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Concurrent infections and socioeconomic determinants of geohelminth infection: a community study of schoolchildren in periurban Guinea-Bissau

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Summary We explored the association between subclinical intestinal helminth infections and other gastrointestinal pathogens in 706 schoolchildren from a poor semirural area while adjusting for socioeconomic risk factors. The study was carried out in two neighbouring areas in the capital of Guinea-Bissau in West Africa. Children aged 4—12 years were visited and one child per mother was invited to participate in the study. Among the 706 children included in the study, helminths were detected in 44.2%, enteropathogenic bacteria in 13.7%, protozoans in 51.1% and rotavirus in 5.9%. A total of 76.1% had an infection of some sort and 41.8% were concomitantly infected with more than one and up to five gastrointestinal pathogens. After adjustment for possible confounding factors, infection with helminths and *Entamoeba histolytica/E. dispar* remained associated. Other risk factors for helminths included increasing age, male gender, small mid-upper arm circumference and chicken husbandry. Maternal school attendance and belonging to a Muslim family were associated with a lower prevalence of helminths. Investigations of concomitant infections are valuable as they may have implications for control and treatment strategies.

1. Introduction

Among the many and complex determinants of gastrointestinal infections, concurrent infection with other gastrointestinal pathogens may play an important role in the resulting pathology and intensity of each infection. In a study from Kenya, children <5 years of age who had diarrhoea were found to have significant associations between enteropathogenic *Escherichia coli* (EPEC) and *Ascaris lumbricoides*, between *Campylobacter* and *Giardia*, and between *Campylobacter* and EPEC, respectively.[1] In Liberia, an association was found between infection

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with *Campylobacter* and intestinal nematodes, especially *Strongyloides stercoralis*, in children <3 years of age.[2] Additionally, bloody diarrhoea appeared to be more frequent when children were co-infected with heavy *Trichuris* and either *Shigella* or *Salmonella*.[3] However, these associations may all have reflected the transmission of certain pathogens in the same environment rather than biological interactions.

Gastrointestinal infection is a leading cause of morbidity and mortality in children <5 years of age in developing countries,[4] but in older children infections with enteropathogenic bacteria and viruses commonly take a subclinical course due to acquired immunity.[5,6] In contrast, helminth infections are more prevalent and increasingly intense in children >5 years of age.[7,8] Although signs and symptoms associated with low-intensity intestinal nematode infections may be mild or even absent, evidence suggests that in humans such less pronounced nematode infections do induce an effect on immune status,[9] nutritional status[10] and the integrity of the intestinal wall.[11]

The aim of the present cross-sectional study was to explore associations between subclinical intestinal helminth infections and other gastrointestinal pathogens while adjusting for socioeconomic risk factors in children aged 4—12 years.

2. Materials and methods

The study was carried out in Bandim II and Belem, two neighbouring areas in Bissau, the capital of Guinea-Bissau, West Africa. A number of studies of childhood diarrhoea have been conducted in these communities and records are kept of all births and deaths at the Bandim Health Project. The climate is hot and humid with mean temperatures of 25 °C in January to 29 °C in May and a rainy season from May to November.

Children born between January 1989 and December 1996 were identified in the database. One child per mother was invited to participate in the study, which was conducted in the dry season from the end of January to the end of April 2001. A total of 706 children aged 4—12 years participated.

A stool container was handed out for each child after a parent or guardian had given their consent. The container was collected within 24 h and body weight, height and the mid-upper arm circumference (MUAC) of the right arm were measured. A structured interview was conducted with the child and the parent or guardian addressing issues including the family's socioeconomic conditions, such as parents' school attendance and ethnicity, household appliances, sanitation facilities, animal husbandry and disease occurrences. Local field workers conducted the questioning in the local language. (A copy of the questionnaire can be obtained from the corresponding author upon request.)

Collected stool samples were stored in a refrigerator until processing, which took place within 21 h. Six tests were carried out on the stool samples: (i) the concentration McMaster technique with a sensitivity of 20 eggs per gram of faeces (EPG) was carried out on 4 g of faeces[12]; (ii) direct microscopy of a wet mount was performed to conduct a qualitative examination for *Strongyloides* larvae and for trophozoites of *Giardia* and *Entamoeba*; (iii) 1 g of faeces was concentrated using the formol-ether technique[13] and examined for protozoa by microscopy of the iodine-stained sediment and (iv) the sediment was used to demonstrate *Cryptosporidium* oocysts after staining by the modified Ziehl—Neelsen technique[13]; (v) stool specimens were also tested for rotavirus with the IDEA ELISA kit as described by the manufacturer (Dakopatts, Copenhagen, Denmark); and finally, (vi) the samples were examined for gastrointestinal pathogenic bacteria. Isolation and culture of *Shigella*, *Salmonella*, diarrhoeagenic *E. coli*, *Campylobacter* spp., *Yersinia enterocolitica* and *Vibrio cholerae* (the last two not detected) from stool samples were carried out in Bissau as described previously.[14—16] The concentration McMaster technique was chosen due to its high sensitivity, and each faecal sample was stirred until completely dissolved.

Any association between helminths and other pathogens may be due to confounding factors such as socioeconomic and environmental factors. Therefore, an analytical strategy was chosen where univariate analyses were applied to determine (i) the odds ratio (OR) between a helminth and other pathogens and (ii) the OR between helminths and a number of socioeconomic variables representing potential risk factors. Factors significant at the 5% level (based on χ^2 analyses) were candidates for multivariate analyses.

In addition to the socioeconomic variables, nutritional measures were also adjusted for as follows: crude anthropometric measurements (i.e. weight, height and MUAC) were log transformed to obtain a normal distribution. Ordinary linear regression models were then fitted with the log-transformed measurements as a dependent variable and with age, age^2 and gender as independent variables. The residuals (observed—fitted measurement) were calculated for all children. These residuals reflected standard anthropometric scores for each child, adjusted for age and gender. For the risk factor analyses, these residuals were categorised by their quartiles and the results expressed as SD scores. These categorical values were included in the risk factor analyses (ordinary logistic regression) with the lowest 25% representing the reference category.

The association of helminth infection and other pathogens as well as potentially confounding factors, including anthropometric measurements, was finally analysed in a multivariate logistic regression analysis both for helminths and *Entamoeba histolytica*/*E. dispar*. The models were reduced by backward stepwise regression analysis (proc logistic in SAS software). Missing information for a subset of the variables did not exclude the child from being included in analyses of other factors. All analyses were performed in SAS version 8 (SAS Institute Inc., Cary, NC, USA).

3. Theory

A number of experimental studies have been carried out to verify possible synergy between intestinal geohelminths and bacterial infections. It was found that *Trichuris suis* infection increased the pathogenesis of *Campylobacter* in pigs[17] and that heavy oesophagostomiasis increased the tissue content and excretion in faeces of *Salmonella enterica* serovar Typhimurium.[18] The mechanism behind these interactions is thought to be mainly immunological, as a helminth-induced Th2 response is known to impair the production of Th1 cytokines responsible for controlling intracellular organisms such as bacteria.[19] The mechanisms determining the