

Amoebiasis and giardiasis in Bangladesh: parasitological and serological studies

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Summary

To determine the prevalence of infections with *Entamoeba histolytica* and *Giardia lamblia* in Bangladesh, we screened stool specimens from patients with diarrhoea attending an urban (N = 2,246) and a rural (N = 2,791) hospital and a group of healthy urban residents (N = 440). Sera from 200 healthy villagers were also examined for antibody to *E. histolytica* using the immunofluorescent antibody test. The prevalence of *E. histolytica* cysts or trophozoites in all groups assessed by examination of a single stool specimen ranged from 0% among infants (<1 year) to 34% among healthy adults 30 to 44 years old. *G. lamblia* was uncommon in infants and found most frequently in urban hospital patients aged five to nine (21%). 12% of one- to two-year-old children had serum antibody to *E. histolytica* at a titre of 1:40. By age 14 years, 80% of those tested were seropositive, and this prevalence declined thereafter with increasing age. Amoebiasis and giardiasis occur frequently in Bangladeshi populations and the morbidity and mortality associated with these infections need to be assessed.

Introduction

Infections with *Entamoeba histolytica* and *Giardia lamblia* are widespread in some populations living in the tropics and subtropics (WHO, 1980). To determine the prevalence of these two parasites in Bangladesh and to identify the population groups at greatest risk of infection, we examined stool samples from patients attending an urban and a rural diarrhoeal disease hospital and a group of healthy urban residents and conducted a serosurvey of villagers for the prevalence of antibody to *E. histolytica*. These descriptive epidemiological data can serve as a basis for comparison with other studies and are useful for setting priorities and developing hypotheses for further study of infections with these parasites.

Methods

Populations studied

Stool survey: The prevalence of *E. histolytica* and *G. lamblia* was determined in three populations: (i) a 4% systematic sample of patients seeking care for diarrhoea at the Dacca hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B) between December 1979 and November 1980 (STOLL *et al.*, 1982), (ii) family members of 100 of these patients and (iii) all patients visiting the rural field hospital of the ICDDR, B in Matlab between February and August 1977 (BLACK *et al.*, 1980).

Serosurvey: Antibody titres to *E. histolytica* were tested in stored sera taken as part of another study in 1980 from healthy family members of patients with cholera who were seen at the Matlab hospital.

Laboratory methods

Stool microscopy: A single stool specimen was collected from each person in a capped container without preservatives. Specimens obtained from hospital patients were examined immediately and those from the field were examined within four hours of collection. Both a saline and an iodine preparation of each specimen were examined under a light microscope by an experienced laboratory technician for five min. A specimen was considered positive for *E.*

histolytica or *G. lamblia* if either trophozoites or cysts were present. Neither concentration methods nor ocular micrometry was used.

Serological test: Sera were tested for antibody to *E. histolytica* using the immunofluorescent antibody (IFA) test. From each individual 0.1 ml of blood was collected by finger prick into a capillary tube and diluted 1:10 into a vial containing 0.9 ml of physiological saline. After spinning off the red blood cells, the sera were stored at -20°C until they were processed.

The antigen for the IFA test was prepared in suspension from *E. histolytica* trophozoites (HK 9) grown axenically (DIAMOND, 1968). The suspension was placed in wells of an IFA slide in a concentration of 1×10^4 trophozoites per well. The slides were dried at 37°C for 5 to 10 min and stored at -20°C. Before use, the IFA slides were brought to room temperature and air-dried for 10 to 15 min. Each serum was titrated in two-fold dilutions from 1/20 to 1/320. 25 ml of each dilution was placed in an antigen well and incubated in a moist chamber for 30 min at room temperature. After three washes with phosphate-buffered saline (PBS), 20 ml of a 1/20 dilution of fluorescein-labelled sheep anti-human IgG conjugate (Wellcome) was added to each well and incubated in a moist chamber for 30 min at room temperature. After three washings with PBS, the slides were counterstained for 10 min in an aqueous solution of Evan's blue (1/500), washed three times in PBS, and sealed with a drop of buffered glycerine and a cover-slip. For each batch of sera, positive and negative controls were run. The slides were read with a fluorescent microscope. A titre of 1:40 was considered positive.

Results

Stool survey: Single stool specimens were examined from 2,791 (61%) of 4,555 patients visiting the Matlab hospital, 2,246 (63%) of the 3,550 patients visiting the Dacca hospital, and 440 (80%) of 553 healthy controls. The prevalence of *E. histolytica* was lowest

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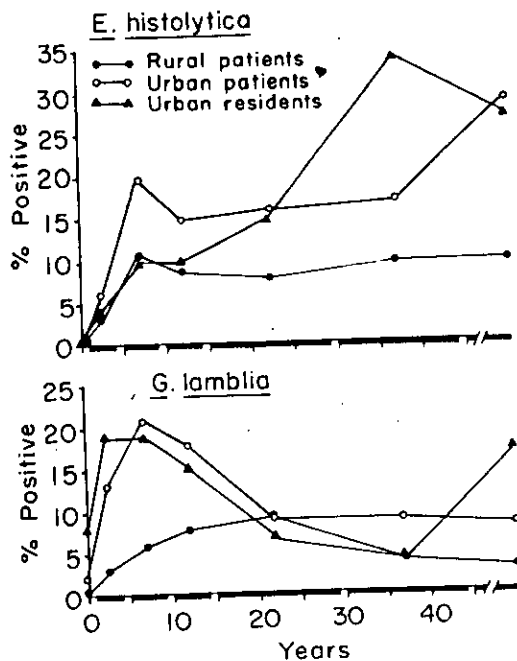


Fig. 1. Age-specific stool-positivity rates for *E. histolytica* and *G. lamblia*, Bangladesh 1977-80.

