PREVALENCE OF INTESTINAL PARASITE INFECTIONS WITH SPECIAL REFERENCE TO *ENTAMOEBA HISTOLYTICA* ON THE ISLAND OF BIOKO (EQUATORIAL GUINEA)

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Abstract. The prevalence of intestinal parasitic infections was assessed (1993 through 1995) among two different groups of persons on the island of Bioko, Equatorial Guinea. In the first group, parasitologic examinations were performed on stool specimens from a household-based sample of 557 dwellers from the rural area of the island. In the second group, 1,633 inpatients and outpatients at the General Hospital of Malabo (the capital of the country) were studied. All age groups were represented in both groups. The average prevalence of the most common protozoan and helminthic intestinal infections in rural and urban areas, respectively, was as follows: *Entamoeba histolytica/E. dispar* (14.9% and 32.7%, respectively), *Giardia lamblia* (7.2% and 8.6%), *Ascaris lumbricoides* (45.8% and 31.4%), and *Trichuris trichiura* (25.7% and 36.4%). Seventy-nine sera from patients with amebic liver abscess (suspected by ultrasonography) were studied by an immunohemagglutination assay, with 44 (56%) showing anti-*E. histolytica* titers ≥ 1:32. Of these 79 sera, 71 were studied by an enzyme immunoassay, 86% of which were positive with titers ≥ 1:64. This study showed that parasitic infections in Equatorial Guinea represent a major health problem.

One of the principal factors contributing to child morbidity and mortality in tropical countries is the high frequency of diarrheal episodes due to intestinal infections, which also contributes to childhood growth retardation. Diarrheal diseases are a major cause of mortality in developing countries; their control and prevention are one of the main objectives of the World Health Organization (WHO) within the Division of Diarrheal and Acute Respiratory Disease Control programs in developing countries.

*Entamoeba histolytica* is one of the six amoeba of the genus *Entamoeba* that infects humans. Infection is distributed worldwide, with a high prevalence in tropical countries. Approximately 480–500 million persons are infected and mortality ranges from 40,000 to 130,000 persons/year. The prevalence of *E. histolytica/E. dispar* infections differs from one geographic area to another, and severity varies from one case to another. Approximately 10% of *E. histolytica*-infected individuals show clinical symptoms with intestinal and/or extra-intestinal pathology. The frequency of amebic liver abscess (ALA) is the most reliable measure in determining the morbidity of invasive intestinal amebiasis. This occurs in 2% of adults infected with *E. histolytica* in endemic areas; in outbreaks, this prevalence increases to 4%.

Equatorial Guinea is located in western Africa between Cameroon and Gabon. The estimated population is approximately 400,000. Its social indicators are among the lowest in the world: a death rate of 14.36‰/1,000 individuals, an infant mortality rate of 100.2 deaths/1,000 live births, and a life expectancy at birth of 49 years. According to a WHO–Health Ministry of Equatorial Guinea internal report (unpublished data), malaria, respiratory, and diarrheal infections were the three leading causes of public health problems in Equatorial Guinea in 1993. They affected 22%, 20%, and 13%, respectively, of the population and were responsible for 32%, 20%, and 13% of overall mortality in all age groups. With respect to nutritional status, caloric and protein intake are adequate (recommended values) in only 25% of the population. One percent of the children less than five years of age have severe malnutrition, 40% have chronic malnutrition (height/age), and 1.8% have acute malnutrition (weight/age) (data collected by the Project Nutrition, March–June 1996, Ministry of Health-Services Conseils Education Nutrition).

The aims of this study were 1) to determine the prevalence and types of intestinal parasites in the population of Bioko, and 2) to calculate seroprevalence rates of anti-*E. histolytica* antibodies in the general population and in patients suspected of having ALA.

