EPIDEMIOLOGY OF AMEBIASIS IN A REGION OF HIGH INCIDENCE OF AMEbic LIVER ABSCESS IN CENTRAL VIETNAM

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Abstract. The recent identification of Entamoeba dispar as a separate species, which is nonpathogenic for humans but morphologically indistinguishable from Entamoeba histolytica, has prompted the World Health Organization to recommend reinforced efforts for reassessment of the epidemiology of amebiasis and, in particular, of E. histolytica. In this regard, the distribution of amebic liver abscess (ALA) cases was analyzed in the province of Thua Thien Huế (TT Huế) in central Vietnam, a region known for its high incidence of invasive amebiasis. In addition, in a particular area of Huế City, a parasitologic and seroepidemiologic survey was performed to identify possible risk factors for transmission of E. histolytica. Based on the analysis of hospital charts from April 1990 to April 1998, 2,031 cases of ALA were identified, indicating an ALA incidence of at least 21 per 100,000 inhabitants per year. Incidence varied substantially between the various districts of TT Huế and directly correlated with population density. The risk for ALA was significantly higher in summer and was age and sex dependent because 95% of the cases were adults, of which more than 80% were males. There was no clustering of cases within households and recurrent cases of ALA occurred more frequently than predicted in the study population. Despite the higher incidence of ALA in males, the parasitologic and seroepidemiologic survey revealed a significant higher infection rate for intestinal protozoan parasites, including E. histolytica in females. Besides level of education and access to a toilet or tapwater, use of river water was identified as an important risk factor for E. histolytica infection.

INTRODUCTION

Amebiasis is defined as a human infection with the fecally-orally spread protozoan parasite Entamoeba histolytica, regardless of the clinical outcome.1 Normally present in the large bowel, the ameba usually persist for months or even years as asymptomatic luminal gut infections. However, occasionally the parasite penetrates the intestinal mucosa and induces colitis or disseminates to other organs, most commonly to the liver, where it causes abscess formation. At the present time, the factors that trigger ameba invasion are unknown.

Despite its medical importance and the fact that E. histolytica is endemic in large parts of the world, little is known about the epidemiology of amebiasis. The last estimate on the global magnitude of the disease was made approximately 15 years ago.3 At that time, based on a comprehensive analysis of the current literature, it was suggested that approximately 500 million people are infected worldwide with E. histolytica. However, this estimate was made before the identification of E. dispar as a separate species, which also colonizes the human gut but which is morphologically indistinguishable from E. histolytica.4 Unlike E. histolytica, E. dispar has never been found to be associated with amebic disease, and this ameba species is therefore considered to be a harmless gut commensal. Accordingly, most of the earlier data on the worldwide distribution of E. histolytica are of limited value.

Recently, the World Health Organization has recommended reassessment of the epidemiology of E. histolytica using test systems, which are able to differentiate between the two ameba species.1 From the few studies that have been conducted so far, it is evident that E. dispar is substantially more prevalent than E. histolytica,4-8 and that only a small proportion of individuals specifically infected with E. histolytica will progress into having amebic disease.8 In addition to the need to further differentiate ameba species, there are two major problems for studying the epidemiology of amebiasis. The first is that although E. histolytica is endemic in most tropical and subtropical countries, the number of infected individuals is usually low and does not exceed 1% of the population.4-8 Thus, studies on the prevalence of the parasite and on the outcome of infection requires the enrollment of a fairly large number of individuals. The second is that the clinical spectrum of E. histolytica-associated intestinal symptoms is broad, ranging from mild diarrhea to severe hemorrhagic colitis.9 However, in most areas endemic for amebiasis, diarrhea is a common disease due to the high prevalence of other diarrhea-causing infectious agents, and therefore, it is difficult to assess whether intestinal complaints are indeed the result of a present E. histolytica infection.

