Immunological Methods of Diagnosis in Giardiasis: An Overview

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ABSTRACT

The epidemiology and clinical presentations of giardiasis are reviewed to provide a basis of understanding the laboratory aspects of this parasitic infection. Diagnosis is then discussed in light of recent studies that challenge our overall approach to the diagnosis of enteric parasites and demonstrate the need for further evaluation on the basis of cost-effectiveness as well as reliability and clinical practicality. Overall effectiveness as well as the difficulties with present standard diagnostic methods in giardiasis are reviewed. This is followed by a discussion of the more recently developed immunological approaches to diagnosis. The role of these tests in the parasitology laboratory is also discussed.

Introduction

*Giardia* is a single-celled parasite capable of infecting the gastrointestinal tract of man and animal. It is found world-wide. Whilst mortality is minimal, on a global scale the morbidity associated with giardiasis is considerable. Large numbers of infections are reported annually in first-world countries, particularly in children. In third-world countries where laboratory confirmation is less likely and under-reporting more common, *Giardia* adds significantly to the burden of diarrheal disease produced by a whole cadre of infectious agents, viruses, bacteria, or parasites.

There is presently some interest in the diagnosis of giardiasis, although not too long ago infection with *Giardia* was considered to be of little clinical consequence. This revival of interest is probably based on the growing appreciation for *Giardia*'s potential for pathogenicity and its ubiquity. For these and other reasons, increasing numbers of cases of giardiasis are being identified by clinicians and confirmed by the laboratory. Giardiasis is one of the few enteric parasitic infections for which non-microscopic detection methods have been described, and the only one at present for which commercially available diagnostic products have been developed.
Epidemiology

At the present time, giardiasis is the most frequently reported gastrointestinal parasitic infection in many parts of the world including the U.S.A. and Canada.\textsuperscript{25,32,17} It has recently been the most common of all reportable enteric (bacterial or parasitic) infections in the western Canadian province of British Columbia.\textsuperscript{11} The prevalence of infection in other parts of North America varies; where sociological factors or inanimate vehicles of transmission with potential for their contamination are present, infection is common.

Transmission is by the fecal-oral route. Different forms of direct or person-to-person spread are likely to occur where standards of personal hygiene are poor. Transmission in day-care centres,\textsuperscript{24} in institutions for the mentally handicapped,\textsuperscript{36} and in other situations where poverty or ignorance preclude good personal hygiene\textsuperscript{31,5} are considered to be due to person-to-person spread. Sexual transmission is also reported.\textsuperscript{33}

The indirect route of transmission occurs where food or drinking water is contaminated by viable cysts from a strain of \textit{Giardia} capable of infecting a human host. Many waterborne outbreaks have been reported\textsuperscript{10,23} in North America and \textit{Giardia} now has the dubious honour of being the most frequently identified agent of epidemic gastroenteritis in the U.S.A.\textsuperscript{28} Endemic waterborne transmission is probably also common, but laboratory confirmation of this or other forms of indirect transmission is difficult to obtain since laboratory techniques used to identify \textit{Giardia} cysts in environmental samples are relatively insensitive. Because the number of cysts present in water or food cannot be amplified in the laboratory, microscopic identification of small numbers of this organism in large volumes of drinking water is both time-consuming and difficult. Two relatively small food-borne outbreaks have also recently been reported.\textsuperscript{38,40}

Clinical Presentations

\textit{Giardia} is now considered to be a non-invasive protozoan, but the pathophysiology of infection is not well understood. Villous atrophy, prostaglandin secretion, brush border injury, and host immune response are all factors that may be important in pathogenesis.\textsuperscript{45}

The spectrum of clinical presentations ranges from asymptomatic passage of cysts to severe, incapacitating diarrhea.\textsuperscript{53} Results of malabsorption may be debilitating, and a failure to thrive syndrome has been documented in infants.\textsuperscript{29} Malabsorption of fat, lactose, and Vitamin B\textsubscript{12}, a protein-losing enteropathy and a Vitamin A deficiency state have also been associated with severe symptomatic giardiasis.\textsuperscript{18,30} Compromised hosts such as children with immunodeficiency disorders or with underlying protein calorie malnutrition\textsuperscript{1} are particularly susceptible to the gastrointestinal insults of symptomatic infections. Janoff et al\textsuperscript{21} note that the severe, prolonged diarrhea, so often seen in the immunocompromised patients with AIDS, has not been associated with this infection.