**MATERIALS AND METHODS**

Area and population studied. Between May 1993 and December 1995, two parasitologic studies were carried out on the island of Bioko, Equatorial Guinea, 45 km from the Cameroon coast in the Gulf of Guinea. Ethical clearance for the study was provided by the Ethical Committee of the Ministry of Health of Equatorial Guinea, the Head of the Centro Hispano-Guineano de Enfermedades Tropicales in Malabo, and the Ethical Committee of the Instituto de Salud Carlos III in Spain. Written informed consent was obtained from all adults and the parents of children before their participation in the study.

In the first study, performed between 1993 and 1994, 557 stool specimens from a 160 randomly chosen rural households of 16 villages (10% of the total in the rural area covered) were analyzed. Stools specimens were collected from 91% of all residents in the selected households. Age, gender, clinical symptoms, and the quality of drinking water treatment were obtained from a short questionnaire given to all families. A history of receiving anthelmintics was not included in the questionnaire because the National Primary Health Program provided mebendazole to all children indiscriminately every three months. In spite of the fact that nearly all adults had been treated with ivermectin (the onchocerciasis prevalence in rural areas ranges between 50% and 75%), only 20% of the adults included in the study had been treated. Therefore, a previous anthelmintic treatment was not an exclusion criterion.

In the second study, conducted between 1993 and 1995,
all the samples were obtained from patients attending the General Hospital of Malabo (the capital of the country); a three-year survey of 1,633 stool samples was carried out on inpatients and outpatients. All the samples were obtained from patients with diarrhea and/or gastrointestinal symptoms, including pain, vomiting, or clinical suspicion of intestinal parasitic infections. We selected all patients seen in the hospital, an average of 3–5 per day. Each patient was asked to bring two stools samples, but since compliance was poor (< 15%), the study was confined to the examination of a single sample. Patients with previous anthelmintic therapy were not excluded.

During this period, 79 patients with suspected ALA/invasive amebiasis were diagnosed by ultrasonography in the Internal Medicine Service. Sera from these patients were analyzed by an immunohemagglutination assay (IHA) in the hospital laboratory in Malabo, and 71 of these analyzed by an enzyme immunossay (EIA) in the laboratory in Spain (Parasitology Department, Centro Nacional de Microbiología, Instituto de Salud Carlos III, Madrid, Spain).

Two serologic surveys were performed. In one, 564 sera from Bioko and the mainland were studied (Bata, the capital of the mainland region, is located on the coast, and Ebebiyin, in the interior, borders Cameroon). These sera, obtained from blood donors and pregnant women, were donated by the National Acquired Immunodeficiency Syndrome Control Program (NACP). Of these, 328 sera were from Malabo (223 from blood donors and 105 from pregnant women), 207 from Bata (133 from pregnant women and 74 from blood donors) and 29 from Ebebiyin (all blood donors).

Procedures. Coproparasitology. Feces were examined between 15 min (for samples from the hospital) and 2 hr (for samples from villages) of being passed. If amebic dysentery was suspected, the specimen was examined as soon as possible and kept in a refrigerator (3–5°C) until examined. The report included a description of the appearance of the specimen, including color, consistency, and whether it contained blood, mucus, or pus. Direct microscopic examination of fecal specimens was performed in fresh physiologic saline with Dobell’s iodine and eosin stain.8 Although counting of eggs was not carried out using a counting technique (e.g., Stoll’s technique or the Kato thick smear technique), which determines the number of eggs per gram, direct microscopic examination of fecal specimens was performed according to the following steps. 1) A drop of fresh physiologic saline was placed on one end of a slide and a drop of Dobell’s iodine was placed on the other. If amebic dysentery was suspected, eosin stain was also used. Using a wire loop, 2 mg of specimen was mixed in each solution and smooth thin preparations were made, and a coverslip was applied to the slide. Material from the interior of the fecal sample, as well as the surface, was included. When the specimen consisted mainly of blood and mucus, it was examined directly with a cover slip without saline. 2) All three preparations were examined microscopically using 10× objectives. Several microscope fields were examined with a 40× objective before reporting a specimen as no parasites found. 3) The number of each type of parasite found in the preparation was counted in the 40× field as follows: – = negative in an average of 100 fields with direct smear and stool concentration; + = less than one parasite in 10 microscopic fields; ++ = 1–10 parasites in 10 microscopic fields; +++ = 1–10 parasites per microscopic field; ++++ = more than 10 parasites per microscopic field. This counting method is not optimal, but comparable data can be obtained with it.

