COMPARISON OF AUTOFLUORESCENCE AND IODINE STAINING FOR DETECTION OF ISOSpora BELLi IN FECES

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Abstract. To evaluate the sensitivity of autofluorescence for detection of Isospora oocysts, wet preparations of 192 stool samples from patients with chronic diarrhea were examined by fluorescence microscopy and by light microscopy after iodine staining used for routine screening for ova and parasites. Silicon-chambered glass coverslips were used for fluorescence microscopy. Isospora oocysts were detected in 46 iodine-stained concentrated stool samples; 91 samples were positive by autofluorescence. According to the maximum likelihood estimates, examination by fluorescence (95.7%; 95% confidence interval [CI], 85.2–99.5) was significantly more sensitive than iodine staining (48.4%; 95% CI, 37.7–59.1). Examination for autofluorescence is a simple, highly sensitive, inexpensive, and easily applicable method to detect Isospora spp. oocysts in feces.

INTRODUCTION

The coccidian Isospora belli was named after the period when it was first described as a cause of diarrhea (lat. bellum, war; here First World War). Only a few hundred cases of human isosporiasis were described until it became an opportunistic infection in immunocompromised, predominantly human immunodeficiency virus–infected patients. Transmission occurs via fecal–oral route, mainly by ingestion of infectious oocysts from contaminated food and water. Like other members of the class Coccidia, I. belli causes a self-limited gastrointestinal disease characterized by watery diarrhea. The disease rarely becomes chronic in immunocompetent patients, but it does often become chronic in patients with acquired immunodeficiency syndrome (AIDS). In tropical and subtropical regions, up to 20% of AIDS patients with diarrhea experience isosporiasis. Treatment—for example, with trimethoprim–sulfamethoxazole—is mandatory in immunocompetent patients who do not experience spontaneous resolution. In patients with AIDS, a continuous prophylaxis is required if CD4 cell counts cannot be increased by antiretroviral therapy.

The 10–20- by 20–30-μm-sized oocysts are found in feces; sometimes they are also found in duodenal aspirates and bile. Standard method of detecting human intestinal Coccidia is examination of concentrated stool. Whereas acid-fast stain is recommended for Cryptosporidium and Cyclospora, Isospora oocysts are stained by iodine, which is commonly used to screen for ova and parasites. Isospora and Cyclospora oocysts fluoresce a bluish violet color under ultraviolet excitation (365 nm), and they become a fluorescent bright green under violet excitation (405 nm). Although examination of wet preparations by fluorescence has become a standard method to detect intestinal Coccidia, the degree of sensitivity has not been well established.

MATERIAL AND METHODS

Stool samples were obtained from immunocompromised bone marrow transplant recipients, patients with human immunodeficiency virus, immunocompetent travelers returning from tropical countries of all age groups with chronically (> 4 weeks) persisting diarrhea, and from one patient with chronic isosporiasis. Each sample was prepared twice by a modified merthiolate–iodine–formaldehyde concentration technique, once with and once without iodine for staining. In contrast to the original description, the toxic merthiolate step was skipped. A wet preparation of the iodine-stained sediment was then screened by light microscopy for typical oocysts and other parasites; ×200 to ×1,000 magnification was used.

For detection of autofluorescence, 100 μL of unstained sediment was filled into wells formed by glass coverslips and commercially available silicon gaskets with 12 holes (Flexiperm Mikro 12; Vivascience AG, Hannover, Germany). The silicon completely adheres to glass to form wells without leakage. These chambered coverslips are commonly used for cell cultures because the glass bottom of each well allows for inverse microscopy up to ×1,000 magnification. In contrast, microtiter plates made of polystyrene cannot be examined beyond ×100 magnification. An inverse Olympus fluorescence microscope with ultraviolet (365 nm) and violet excitation (405 nm) was used to screen stool samples for autofluorescence.

