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Comparison of Three Methods for Detection of *Cryptosporidium* Oocysts in a Low-Prevalence Population

ELLEN JO BARON,[1,2]†* CATHY SCHENONE,[1] AND BEVERLY TANENBAUM[1]

North Shore University Hospital, Manhasset, New York 11030,[1] and Cornell University Medical College, New York, New York 10021[2]

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Four hundred fecal specimens which had been received for routine ova and parasite examination were concentrated by Formalin-ether sedimentation. Sediments were examined as saline and iodine-stained wet preparations and were stained with rhodamine-auramine O and a commercially available monoclonal fluorescent-antibody stain for oocysts of *Cryptosporidium* species. Examination with the fluorescent stains detected cryptosporidia in both positive specimens (0.5% prevalence), and routine direct wet-preparation examination detected cryptosporidia in one of them. Detection of only low numbers of positive specimens in our nonrisk population argues against routine use of specific and expensive stain reagents.

Oocysts of *Cryptosporidium* species can be identified in human and animal feces by their acid-fast property. Although Sheather sugar flotation may result in increased concentration of the cysts, this method is cumbersome and does not lend itself to convenient incorporation within the routine concentration and staining procedures favored in most clinical laboratories (3, 5, 8). Any acid-fast stain (including fluorochromes) will be taken up by cyst walls, but the time required to prepare and examine acid-fast stains on all stool samples received for routine parasitology would not be cost effective unless the prevalence of cryptosporidiosis was shown to warrant such effort (5, 6).

According to published studies from developed countries, including Australia, Canada, England, and the United States, prevalence among patients without known risk factors, such as acquired immunodeficiency syndrome (AIDS) or intimate animal contact, has been extremely low (1, 2, 7, 9, 10). The purpose of our study was twofold: (i) to determine the prevalence of cryptosporidiosis among patients from a sophisticated urban and suburban setting whose stool samples were submitted for routine parasitologic examination, and (ii) to ascertain whether either of two tests (one stain which is specific for oocysts and one stain which enhances visibility of oocysts) would offer an advantage, in addition to routine stool sample examination procedures, for the detection of cryptosporidia in our patient population.

Although *Cryptosporidium* prevalence has been studied, authors disagree as to whether special methods should be used in a search for oocysts along with routine parasitologic examination methods (13; I. Nachamkin, Reply, J. Infect. Dis. **156**:47, 1987). Because the incidence in humans is higher in summer and autumn than at other times, some workers have advocated an extended period of observation to ascertain the value of routinely performing specific assays for this parasite (12). We examined a total of 400 stool samples received during the periods from mid-June through late July and from mid-September through late October. In this way, we were able to include stool samples from the time periods thought to include seasonal peaks in incidence in the United States.

All stool samples were examined directly by emulsifying a small portion (approximately 0.1 ml) in a drop of saline on a microscope slide and another portion in a drop of Lugol iodine on a separate slide. These wet preparations were covered with a glass cover slip and examined visually under magnifications of ×100 and ×400 with reduced light for a minimum of 3 min per preparation, covering approximately 100 fields. Oocysts were visible as round or oval refractile particles with diameters of 2 to 6 μm. They failed to take up iodine; this is helpful in distinguishing them from yeast cells, which they resemble on wet preparations (3). In addition, a walnut-sized portion of feces was concentrated by using the Fecal Parasite Concentrator system (Evergreen Scientific, Los Angeles, Calif.). Concentration has been shown to increase the number of oocysts visualized (13). All concentrated stool samples were examined by using routine methods (wet preparation and iodine stain). A portion of each unformed stool sample (approximately 65% of all stool samples received for ova and parasite studies) was stained with a Trichrome-Wheatley stain and examined by using standard procedures (3). If particles suspected of containing *Cryptosporidium* species were found at any time during the routine examination, a modified acid-fast stain of the material (Kinyoun carbolfuchsin) was examined (3). The concentrates from all stool specimens were then renumbered to randomize them, and portions were stained with a rhodamine-auramine O acid-fast method (Difco Laboratories, Detroit, Mich.) and with the Meridian Merifluor monoclonal antibody fluorescein-conjugated stain (Meridian Laboratories, Cincinnati, Ohio) according to the recommendations of the manufacturer (4). Rhodamine-auramine O-stained slides were examined at a magnification of ×100 for at least 1 min, covering a minimum of 30 fields of view, although those slides showing suspicious particles often required examination for a longer period (up to 5 min).