When amebae and flagellates were found, feces were preserved using a polyvinyl alcohol fixative solution. A permanent smear stained using the trichrome technique (Fekal Accustain, Trichrome stain LG solution; Sigma, St. Louis, MO) was required for all specimens with E. histolytica. dispal to confirm the direct examination of wet fecal preparations for the study of protozoan nuclear detail. Quality control of the samples was carried out in the Parasitology Department of the Centro Nacional de Microbiología in Madrid. First-stage larvae of Strongyloides stercoralis were specifically identified on the basis of their morphologic characteristics.9 All persons found positive for pathogenic protozoa or helminths were given appropriate chemotherapy.

Immunodiagnostic tests. The 79 serum samples from suspected ALA patients were tested by an IHA with the Celllognost Amoebiasis EIA (Melotec, S.A., Barcelona, Spain). Qualitative analysis was performed by determination of optical density values at 450 nm; a dilution of 1:64 was used for all serum samples. The 564 sera donated by the NACP were analyzed with the Melotest Amoebiasis EIA according to manufacturer’s instructions and with complete confidentiality, according to the NACP Sentinel Surveillance System policy.

Data analysis. Chi-square and Fisher’s tests were used to study significant associations or differences with the SAS/STAT program.10 The study population was classified into four age groups: < 1 year, 1–4 years, 5–14 years, and > 14 years of age.

RESULTS

Prevalence of intestinal parasite infections. In the rural survey, we detected intestinal parasites in 400 (71.8%) of 557 specimens. The overall infection rate in the hospital-based study was of 75.1% (1,227 of 1,633 specimens examined). The parasite prevalences in both surveys are shown in Table 1. Entamoeba histolytica E. dispar was the most common protozoan parasite in stools, while the roundworms Trichurus trichiura and Ascaris lumbricoides were the most abundant intestinal nematodes. Eggs and larvae of other nematodes (Strongyloides stercoralis and Ankylostoma duodenale) were detected in rural and urban areas. Schistosoma intercalatum was detected on Bioko only in outpatients at the General Hospital of Malabo, and always in individuals of the Fang ethnic group, who originated from the mainland area (between Cameroon and Gabon). Entamoeba histolytica E. dispar prevalence increased with age, while Giardia lamblia prevalence decreased ($\chi^2 = 12.4$, $P < 0.05$) (Table 2).

Table 3 shows the epidemiologic features for E. histolytica E. dispar and Giardia lamblia. There were no significant differences in infestation rates by sex, either overall or
TABLE 1

<table>
<thead>
<tr>
<th>Protozoa</th>
<th>Rural surveys (n = 557) No. (%)</th>
<th>Hospital surveillance (n = 1,633) No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Balantidium coli</strong></td>
<td>0 (0.0)</td>
<td>8 (0.5)</td>
</tr>
<tr>
<td><strong>Blastocystis hominis</strong></td>
<td>9 (1.6)</td>
<td>43 (2.6)</td>
</tr>
<tr>
<td><strong>Chilomastix mesnili</strong></td>
<td>0 (0.0)</td>
<td>14 (0.9)</td>
</tr>
<tr>
<td><strong>Endolimax nana</strong></td>
<td>0 (0.0)</td>
<td>6 (0.4)</td>
</tr>
<tr>
<td><strong>Entamoeba coli</strong></td>
<td>57 (10.2)</td>
<td>182 (11.1)</td>
</tr>
<tr>
<td><strong>Entamoeba histolytica/E. dispar</strong></td>
<td>83 (14.9)</td>
<td>534 (32.7)</td>
</tr>
<tr>
<td><strong>Entero monas hominis</strong></td>
<td>0 (0.0)</td>
<td>7 (0.4)</td>
</tr>
<tr>
<td><strong>Giardia lamblia</strong></td>
<td>40 (7.2)</td>
<td>141 (8.6)</td>
</tr>
<tr>
<td><strong>Iodamoeba bütschlii</strong></td>
<td>2 (0.4)</td>
<td>14 (0.9)</td>
</tr>
<tr>
<td><strong>Trichomonas hominis</strong></td>
<td>6 (1.1)</td>
<td>98 (6.0)</td>
</tr>
</tbody>
</table>