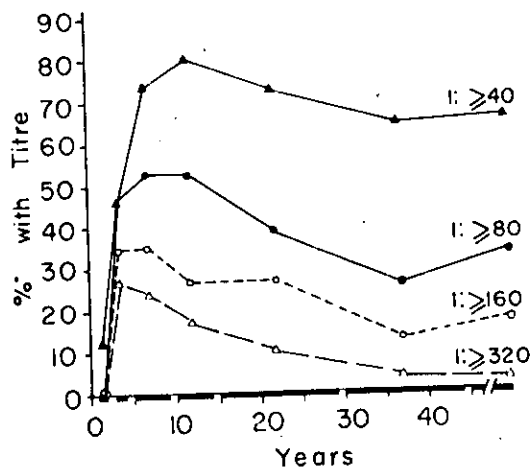


Fig. 2. Age-specific prevalence of serum indirect immunofluorescent antibody to *E. histolytica* Bangladesh, 1980*.

*No. of persons tested: age group 1-2 years, 17, other age groups, 23 or more.

(<1%) among infants (<one year) in all populations (Fig. 1) and increased with age. Urban adults aged 15 years or more were most frequently infected (15 to 34%) and had a higher prevalence of amoeba than rural patients of the same age.

G. lamblia was uncommon in infants and most prevalent among urban residents and patients aged

one to 14 and rural hospital patients aged 15 to 29 years (Fig. 1). The high prevalence of *Giardia* seen in urban children was not found in rural children.

Serosurvey: Serum samples of 200 Matlab residents were examined for antibodies to *E. histolytica*. At a screening titre of 1:40, 12% of one- to two-year-old children already had acquired antibody and by 14 years, 80% of those tested were seropositive (Fig. 2). The prevalence of a high antibody titre (1:≥320) suggestive of recent disease was greatest in three to four-year-old children and declined with increasing age.

Discussion

All population groups that we have examined in Bangladesh have had a high prevalence of amoebiasis and giardiasis. Infection with *E. histolytica* is acquired rapidly by children less than 10 years of age compared to older children and adults. This is consistent with our understanding of the faecal-oral route of transmission of this infection which could affect children primarily. While our population comprised individuals who differed by place of residence (urban v. rural) and health status (ill v. well), the prevalence of *E. histolytica* by stool examination was comparable. The high prevalence of infection among healthy urban residents may represent a selection bias due to difficulties in collecting specimens from adults who were well.

Giardiasis was most common in young urban patients, an observation reported in other areas (MEYER & JARROL, 1980). The lower prevalence and older age distribution among rural patients perhaps reflect differences in the intensity of infection and the mode of transmission of the parasite.

Stool surveys of *E. histolytica* are useful to determine the point prevalence of infection whereas serosurveys can be used to assess a population's cumulative experience with invasive disease since antibodies may persist for years (KRUPP & POWELL, 1971). In the Matlab population, by age 14, 80% of individuals had circulating antibody to *E. histolytica* at titres of 1:≥40. A seropositivity rate higher than the stool prevalence, as found in Matlab, has been noted in residents of Calcutta and Bangkok (MEEROVITCH *et al.*, 1978), rural Malaysia (THOMAS & SINNIH, 1982) and El Salvador (SPENCER *et al.*, 1981). Our observation that the prevalence of antibody to *E. histolytica* declines in adults suggests that older individuals are less exposed, that they are less susceptible to invasive disease, or that they are less likely to develop a detectable antibody response to infection. While some epidemiological (WHO, 1980) and immunological (BRAY & HARRIS, 1977) evidence of acquired resistance against amoebiasis exists, other studies (KRUPP, 1970) have documented reinfection of successfully treated patients even though they had high levels of serum antibody to *E. histolytica*.

For practical and economic reasons, direct smear examination of a single stool specimen has been used widely in surveys to screen for amoebiasis and giardiasis even though this technique may underestimate the true prevalence of infection. SAWITZ & FAUST (1942) found that when a single iodine or haematoxylin-stained faecal film was examined, the chances of finding infection with *E. histolytica* was less than one in five although for heavy infections and

dysentery detection rates were much greater. Some overdiagnosis of amoebiasis may result when micrometry and special stains are not used, since smaller *E. hartmanni* and other protozoan parasites or faecal leucocytes can be mistaken for *E. histolytica* (SPENCER *et al.*, 1981).

Although our populations were not selected on a true random basis, we believe our results do depict the general pattern of *E. histolytica* and *G. lamblia* infections in these communities. The morbidity and mortality attributable to these infections, their modes of transmission and the risk factors for infection should be assessed through further studies.

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