In contrast to intestinal amebic disease, the diagnosis of amebic liver abscess (ALA) seems to be more reliable, since only a few but uncommon diseases such as pyogenic liver abscess present the typical combination of ALA-associated clinical symptoms, sonographic findings, and a good response to treatment with metronidazole.9 Although no precise data are available, it appears that the incidence of ALA differs between various amebiasis-endemic areas. A comprehensive review of the current literature on amebic diseases from 1929 to 1997 listed 5,642 reported cases of ALA, most of them from Mexico and southeast Asia.10 Recently, a region around Huế City in central Vietnam was identified as an area with an extraordinary high number of ALA patients,11 which might constitute an excellent site for studies on the epidemiology of amebiasis. Here we report on the analysis and distribution of more than 2,000 ALA cases in the province of Thua Thien Huế (TT Huế), as well as on a parasitologic and seroepidemiologic survey conducted in a particular area of Huế City.

MATERIALS AND METHODS

The study has been approved by the Scientific Council of Education, Training and Ethics of Huế Medical School on September 11, 1998.

ALA patients. The files of the Central Hospital of Huế City from April 1990 to April 1998 were analyzed and all 2,031 charts of patients treated for ALA were selected. The diagnosis of ALA was based on clinical signs (e.g., weight loss,
fever, abdominal pain and tenderness), ultrasound results (space-occupying, round, hypoechoic liver lesions), and a good response to therapy with metronidazole or dehydroemetine as shown by disappearance of clinical symptoms and reduction in abscess size. Serologic analysis for *E. histolytica* using a commercially available enzyme-linked immunosorbent assay (ELISA) was conducted on 200 of these patients, of which 189 (94.5%) were positive. The address, age, gender, and date of admission of the various ALA patients were obtained based on information listed in their hospital charts. The male and female distribution in the various age groups was calculated on the basis of the 1989 Vietnam census. The area and the number of inhabitants of the various districts of the province of TT Huế and the commune of Phu Cat, Huế were obtained from the 1999 statistical yearbook of the TT Huế statistical office.12

**Parasitologic and serologic survey.** According to the information obtained from the hospital files, Phu Cat, one of the 25 communes into which Huế City is divided, was identified as the area of highest ALA incidence. A closer look revealed that a considerable number of ALA patients were residents of an area within Phu Cat that comprised a very dense populated triangle between Chi Lang Street, Bach Dang Channel, and the Perfume River. All 961 households within the triangle were randomized and 50% of the households were visited between January and July 1999. After informed consent was obtained, all adult household members between 20 and 55 years old were interviewed using a structured questionnaire to obtain information about household characteristics, living conditions, personal habits, and educational level. In addition, stool and serum samples were collected. Stool samples were analyzed using the *E. histolytica*-specific lectin coproantigen ELISA (TechLab, Inc., Blacksburg VA) according to the manufacturer’s instructions. In addition, stool samples were examined microscopically for the presence of protozoan parasites and helminth eggs using the formol-ether-concentration technique and subsequent staining with Lugol’s iodine solution.13 Serum samples were tested for the presence of antibodies to *E. histolytica* using an ELISA based on a recombinant *E. histolytica* surface antigen as previously described.14 This assay has been shown to be highly specific, even when used in amebiasis-endemic countries, and it is able to detect antibodies to *E. histolytica* for approximately 6–12 months after successful anti-amebic therapy.15

**Statistical analysis.** All data collected were computer-coded and analyzed by use of Jandel Sigma Stat (Chicago, IL), SPSS (Chicago, IL), or StatView (Cary, NC) software. Binomial or chi-square tests were used for comparisons between two groups. Multiple logistic regression was used to test for the simultaneous involvement of several factors that might influence anti-amoeba antibody status. A *P* value < 0.05 was considered to be statistically significant. For the determination of relative risks (RRs), the program RELRISK (Ott J, http://linkage.rockefeller.edu) was used. Confidence intervals and standard errors were determined by standard methodology.