Fungi

* Candida albicans 4 (0.7) 59 (3.6)

Helminths

Nematodes

* Ancylostoma duodenale 5 (0.9) 47 (2.9)
* Ascaris lumbricoides 255 (45.8) 512 (31.4)
* Strongyloides stercoralis 1 (0.2) 13 (0.8)
* Trichuris trichiura 143 (25.7) 595 (36.4)

Cestodes

* Hymenolepis diminuta 0 (0.0) 2 (0.1)

Trematodes

* Schistosoma intercalatum 0 (0.0) 3 (0.2)

* Considered nonpathogenic.
† Considered nonpathogenic.

for specific parasitic species. We found an association between positive stools and the quality of drinking water treatment. There were no differences in the hospital samples between the presence or absence of these parasites and the patient’s symptomatology. However, there were differences in the rural samples. Contrary to expectations, the prevalence of *Giardia lamblia* was not associated with presence or absence of diarrhea, but a significant association with diarrhea was observed for *E. histolytica*. The presence of *E. histolytica* trophozoites in stool samples from children was strongly associated with diarrhea ($P = 0.03$ for the rural surveys and $P < 0.001$ for the hospital survey, by Fisher’s exact test).

Seroprevalence survey and immunology tests to detect ALA. A total of 328 sera from Bioko donors and pregnant women were analyzed with the EIA. This assay detected 202 positive samples (61.5%). In the two mainland region areas, the prevalences were 78% (Bata) and 31% (Ebebiyin). Of 79 sera from ALA patients diagnosed by ultrasonography and analyzed by the IHA, 56 (70%) had anti-*E. histolytica* antibody titers ≥ 1:32. Seventy-one of these 79 sera were tested by the EIA, and 66 (86%) of 71 positive samples had titers ≥ 1:64. As expected, the ALA-positive samples were diagnosed in adults (age range = 21–56 years old), with a 2:1 (male:female) sex ratio.

**DISCUSSION**

To date, no enteroparasitologic surveys have been carried out in Equatorial Guinea; individual studies had been performed in the country’s principal hospitals, but no published studies have demonstrated the existence of the main intestinal parasites present in this tropical area.

A clear association exists between intestinal helminth infection and growth retardation.11 The majority of human nematode infections, with the exception of the filarial worms, are diagnosed by finding characteristic eggs in feces. Eggs of the hookworm *Ancylostoma duodenale*, as well as the roundworm *Ascaris lumbricoides* and the whipworm *Trichuris trichiura*, were detected in feces. The prevalence of these infections is similar to that found in several developing tropical countries, with average prevalences in African countries (Nigeria, Togo, and Guinea-Bissau) between 5% and 60%. Hookworm, roundworm, and whipworm are the three main nematode infections implicated in growth retardation of children in tropical countries.16 Hookworm causes systemic secondary effects related to iron deficiency, anemia and possibly malnutrition or malabsorption. Ascaridiasis causes vitamin A deficiency and possibly malnutrition as a secondary effect. Trichuriasis causes iron deficiency, anemia, and possibly malnutrition.

Rhabditid larvae of *Strongyloides stercoralis* were detected in fresh fecal samples. This parasite and *Ancylostoma duodenale* were detected in rural and urban areas. The prevalence of strongyloidiasis and Old World hookworm infections may implicate these two helminth infections in growth retardation in children due to competition for nutrients in the digestive tract. In Equatorial Guinea, where approximately 43% of the children are malnourished, demonstrating the role of these nematode infections in growth retardation is difficult. The most useful tool in developing countries to measure nutrient absorption failure and malnutrition is detection of severe anemia. This can be masked by malaria (60–80% of children less than 10 years of age show parasites in the blood), which is the main cause of severe anemia in Equatorial Guinea.