Monoclonal antibody-stained slides could be examined in approximately 1 min, and positive results were often apparent immediately. This stain has been shown to be highly specific for *Cryptosporidium* oocysts with virtually no cross-reactivity (4). The technologist examining smears by any method was blind to the results achieved with other methods.

* Corresponding author.

† Present address: Clinical Anaerobic Bacteriology Research Laboratory, Wadsworth Division, Veterans Administration Medical Center, Wilshire and Sawtelle Blvds., West Los Angeles, CA 90073.

A second smear was prepared from the concentrates of stool samples thought to be positive by either fluorescence method and was restained with the Merifluor fluorescent-antibody stain and with modified Kinyoun carbolfuchsin. The original positive smear was cleaned with xylene and overstained with modified Kinyoun carbolfuchsin as well.

The Merifluor stain detected four positive stools, although two of these were not confirmed on reexamination of the sediment. Rhodamine-auramine O yielded 101 smears that exhibited nonspecific fluorescence to such a degree that a definitive determination could not be made initially. Confirmatory restaining with Kinyoun carbolfuchsin and the monoclonal antibody stain detected only two true positives from this group of 101 smears. Routine examination (wet preparations) of all stools detected one of the positive results (from a patient with AIDS). Previous studies have shown that symptomatic patients shed moderate-to-large numbers of oocysts in their stools, increasing the likelihood of detection by routine methods (11). Specificity was 80% for rhodamine-auramine O and 99.5% for Merifluor in our low-prevalence population.

The Merifluor stain procedure yielded a clean slide with a clear-cut result, in contrast to numerous ambiguous results obtained with rhodamine-auramine O. Kinyoun acid-fast stain also yielded interpretable results, but the smears required additional time for examination (at least 3 min each). The two false-positive specimens detected with Merifluor were thought to be the result of spillover from a positive control specimen on the same slide. We recommend that a separate slide be used to stain the positive control, which contains numerous oocysts. The Merifluor monoclonal antibody fluorescent stain for *Cryptosporidium* oocysts is a reliable and specific stain; we recommend its use as a confirmatory method for ambiguous results obtained by routine methods and as a standard procedure for specimens from patients with a high probability of having cryptosporidiosis, such as immunosuppressed patients, those with chronic diarrhea of unknown etiology, and those with traveler's diarrhea, and specimens from epidemic diarrhea outbreaks (such as those involving institutionalized patients, children in day care centers, and patients with veterinary animal contact) (12).

Over the 3-month study period, encompassing what are thought to be times of increased prevalence in the United States, we found only 0.5% prevalence of detectable oocysts from our unselected patient population. Although the total number of stool samples examined was small, it represents the types of specimens routinely submitted to similar medical centers in the northeastern United States. We did not skew our expected results by selecting stool samples from patients with AIDS or other immunosuppression; only 15 of the specimens examined were received from patients diagnosed as having AIDS. One of the two positive results was from a patient with AIDS, and the other was from an elderly female patient without recent travel history. Given these results, we do not recommend routine use of an expensive and time-consuming procedure such as the fluorescent stains or the acid-fast stain for the detection of cryptosporidiosis in patient populations similar to ours, although an occasional specimen with low numbers of oocysts may be missed by a routine visual examination with and without iodine. It may be prudent for individual laboratories to undertake a similar survey to determine the prevalence of this parasite in the patient population that they serve before choosing routine methods for the detection of *Cryptosporidium* species.

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