**RESULTS**

**Association of the incidence of ALA with population density and its predominance in adult males.** Based on the information found in the hospital files, 2,031 patients with ALA were treated at the Central Hospital of Huế between April 1990 and April 1998. Over the eight-year observation period, the number of ALA patients admitted to the hospital was relatively stable, approximately 260 cases per year, with a slightly but not significantly higher number in 1993 (Figure 1). However, a significant difference in the number of ALA admissions was found between the dry, hot summer season from April to September (56% of cases) and the rainy and cooler winter season from October to March (44%; *P* < 0.001).

Complete addresses were available for 1,813 of ALA patients, which indicated that the majority of the cases (1,763) were residents of the nine districts in the province of TT Huế (Figure 2). Only a few cases (50) were from the neighboring provinces of Quang Tri and Quang Nam. The highest incidence of ALA was found in the district of Huế City and lowest incidence was found in the districts of A Luoi and Nam Dong. The latter two districts are located in the mountain area along the border with Laos. With the exception of Quang Dien, the incidence of ALA in the various districts showed a direct correlation with population density (Table 1).

Data on gender were available for 2,023 ALA patients and data on gender and age were available for 1,911 patients. The results indicate that ALA was rare in children, since more than 95% of all ALA patients were adults (> 15 years old) with a peak incidence in the 30–49-year-old age groups. In addition, incidence of ALA was sex dependent and was much higher in males. Overall, the male to female ratio was 4 to 1. However, the ratio varied with age. Whereas in children no substantial sex differences were found for the incidence of ALA, the male to female ratio of adult ALA patients was approximately 7:1 for the 30–49-year-old age groups and approximately 2.5:1 for the age groups more than 60 years old (Figure 3). There were no substantial differences in the age and sex distribution of ALA patients between the various districts.

**Lack of induction by ALA of long-lasting immunity against development of recurrent ALA.** In patients living in Huế City, nine current ALA, were reported, all of which occurred in males. The average time between the first and the second abscess was 27 months (range = 6–40 months). The mean observation period for all 666 males with ALA from Huế City was 4.13 years. Thus, the risk for a man to develop a second ALA within one year was 0.33%, which is more than five times higher than the risk for males to develop an ALA.
In addition, the hospital files suggested that there was no clustering of ALA cases within households. Of the 1,813 patients for whom complete addresses were available, 1,811 were from different households. Only in two cases did two members from the same family develop ALA within the eight-year observation period.

Lack of an association between higher incidence of ALA in adult males and an increased rate of infection with *E. histolytica*. To investigate whether the higher incidence of ALA in adult males is due to an increased infection rate with *E. histolytica*, a parasitologic survey was performed in an area of extraordinary high ALA incidence, but with an age and sex distribution of ALA patients similar to that found for the entire province. The selected study area was located within the commune Phu Cat of Hue City along the confluence of Bach Dang Channel and the Perfume River. It is characterized by a relatively high population density and contains a considerable number of households with poor sanitary conditions and without direct access to reasonable drinking water, such as tap water. The incidence of ALA was 106 cases per 100,000 inhabitants per year, thus being three times higher than the average ALA incidence for all of Huế City.

All 961 households in the study site were randomized and 50% of these households were visited. Stool samples were collected from all adult household members between 20 and 55 years old. A total of 1,187 stool samples were obtained, which represented 82% of the expected sample size. In addition, blood samples were taken from approximately one-third of the individuals (416) to test for the presence of antiamoebic antibodies in serum. Microscopic analysis of the stool samples showed a considerably high prevalence of infections with orally transmitted intestinal parasites (Figure 4). A total of 71.4% of the individuals were infected, 41.3% harbored at least one of the various protozoan species, and 57.7% had helminth infections, in particular, *Ascaris* or *Trichuris*. With regard to *E. histolytica*, 0.9% of the stool samples tested positive for *E. histolytica* coproantigen and 12.7% of

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**TABLE 1**

Population density and incidence of ALA in the various districts of the province of Thua Thien Huế