Examinations and comparison of results were performed without any correlation to the patients’ data. A sample was recorded positive if oocysts were found by at least one method; otherwise, it was regarded negative. Assuming that the detection of typical oocysts by any of the 2 methods has to be regarded as evidence for Isospora, no false-positive result will be found, and a specificity of 100% of each method can be considered. The maximum likelihood method was used to estimate the sensitivity of each method for detection of Isospora oocysts in stool samples. In addition, we provide the exact 95% confidence intervals (CIs) based on the binomial distribution. The sensitivities were compared by the sign test.

RESULTS

Out of 192 stool samples examined, 93 were positive by at least one method, and 99 were recorded negative by both methods (Table 1). A positive result by both methods was obtained in 44 samples; 2 were positive only by iodine staining, and 47 samples were positive only by the autofluorescence technique. According to the maximum likelihood method, an Isospora prevalence of 49.6% in the study group was calculated, which is slightly above the 48.4% prevalence found by microscopy. Thus, only 2 false-negative results by both methods are expected by the model. The sensitivity of iodine staining was 48.4% (95% CI, 37.7–59.1), but a sensi-
tivity of 95.7% (95% CI, 85.2–99.5) was calculated for auto-
fluorescence ($P < 0.0001$ according to the sign test). The odds ratio of the 2 sensitivities is 23.5 (95% CI, 7.3–143.8).

**DISCUSSION**

The detection of autofluorescent oocysts is simple and fast; it is based on the distinct green or bluish color. The time needed to screen 100 μL of a wet preparation is identical to that needed to examine iodine-stained fecal sediments ~ 2–5 μL in volume. Identical volumes of feces were used for examination by autofluorescence and examination of Kinyoun acid-fast–stained smears in a former study. No difference in sensitivity was found, but autofluorescence was recommended because of its simple preparation and the deletion of staining procedures. The significantly higher sensitivity in the present study can be attributed to the larger volume of 100 μL examined by fluorescence. However, in a previous study of stool samples from swine, autofluorescence disclosed a significantly higher number of infections as well as higher numbers of *Isospora suis* oocysts per sample in spite of the use of identical volumes for both methods. The difference was due to easy and clear-cut identification of autofluorescent oocysts against a dark or nonspecific greenish background; fecal components, on the other hand, disturb visualization of iodine-stained oocysts by light microscopy. In the present study, wet preparations used for autofluorescence were not translucent. Therefore, examination by light microscopy was impossible. Whereas fecal debris hinders detection of iodine stained *Isospora* oocysts by light microscopy and thus limits the volume to be examined, autofluorescence shines through even large amounts of fecal material, as demonstrated here. The chambered coverslips used in the present study are comfortable to screen 100-μL samples. The silicon gaskets can be reused after disinfection, although this is not recommended. This procedure’s requirement of an inverse fluorescence microscope might be disadvantageous. Screening of wet preparations by epifluorescence microscopy is a well-established method, but the volume to be screened is limited.4

The PCR method is another sensitive way to detect *Isospora* in clinical specimens. It was not included in this study because it cannot be regarded as an alternative for routine use: it requires special skill and special machines to perform, and it is expensive.

Autofluorescence of the oocyst wall under ultraviolet or violet illumination has been detected in all intestinal coccidian human pathogens, although the autofluorescence is so weak in *Cryptosporidium* that it cannot be used to enhance diagnosis.6 A differentiation from autofluorescent plant and fungal spores is not possible because of identical fluorescence and size. Thus, examination of wet preparations for autofluorescence of *Isospora* and *Cyclospora*, but not *Cryptosporidium* oocysts, in fecal samples has to be regarded as a highly sensitive screening method. It is especially recommended in regions in which both opportunistic infections are common.

In conclusion, we demonstrate examination of wet preparations by autofluorescence to be significantly more sensitive than iodine staining for the detection of *I. belli* oocysts in concentrated stool samples (odds ratio, 23.5; 95% CI, 7.3–143.8).

Acknowledgments: The data presented here are part of the doctoral thesis of Nicole Binder.

Financial support: This study was supported by a financial grant from Förderverein AIDS im Kindesalter e.V., Bonn, Germany.

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