaris lumbricoides, 1 (0.3%) Trichuris trichiura, 2 (0.6%) Blastocystis hominis, 4 (1.2%) Trichomonas

Table 1

Results of EIA ProSpecT Giardia/Cryptosporidium, Giardia, and Cryptosporidium Microplate Assays.

<table>
<thead>
<tr>
<th>EIA Microplate Assays</th>
<th>No. of stool specimens</th>
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<tbody>
<tr>
<td>Giardia/Cryptosporidium positive results</td>
<td>84</td>
</tr>
<tr>
<td>Giardia positive results alone</td>
<td>62</td>
</tr>
<tr>
<td>Cryptosporidium positive results alone</td>
<td>7</td>
</tr>
<tr>
<td>Both Giardia and Cryptosporidium positive results</td>
<td>9</td>
</tr>
<tr>
<td>Both Giardia and Cryptosporidium negative results</td>
<td>6</td>
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hominis, and 6 (1.7%) Cyclospora spp. These organisms showed no evidence of cross reactivity with Giardia lamblia or Cryptosporidium spp by EIA.

DISCUSSION

By virtue of its rural location, Sangkhla Buri is characterized by a high incidence of both G. lamblia and Cryptosporidium spp, 18.4 and 3.4%, respectively (Boonchai et al, 2002, unpublished data). The endemic nature of these intestinal parasites and their ability to be transmitted person-to-person (Black et al, 1977; Garcia, 2001) calls for reliable diagnostic methods. Accurate and rapid diagnosis can lead to implementation of preventative measures and - in cases of giardiasis - treatment to limit the spread of disease.

Choices for diagnostic testing are ever increasing. Historically, a diagnosis of giardiasis relied on detection of cysts and/or trophozoites in the stool by a highly trained microscopist, while a diagnosis of cryptosporidiosis relied on detection of small oocysts, again, by microscopic examination. These examinations are labor-intensive, subjective, require a well-trained microscopist who sees repeated positives, require consistency in staining, and rely on an infected person shedding the cysts, trophozoites, or oocysts, an event which is known to be intermittent.

For G. lamblia detection, advances in laboratory techniques include DFA, CIE, and ELISA. At least one study comparing two of these methods against the “gold standard”-microscopy-found DFA to be no more sensitive than microscopy (Aldeen et al, 1998). Detecting Cryptosporidium is being performed by an array of stains: auramine, DMSO-mAFB (Bronsdon, 1984), DFA, and ELISA. The drawback of DFA is its high cost. In contrast, EIA techniques to detect these two pathogens are recognized as rapid, easy to use, highly sensitive and specific, less labor-intensive and subjective than microscopic methods, and less dependent on expensive equipment (Dagan et al, 1995; Parisi and Tierno, 1995).

Our experience with ProSpecT Microtiter Assays confirmed earlier reports citing their ease of use and increased sensitivity when compared to “gold standard” methods (Dagan et al, 1995; Parissi and Tierno, 1995). In contrast to microscopy, ELISA
detected 30 additional *G. lamblia* cases and 11 additional *Cryptosporidium* cases in an infant and child population. The lack of cross-reactivity with five other parasites highlighted the test’s specificity. Freezing of specimens did not adversely affect test results. Additionally, high correlation between visual and OD readings made this group of three ELISA tests independent of equipment expenditures. Thus, these ELISA tests served as reliable and sensitive adjuvants to microscopic ova and parasite examinations.

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**REFERENCES**