<table>
<thead>
<tr>
<th>District</th>
<th>Inhabitants*</th>
<th>Inhabitants (km²)*</th>
<th>ALA cases</th>
<th>Incidence [95% conf. interval]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huế City</td>
<td>292,169</td>
<td>4,116</td>
<td>858</td>
<td>37 [30–40]</td>
</tr>
<tr>
<td>Phú Vang</td>
<td>169,188</td>
<td>604</td>
<td>321</td>
<td>24 [16–32]</td>
</tr>
<tr>
<td>Huong Tra</td>
<td>108,850</td>
<td>209</td>
<td>183</td>
<td>21 [13–31]</td>
</tr>
<tr>
<td>Huong Thuy</td>
<td>87,957</td>
<td>192</td>
<td>113</td>
<td>16 [8–26]</td>
</tr>
<tr>
<td>Quang Dien</td>
<td>89,641</td>
<td>550</td>
<td>94</td>
<td>13 [6–23]</td>
</tr>
<tr>
<td>Phú Lộc</td>
<td>142,373</td>
<td>196</td>
<td>101</td>
<td>9 [4–15]</td>
</tr>
<tr>
<td>Phong Dien</td>
<td>99,293</td>
<td>104</td>
<td>69</td>
<td>9 [3–16]</td>
</tr>
<tr>
<td>Nam Dong</td>
<td>20,796</td>
<td>32</td>
<td>11</td>
<td>7 [0–31]</td>
</tr>
<tr>
<td>A Luoi</td>
<td>34,867</td>
<td>28</td>
<td>13</td>
<td>5 [0–19]</td>
</tr>
</tbody>
</table>

province TT Huế 1,045,134 207 1,763 21 [18–24]

* Data were obtained from the statistical year book 1999, TT Huế statistical office.† Only those cases from 4/90–4/98 are considered for which complete patient addresses were available.‡ Number of ALA cases per 100,000 inhabitants per year.
the individuals had anti-\textit{E. histolytica} antibodies in serum. Interestingly, in contrast to the sex distribution of ALA patients, protozoan parasites as well as anti-amoebic antibodies were significantly more prevalent in females (Figure 4).

**River water as a source of \textit{E. histolytica} infections.** To identify possible risk factors for \textit{E. histolytica} transmission, anti-amoebic antibody status of individuals was compared with household characteristics and living conditions (Table 2). The results indicated no association between the outcome of serology and the number or density of people per household. In contrast, a strong association was found between the absence of anti-amoebic antibodies and a higher educational level, access to toilets, or access to tap water. In addition, anti-amoebic antibodies were significantly more prevalent in individuals who regularly use river water. Interestingly, the latter finding was independent from whether or not these individuals had access to toilets or tap water, suggesting that river water constitutes an important source for \textit{E. histolytica} infections. This result was further confirmed by multiple logistic regression analysis. When age, gender, educational level, number of household members, living area, access to a toilet or tap water, and use of river water were used as explanatory variables significant influences on antibody positivity were found only with the use of river water \( (P < 0.03, \text{RR} = 2.540) \) and gender \( (P < 0.02, \text{RR} = 0.471) \). All other variables were not significant in this analysis.

