Etiology of Acute Diarrhea in Adults in Southwestern Nigeria

Okeke et al. (5) recently reported that *Entamoeba histolytica* was by far the most frequently encountered pathogen found in association with bloody diarrhea. The method used to come to that conclusion consisted of microscopic examination of a simple wet smear, which is nonspecific in the diagnosis of amebiasis. Moreover, multiple infections with other enteric bacteria were very common, which might further confound the conclusion. It is in fact common knowledge that *E. histolytica* is not commonly seen with acute diarrhea; it is rather chronic in nature most of the time.

As of 1997 it was officially accepted by the World Health Organization (WHO) (7) that *E. histolytica* sensu lato is composed of two morphologically identical species: the pathogenic *E. histolytica* and the nonpathogenic, but very common, *Entamoeba dispar*. These two parasites cannot be correctly distinguished by microscopy alone unless the trophozoites in question are seen engulfing red blood cells. In that case microscopy could be diagnostic, but even the correct diagnosis appears difficult (2).

Finding of entamoeba trophozoites or cysts in bloody diarrhea is not enough to draw conclusions on causality. Our extensive experience with the diagnosis and epidemiology of amebiasis in Ethiopia revealed that, in contrast with the general belief, *E. histolytica* is quite rare (2–4). Not only in asymptomatic cases, but even in patients with bloody diarrhea, the great majority of trophozoites and cysts found appeared to belong to either *E. dispar* or altogether different intestinal amoeba (2). Similar results were reported from Cote d'Ivoire (1) and Ghana (6). Unless powerful species-specific diagnostic tests are used, we have to believe that most of these reported cases of "*E. histolytica*" are in fact cases of *E. dispar* or altogether different amoeba. We would like to point out that it is incorrect to report *E. histolytica* using conventional microscopy alone to come to such a sweeping conclusion. Important conclusions, both at the individual level and at the population level, require inclusion of species-specific tests like PCR.

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Amha Kebede*

Ethiopian Health & Nutrition Research
Institute/Ethio-Netherlands AIDS
Research Project, P.O. Box 1242
Addis Ababa, Ethiopia

Anton M. Polderman

Department of Parasitology
Leiden University Medical Center
Wassenaarseweg 62, Postbus 9605
2300RC Leiden, The Netherlands

*Phone: 251-1-751522

E-mail: amha@enarpc.com

Authors' Reply

While our study, published in 2003 (2), was focused on diarrheagenic *Escherichia coli*, we felt that it was necessary to report other presumptive pathogens that we encountered. Criteria that were used to define *Entamoeba histolytica* were morphological features, the presence of engulfed red cells, and motility. Since these criteria are more discriminatory than the use of morphology alone (5, 7), it is unlikely that similar but unrelated amoebae would be mistaken for the *E. histolytica* complex. However, as Kebede and Polderman observe, our wet-mount methodology could fail to delineate pathogenic *E. histolytica* from morphologically identical, but nonpathogenic, *Entamoeba dispar*. Following the reclassification of *E. histolytica* into pathogenic *E. histolytica* sensu stricto and *E. dispar* Brumpt, recent and in-press papers (including those cited above) have detailed more-discriminatory molecular methods. As stated by Kebede and Polderman, these methods, in the main published after our study was conducted, are required for precise delineation of these strains in epidemiological studies. They have been used in some, but not all, recent studies and are predicted to completely replace microscopy in the near future.

We are in agreement with Kebede and Polderman on the methodological limitations of that part of our study, but we find insufficient basis to speculate that most of the amoeba reported were nonpathogenic. The epidemiology of *E. histolytica* and other intestinal pathogens is known to vary geographically (1, 3, 4), and the conclusions drawn from comparisons with data from considerably dissimilar studies are not in any way supported by our findings. Recovery of *Entamoeba* was very strongly associated with diarrhea in our study ($P < 0.0002$), and the *E. histolytica* complex was the most common agent detected in bloody diarrhea specimens in which only one agent was found. Therefore, our data do suggest an association of the *E. histolytica* complex with diarrheal disease, even if the specific contribution of *E. histolytica* sensu stricto cannot be assessed. The claim that *E. histolytica* is unusual in acute infection is not supported by our data or by other published literature, including recent studies employing discriminatory tests. For example, Haque et al. recently demonstrated that *E. histolytica* is an important cause of acute and bloody childhood diarrhea (1). Thus, we feel that these should be areas of future

investigation, rather than speculation, and prefer to restate that there was an association of the *E. histolytica* complex (*E. histolytica* and *E. dispar*) with disease.

WHO issued a statement a few months before our study was conducted on the potential significance of nondelineation of *E. histolytica* from *E. dispar* and recommended the development of suitable methodology (6). Notably, the most recent version of the WHO Manual of Basic Techniques for a Health Laboratory, published 6 years later (7), describes only wet-mount microscopy even though it mentions the problems with specificity. This practical consideration arises from the undeniable fact that stool microscopy continues to be the only technology available to many laboratories in areas of endemicity (5). Immunological tests offer greater portability but are often too expensive to be employed routinely in developing countries. They also require cold-chain transportation that may be unavailable and, in the case of antibody tests, fail to distinguish between current and past infections (4, 5). Molecular epidemiology studies conducted in Africa, including our study and the work cited by Kebede and Polderman, have invariably involved partnerships with industrialized countries. This approach, while useful for small research studies, is inadequate and unsustainable for much-needed larger studies or for clinical diagnostics. Thus, as in our paper, Kebede and Polderman emphasize the necessity for capacity building for molecular diagnostics in sub-Saharan Africa. Definitive identification of important diarrheal pathogens—*E. histolytica* as well as diarrheagenic *E. coli*, the central focus of our study—cannot be conducted without it.

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Iruka N. Okeke*
Department of Biology
Haverford College
370 Lancaster Ave.
Haverford, PA 19041

Oladipupo Ojo
Novartis PharmaServices
Lagos, Nigeria

Adebayo Lamikanra
Faculty of Pharmacy
Obafemi Awolowo University
Ile-Ife, Nigeria

James B. Kaper
Department of Microbiology and Immunology
University of Maryland Medical School
Baltimore, Maryland

*Phone: (610) 896-1470
 Fax: (610) 896-4963
 E-mail: iokeke@haverford.edu