Helminth infection prevalence is measured in most field studies by determining the proportion of individuals with parasite eggs in a stool sample. Technical problems in sample examination, as well as the diversity of diagnostic coprologic techniques used, indicate that the presence of eggs in stool samples is a marker that underestimates true infec-
tion frequency, as reported by several investigators for ascariasis
and Necator americanus. This and the limited number of samples examined (one per patient in this study) suggest that we have underestimated the prevalence of helminth and protozoan infections. It is well known that examination of three or more stool samples maximizes cyst detection rates, but for reasons of compliance and logistics, additional examinations could increase prevalence detection rates, as reported by other investigators.

Three Schistosoma species have been detected in Equatorial Guinea: S. mansoni, S. haematobium, and S. intercalatum. All schistosomiasis cases (S. intercalatum) in this survey were found in members of the Fang ethnic group from the mainland region, the focus of this disease in Equatorial Guinea.

The other helminthic parasite detected was the human cestode Hymenolepis diminuta, which was well differentiated in this survey from Hymenolepis nana. Eggs of the former are much larger and in all cases were 60–80 mm in diameter. These cestode infections are associated with rodent reservoirs. Eggs are passed in the feces and must be ingested by rat fleas or flour beetles, which act as intermediate hosts. Rats constitute an important part of the human diet in Equatorial Guinea.

Entamoeba coli was present in more than 10% of the stools examined. This high prevalence rate may reflect the state of poor environmental sanitation in Equatorial Guinea. Although the pathogenicity of Blastocystis hominis remains controversial and infection is not associated with any clinical condition, all samples in which this parasite was detected were from patients with diarrhea. It was found along with other enteroparasites such as E. histolytica/E. dispar or Giardia lamblia. Several reports claim that this parasite is pathogenic in children. Although association with diarrhea does not confirm pathogenicity, this survey corroborates the association of Chilomastix mesnili, Hymenolepis diminuta, and Trichomonas hominis in Kenya with unformed stools and diarrhea. The prevalence of E. histolytica/E. dispar was 14% in the cross-sectional surveys and 33% in the hospital survey. Each was higher than that reported in other African countries.

A clear association with diarrhea was corroborated for E. histolytica, but not for Giardia lamblia. In the hospital survey, these two infections showed a standard age distribution in which the prevalence of E. histolytica/E. dispar increases with age, while that of Giardia lamblia decreases after adolescence. Curiously, in the cross-sectional surveys, E. histolytica/E. dispar was most prevalent in those less than one year of age. This may indicate a transmission route different from that for 2–14-year-old children, in which it is probably person-to-person. In the rural areas, only 20% of the adults have been treated with ivermectin (the onchocerciasis prevalence rates in rural areas vary between 50% and 75%). The treatment of the people more than six years of age could be a cause of the results observed in the cross-sectional surveys in rural areas. In addition, the low level of health education about water treatment and the use of other milk prepared with water in these rural areas could be the cause of the high prevalence in this age group.

### Table 3

Epidemiologic features related to Entamoeba histolytica/E. dispar and Giardia lamblia