Symptoms of acute giardiasis include nausea, anorexia, malaise, and explosive diarrhea. Foul-smelling, watery stools are often associated with increased flatulence and abdominal cramps. This acute stage may resolve spontaneously after a few days, but some patients go on to become asymptomatic cyst passers while others have intermittent recurrence of symptoms. Chronic giardiasis is associated with the passing of frequent and foul-smelling, semi-formed stools often accompanied by increased flatulence, abdominal cramps, nausea, belching, heart-burn, anorexia, and weight loss.
The host may eliminate the parasite after a variable length of time without therapy, but two therapeutic agents, metronidazole and quinacrine, are both relatively effective.9

Diagnostic Approaches

In the face of rising health care costs, medical parasitology laboratories need to reassess their approach to the diagnosis of enteric parasites. Incorporation of new or well-known laboratory procedures into a routine for specimen examination should be based on cost-effectiveness, the clinical significance of results, as well as the sensitivity and specificity of the test. At present, all gastrointestinal parasites including *Giardia* are identified by use of the same procedure; microscopic examination of preparations from two or three fecal samples submitted by the patient.

The recommendation that several specimens routinely be submitted for “O + P” examination arose out of studies in which less efficient diagnostic methods were used than are available now. It has recently been reported that diagnosis of the majority of clinically significant infections, including giardiasis, may be made on the first of this series of fecal examinations. Montessori35 and others44 have suggested that physicians should consider ordering examination of a second or third fecal specimen for parasites only when the result from the first examination is negative, the patient’s symptoms persist, and other causes for the diarrhea have not been found. The usefulness of such a selective processing of fecal specimens will only succeed, however, where information flow between clinician and laboratory is efficient and bi-directional.

How far should cost-containment go? Should medical parasitologists also reevaluate whether or not permanent stained preparations, such as iron hematoxylin or Gomori’s trichrome stain, need be made on every stool specimen submitted to the laboratory? At present, many experts in North America advocate examination of stained preparations on all specimens.2,12 In one recent study of pooled versus multiple separate specimens (testing for all “ova and parasites”) carried out in an attempt to address control of laboratory costs, the authors stated that preparing stained slides on all specimens was necessary.39 In other parts of the world, however, expert diagnostic parasitologists suggest that the laboratory should again be directed towards a more selective use of stained preparations depending primarily on the clinical presentation of the patient. These and other questions need to be studied further.

Perceived if not real difficulties in the diagnosis of giardiasis have spawned development of new laboratory approaches for this infection. Reference is often made to the study by Danciger;8 in this group of children studied, the number of *Giardia* passed daily in feces was noted to be either high or low, or the number fluctuated. Intermittent shedding of cysts has been documented in the gerbil animal model for giardiasis.3 Clinicians also have long recognized the occurrence of “cryptic” cases of giardiasis where stool examinations have been negative but subsequent examination of a small bowel specimen confirmed the presence of the parasite.

The notion of the superiority of examination of small bowel specimens possibly arose from experiences with this group of “cryptic” cases; however, in a recent study14 it was noted that examination of feces using reliable routine methods was a more efficient diagnostic procedure than examination of small bowel specimens. In this study, *Giardia* was identified on examination of the first fecal
specimen in 73 percent of cases and in 85 percent of cases after examination of three fecal specimens. Only 15 percent of cases were identified by examination of small intestinal aspirates alone, and *Giardia* trophozoites were identified in only 44 percent of all aspirates from cases also confirmed by fecal examination.