**DISCUSSION**

In an attempt to obtain more precise data on the epidemiology of amebiasis, we have analyzed the distribution of ALA cases in the province of TT Huế in central Vietnam, a region of high incidence of invasive amebiasis. In addition, in a specific area of Huế City, a parasitologic and seroepidemiologic survey was performed to identify possible risk factors for \textit{E. histolytica} transmission. The ALA cases were analyzed by making use of the hospital charts of patients admitted to the Central Hospital of Huế between April 1990 and April 1998. The diagnosis of ALA was most likely correct for the vast majority of cases included into the study because all patients had corresponding clinical signs, space-occupying, round, hypoechocic liver lesions, and showed a good response to anti-amoebic treatment. In addition, these patients had a 94.5% prevalence of anti-amoebic antibodies. Moreover, in a recent study, liver abscess aspirates of 50 consecutive ALA-suspected patients were cultured for the presence of aerobic or anaerobic bacteria, of which 49 (98%) were shown to be sterile, excluding the differential diagnosis of pyogenic abscesses (Blessmann J and others, unpublished data). The period between April 1990 and April 1998 was chosen as because it represented the time span when the Central Hospital of Huế was the only medical care unit in the entire province where ultrasound was available. Since treatment of ALA in central Vietnam is generally performed by a combination of abscess puncture and metronidazole therapy,\textsuperscript{11} all suspected ALA cases from various parts of the province were usually sent to the Central Hospital of Huế. However, according to information provided by local physicians and health authorities, some less severe cases of ALA may have been treated with metronidazole alone and were not sent to the hospital. Thus, the data from the hospital charts were suitable to determine the distribution of ALA cases with respect to gender, age and living site, but probably underestimated the true incidence of ALA within the TT Huế population. Nevertheless, based on the hospital charts, the incidence of ALA was rather high since more than 2,000 cases in a population of approximately one million inhabitants were recorded, which to our knowledge represents the highest incidence of ALA on a regional level ever reported.

Over the eight-year study period, the annual number of ALA cases was rather constant, suggesting a stable epidemiologic situation for the transmission of \textit{E. histolytica} and the development of ALA in the studied population. Conversely, incidence of ALA varied substantially between the various districts of the province TT Huế, but was found to correlate directly with population density. Although information is currently lacking about the relationship between population density and incidence of invasive amebiasis, our results are in agreement with observations from the Gambia.\textsuperscript{16} Here, in a nationwide survey, the prevalence of intestinal \textit{E. histolytica}/ \textit{E. dispar} infections also directly correlated with population density. Interestingly, in the same study, a higher prevalence

<table>
<thead>
<tr>
<th>Table 2</th>
<th>Correlation between anti-amoebic antibody status and household characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Household characteristics</td>
<td>No. of individuals</td>
</tr>
<tr>
<td>&gt; 5 individuals per household</td>
<td>297</td>
</tr>
<tr>
<td>( \leq 5 ) individuals per household</td>
<td>119</td>
</tr>
<tr>
<td>Living space per person ( \leq 10 \ \text{m}^2 )</td>
<td>209</td>
</tr>
<tr>
<td>Living space per person &gt; ( 10 \ \text{m}^2 )</td>
<td>192</td>
</tr>
<tr>
<td>Education ( \leq 4 ) school years</td>
<td>141</td>
</tr>
<tr>
<td>Education &gt; ( 4 ) school years</td>
<td>273</td>
</tr>
<tr>
<td>No access to a toilet</td>
<td>152</td>
</tr>
<tr>
<td>No access to tap water</td>
<td>264</td>
</tr>
<tr>
<td>Access to a toilet</td>
<td>80</td>
</tr>
<tr>
<td>Access to tap water</td>
<td>336</td>
</tr>
<tr>
<td>Use of river water</td>
<td>198</td>
</tr>
<tr>
<td>No use of river water</td>
<td>218</td>
</tr>
<tr>
<td>Access to a toilet and tap water but use of river water</td>
<td>65</td>
</tr>
<tr>
<td>Access to a toilet and tap water but no use of river water</td>
<td>190</td>
</tr>
</tbody>
</table>

* RR = relative risk.
of the parasite was found in the western part of the Gambia, which has lower temperatures and a higher humidity compared with the eastern part of the country. This appears to contradict our results, which indicated substantially more cases of ALA during the dry summer season. However, if one considers that the average time between infection with *E. histolytica* and onset of ALA is approximately five months, the higher incidence of ALA in the Vietnamese population during summer might be the result of increased infection rates with *E. histolytica* during the winter.