<table>
<thead>
<tr>
<th>Rural surveys</th>
<th>Total (%)</th>
<th>E. histolytica/E. dispar</th>
<th>G. lamblia</th>
<th>P-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>251 (45)</td>
<td>36 (14)</td>
<td>21 (8)</td>
<td>0.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Female</td>
<td>306 (55)</td>
<td>47 (15)</td>
<td>19 (6)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Water</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not treated</td>
<td>522 (94)</td>
<td>71 (14)</td>
<td>36 (7)</td>
<td>0.002</td>
<td>0.3 (F)</td>
</tr>
<tr>
<td>Treated</td>
<td>35 (6)</td>
<td>12 (34)</td>
<td>4 (11)</td>
<td>0.3 (F)</td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>57 (10)</td>
<td>21 (37)</td>
<td>10 (18)</td>
<td>0.001</td>
<td>0.004 (F)</td>
</tr>
<tr>
<td>No</td>
<td>500 (90)</td>
<td>62 (12)</td>
<td>30 (6)</td>
<td>0.004 (F)</td>
<td></td>
</tr>
<tr>
<td><strong>Diarrhea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>17 (3)</td>
<td>6 (35)</td>
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<td>77 (14)</td>
<td>39 (7)</td>
<td>1.0</td>
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<tr>
<td><strong>Hospital surveillance</strong></td>
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<td></td>
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</tr>
<tr>
<td><strong>Gender</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>797 (49)</td>
<td>268 (34)</td>
<td>70 (9)</td>
<td>0.03 (F)</td>
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<tr>
<td>Female</td>
<td>836 (51)</td>
<td>266 (32)</td>
<td>71 (8)</td>
<td>0.9</td>
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<tr>
<td><strong>Water</strong></td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>Not treated</td>
<td>1,264 (78)</td>
<td>426 (34)</td>
<td>106 (8)</td>
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</tr>
<tr>
<td>Treated</td>
<td>358 (22)</td>
<td>106 (30)</td>
<td>34 (9)</td>
<td>0.004 (F)</td>
<td></td>
</tr>
<tr>
<td><strong>Symptoms</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>1,455 (89)</td>
<td>486 (33)</td>
<td>122 (8)</td>
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<td>0.4</td>
</tr>
<tr>
<td>No</td>
<td>178 (11)</td>
<td>48 (27)</td>
<td>19 (11)</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Diarrhea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>733 (45)</td>
<td>297 (41)</td>
<td>57 (8)</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>No</td>
<td>900 (55)</td>
<td>237 (26)</td>
<td>84 (9)</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Yates’ corrected. F = Fisher’s exact two-tailed P-value. Bold P values indicate statistical significance.
In the two seroepidemiologic surveys indicate a high prevalence of anti- E. histolytica antibodies on Bioko and in the two mainland cities where screening was carried out. Since the asymptomatic cyst carriers usually show negative serologic results in immunologic tests, these results (seroprevalences of 31%, 61%, and 78%, respectively) indicate that if a good correlation exists between infection with pathogenic zymodemes and positive serologic test results, the prevalence of symptomatic amebiasis in Equatorial Guinea is very high. However, antibodies may persist up to two years after diagnosis and treatment. The serologic results confirm the underestimation of the prevalence in the coproparasitologic study in which only one fecal examination was carried out. Thus, Equatorial Guinea is in a zone highly endemic for amebiasis. The high seropositivity rate would explain the large number of invasive strains, which in consistent with the increased number of ALAs detected at the hospital in Malabo. Zymodeme, molecular, or immunologic analyses are needed to evaluate the true prevalence of invasive strains. In our study, the possible relationship between asymptomatic carriers and symptomatic patients cannot be corroborated because the serologic and stool surveys were different. The survey confirms the hyperendemicity of amebiasis in three geographic areas of the country, an island and two mainland villages, and may indicate a significant number of extra intestinal amebiasis cases.

In Equatorial Guinea, diagnosis of ALA is not always easy. When a case of ALA is suspected, the patient is immediately treated with metronidazole according to WHO recommendations. Ultrasonography, in addition to serologic tests, is the most useful method for detecting ALA. Ultrasonography has been used in Equatorial Guinea since 1995, a period coinciding with the increased number of suspected ALA cases in Malabo. However, immunologic diagnosis is required to corroborate these cases. The IHA and EIA are two techniques whose results correlate well with the presence of ALA. This study showed a good correlation between these two immunologic techniques and the presence of ALA, since 70–80% of the suspected cases were confirmed. Amebic liver disease was detected only in adults and the sex ratio was 2:1 (male:female), which coincides with that reported since 70–80% of the suspected cases were confirmed. Amebiasis: introduction, current status and research questions. Rev Infect Dis 8: 218–227.


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