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Overdiagnosis of *Entamoeba histolytica* and *Entamoeba dispar* in Nicaragua: A Microscopic, Triage Parasite Panel and PCR Study

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Background. Based on stool microscopy, an *E. histolytica*/*E. dispar* prevalence of 18.6% was found in León, Nicaragua about 10 years ago. Since then, new non-microscopic methods have been developed to discriminate between pathogenic *E. histolytica* and non-pathogenic *E. dispar*. The main objectives of the present study were to evaluate the true prevalence of *E. histolytica* among individuals with diarrhea and to assess the diagnostic procedures carried out at the health center level.

Methods. A descriptive study was carried out on patients with diarrhea. Parasite detection was performed by conventional microscopy on native preparations or concentrated and stained specimens, Triage Parasite Panel and by PCR for both *E. histolytica* and *E. dispar*.

Results. In 134 individuals with diarrhea, the prevalence of intestinal parasites was 69% as detected by direct stool examination. *E. histolytica*/*E. dispar* was found in eight (6%) of the samples, but the health centers reported 24%. In the Triage Parasite Panel only one case of *E. histolytica*/*E. dispar* was found. Analysis by PCR showed *E. dispar* in ten (7.5%) and *E. histolytica* in two cases (1.5%). The detection of intestinal coccidia and *Dientamoeba fragilis* required additional staining methods.

Conclusions. PCR results showed that *E. histolytica* is a rare finding in patients with diarrhea. At the health centers, *E. histolytica, E. histolytica*/*E. dispar* were clearly overdiagnosed, with the consequence of overtreatment.

Key Words: E. histolytica, *E. dispar*, PCR, Diarrhea, Diagnostic problems.

Introduction

Amebiasis is still one of the major health problems in tropical and subtropical areas and is characterized by low socioeconomic status and poor hygiene that favors the indirect fecal-oral transmission of the infection (1). The last estimate of the global magnitude of the disease was made approximately 15 years ago (2). At that time, based on a comprehensive analysis of the current literature, it was suggested that approximately 500 million people worldwide were infected with *Entamoeba histolytica*. However, this estimate was made before the identification of *Entamoeba dispar* as a separate species, which also colonizes the human gut but is morphologically indistinguishable from *E. histolytica*. In 1993, the formal re-description of *E. histolytica* resulted in two species: *E. histolytica*, capable of causing amebic colitis and liver abscess and *E. dispar,* a non-pathogenic commensal (3). On the basis of several epidemiological studies performed worldwide, it was determined that the vast majority of infected humans harbor the non-pathogenic species *E. dispar*, whereas only 10–12% were infected with *E. histolytica* (4). Nevertheless, information on the geographic distribution of *E. histolytica* and *E. dispar* is still incomplete and unreliable because few surveys have been carried out using standardized procedures and do not

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discriminate between the species (5). The laboratory diagnosis of amebiasis, particularly intestinal amebiasis, depends on a number of procedures, any of which can prevent organism recovery if not performed properly. This diagnosis can be one of the most difficult to achieve. Lack of appropriate training and diagnostic tests may lead to missed infections (6). In Ecuador, a recently published study clearly demonstrates that there are diagnostic methods more specific and sensitive than direct microscopy. Antigen detection assays, isoenzyme analysis, and polymerase chain reaction (PCR)-based tests are needed to establish the true distribution of *E. histolytica* and *E. dispar* (7). Studies in Ethiopia and Brazil found that many amebic infections are incorrectly attributed to *E. histolytica* and then treated, unnecessarily, with amebicidal drugs (8–11).

In 1991, a study in Managua, Nicaragua at The National Center of Hygiene and Epidemiology suggested that intestinal amebiasis was being diagnosed excessively by both physicians and laboratory technicians (12). In León, Nicaragua a prevalence study of intestinal parasites showed an *E. histolytica*/*E. dispar* prevalence of 18.6% in an asymptomatic population (13).

As no inexpensive and practical diagnostic procedures are currently available for the identification of *E. histolytica* at the health center level in countries with limited resources, we are left with the common practice of identifying *E. histolytica*/*E. dispar* as *''E. histolytica''*. The likely consequence is overdiagnosis and overtreatment. The main objective of the present study was to assess the prevalence of *E. histolytica* among individuals with diarrhea by means of PCR. We also wanted to evaluate the current routinely used direct microscopy method for its concordance, sensitivity and specificity in detecting *E. histolytica*/*E. dispar* and other parasitic pathogens and to evaluate a non-microscopic detection method for *E. histolytica*/*E. dispar*, *Giardia intestinalis* and *Cryptosporidium* spp.

Materials and Methods

Study Area

The León municipality has an extension of 1114 square kilometers, which covers both urban and rural areas. The climate is warm and humid, with a mean temperature of 32°C. León is the second city in Nicaragua with an estimated number of 200,000 inhabitants. Forty-eight percent of the population is <15 years old and 52% are female. Eighty percent of the population is living in the urban area; only 48% have access to the sewage system. There are on average 5.7 persons per household.

Study Group

The study was conducted in 134 individuals with diarrhea from February to July 2002. Patients >2 years old, presenting with diarrhea at three different health centers in León were enrolled in the study. For the purpose of this study, diarrhea was defined as a change in the stool pattern, characterized by an increased frequency to at least three or more liquid stools per 24 h. Patients had not used any antiparasitic drugs for 1 week before the sampling. Individuals were invited to participate in this study. In case of children, signed informed consent was provided by the child's parents before the study started. The National University's Ethics Committee approved the study.

Collection of Stool Samples and Microscopic Examination of Fecal Specimens

A plastic container without any preservatives was given in order to collect stool samples. Samples were deposited at the health centers. Immediately after delivery, samples were divided into different containers. Part of the specimen was fixed with 70% ethanol (one part stool + four parts of ethanol) for DNA extraction. Another part fixed in SAF (sodium acetate-acetic acid-formalin) fixative for staining methods. The same specimen was examined by direct microscopy with saline and iodine by health center technicians. Stool samples clearly marked by number stickers were transported within 1–2 h to the Microbiology Department at the University of León in order to be re-examined. In addition to direct microscopy of fresh smears, formalin-ethyl acetate sedimentation technique was used for detection of cysts and eggs (14), iron hematoxylin staining for amebas and flagellates and modified Ziehl-Neelsen staining for detection of enteric coccidia (6). No additional tests for viral and bacterial infections were performed.

Antigen Detection

The Triage Parasite Panel (TPP) (Biosite Diagnostic Inc., San Diego, CA), an immunochromatographic assay for the detection of antigens specific for *G. intestinalis*, *E. histolytica*/*E. dispar*, and *C. parvum* was used according to the procedure provided with the kit.

DNA Extraction

DNA was extracted from all samples using the QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to the manufacturer's recommendations. An initial washing step was added: about 0.5 mL of the stool-ethanol suspension was washed once with saline. After centrifugation for 3 min at 6000 $\times$ g, the sediment was re-suspended in an equal amount of saline and 20 μL was used for extraction. DNA samples were analyzed the day of extraction or stored at –20°C until analyzed. One μL of DNA was used for PCR analyses.