It has not been established whether protective immunity to invasive amebiasis truly develops. Recurrent ALAs have been repeatedly described, and in a study from Cambodia as many as 8% of the patients developed a second liver abscess. However, clinicians in endemic areas have made anecdotal observations that liver abscess infrequently recurs. In addition, the analysis of the hospital charts of 1,021 patients cured of ALA in Mexico City between 1963 and 1968 revealed that substantially less of these patients were readmitted during this time with a diagnosis of ALA than predicted in the study population. However, there was no knowledge of the patients’ current whereabouts or of admissions to other hospitals. In contrast, the data from Huế City indicate that recurrent ALA occurs more frequently than predicted in the study population. In addition, the number of recurrent ALAs in our study population is certainly underestimated since knowledge of the clinical course may result in self-treatment of the disease. This became evident during our survey in Phu Cat, where we identified at least three patients with recurrent ALA (confirmed by ultrasound and positive serology) who underwent self-treatment with metronidazole. Thus, the number of recurrent ALAs in the total Huế population is believed to be much higher than that predicted from the hospital charts. Taken together, our findings do not support the concept that ALA induces protection against invasive amebiasis or at least does not induce long-lasting immunity against recurrent ALAs. Furthermore, our results do not support the idea of a genetic trait that may predispose for the development of ALA, since no clustering of cases within families was found. However, the lack of clustering may also indicate that fecal-oral spread of the parasite between family members does not play an important role in transmission of *E. histolytica* in the study population, and that the primary source for *E. histolytica* infection is located outside the household. This latter idea would be consistent with our findings, which indicate that the use of river water in Huế constitutes an important risk factor for *E. histolytica* infection.

One of the unresolved enigmas in amebiasis is that the occurrence of ALA greatly predominates in adult males, with a peak incidence at approximately 40 years of age. This has been observed in all parts of the world where amebiasis is endemic, and was confirmed in this study. However, none of the previous reports comprises such a large collection of ALA cases, obtained from a circumscribed region within a relatively short period of time which makes our data rather robust. In addition, the age and sex distribution of ALA cases in some of the previous reports may be biased due to pre-selection of ALA cases (e.g., no children included, reduced access of women to medical care). Nevertheless, the consistent worldwide observation of a substantial higher incidence of ALA in adult males suggests that this is independent of cultural or ethnic backgrounds. Moreover, the higher incidence of ALA in males is obviously not due to a higher rate of infection with *E. histolytica*. According to the results of our survey in the adult population of Phu Cat, women were found to be more frequently infected with intestinal protozoan parasites and had a significant higher prevalence of amoebic antibodies. The higher prevalence might be explained by an increased exposure to the parasite, since women primarily serve the household, which includes washing of clothes, dishes, and food at the river bank, as well as taking water from the river.

An unexpected finding of our study was the low prevalence of 0.9% for *E. histolytica* as shown by the commercially available *E. histolytica* lectin-specific coproantigen ELISA. Although this value is in agreement with results from other endemic areas, it does not explain the high incidence of ALA in the Huế population. The results might suggest that local factors or habits such as the gut flora or diet possibly contribute to the outcome of *E. histolytica* infection. Conversely, it remains to be established whether the lectin coproantigen ELISA is suitable for the detection of *E. histolytica* in stool samples of the Vietnamese population, since the results of this test showed only a weak association with ameba serology. Similar findings were reported from Fortaleza, Brazil with the same version of the antigen test, whereas a stronger association was found with stool samples from Bangladesh, using a newer version of the test. However, based on previous results using stool culture and subsequent isoenzyme analysis or a polymerase chain reaction (PCR) to differentiate amebae or using a direct *E. histolytica*-specific PCR with stool samples, it is evident that more than 80% of individuals infected with *E. histolytica*, even when asymptomatic, have anti-amoebic antibodies in serum. Thus, further studies are required using other test systems such as a direct stool PCR to prove or disprove our results with the *E. histolytica* coproantigen ELISA.

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