The string test as a method of sampling duodenal fluid has also been reported to be a useful diagnostic procedure and a useful "routine" procedure (diagnostically unnecessary) for obtaining new *Giardia* strains. In one study, 10 of 12 cases of giardiasis were identified on both stool examination and string test examination, one case was identified on stool examination alone, and one case was identified only after carrying out examination of duodenal material obtained by the string test. Clearly, examination of fecal specimens remains the most useful of laboratory methods for the diagnosis of giardiasis although endoscopy or use of the string test (a practical alternative to endoscopy) may be helpful in the small number of cases missed by examination of stool samples.

**Immunological Approaches to Diagnosis**

Principles of serological testing, used to detect anti-*Giardia* antibodies in serum and, more recently, *Giardia* antigen in feces, may also be useful in cases where routine fecal examination fails.

In 1980, Visvesvara et al reported that serum antibodies to *Giardia* which had previously been described in cases associated with malabsorption, could be detected using an immunofluorescence test (IFA). The enzyme-linked immunosorbent assay (EIA) was shown to be a technically superior method for detection of anti-*Giardia* immunoglobulin. Further studies have shown that this test will not distinguish current from past infections and will probably not be useful in diagnosis. Use of an EIA method to detect anti-*Giardia* IgM appeared promising in one study, but confirmation of these results needs to be obtained. These techniques have been shown to be useful in seroprevalence studies. There is at present no commercially available diagnostic test for the detection of anti-*Giardia* antibodies.

Detection of *Giardia* antigen in feces was first demonstrated using the technique of counterimmunoelectrophoresis (CIE). An IFA technique used to detect intact *Giardia* cysts in feces or in water samples was also described, and IFA kits marketed soon thereafter. The use of an IFA method, however, requires fluorescence microscopy; although identification on a morphological basis may be made easier by use of the immunologically-based reaction, this approach is overall more labour intensive than methods of antigen detection which eliminate the need for microscopy and morphological identification altogether. The CIE method of antigen detection appeared sensitive and reliable. However, this technique was superseded by use of the EIA with Ungar et al describing a double-antibody method that not only had a sensitivity of 92 percent and a specificity of 98 percent but data from this and subsequent studies suggested that some microscopy-negative but EIA positive feces were in fact missed cases of giardiasis.

Specimens preserved in polyvinyl alcohol or formalin could not be used in the system described by Ungar, but other EIA methods have since been developed and at least one study described an EIA capable of being used with both formalin-fixed and

* Merifluor Giardia, Meridian Diagnostics, Inc., Cincinnati, OH; Giardia-Cel I.F. Test, Cellabs Diagnostics Pty. Ltd, Brookvale, Australia.
unfixed stools. With this promising data and with an increasing number of cases of giardiasis being reported throughout North America, EIA antigen detection kits have recently become commercially available. An EIA antigen detection method developed in Britain has also been described, and a follow-up publication described its usefulness in an epidemiologically oriented study.

Summary

The laboratory diagnosis of Giardia and other diarrhea-producing protozoans deserves further study. Information on such questions as the cost-effectiveness and reliability of a more selective approach to the number of fecal specimens examined per patient and on the more selective use of staining techniques is needed. Where do the immunologically-based tests fit into the overall diagnostic laboratory scheme at present? Since they represent additional cost, most often duplicating information obtained from procedures already carried out in the search for other enteric parasites, these tests should probably be reserved for use in cases where concentrated specimens examined are negative for Giardia or other diarrhea-causing protozoans.

When complementary diagnostic tests are necessary, the use of EIA antigen detection tests on fecal samples may supersede examination of small bowel specimens if only because they do not require an invasive procedure for the patient. If commercial interest continues to the point of development of EIA reagents for the detection of all diarrhea-causing protozoan, immunological tests will undoubtedly take a more significant place in the daily processing of stools in diagnostic parasitology laboratories.

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