Abstract

Background: The seroepidemiology of Entamoeba histolytica in Mexico has been scantily studied. The aim of the study was to determine the seroprevalence and correlates of E. histolytica antibodies in adults in rural areas in Durango, Mexico.

Methods: Through a cross-sectional study, E. histolytica IgG antibodies were determined in 282 adults living in rural Durango, Mexico using an enzyme-linked immunoassay. In addition, seroprevalence association with the socio-demographic, housing conditions, and behavioral characteristics of the subjects studied was investigated.

Results: One hundred and eighteen (41.8%) of the 282 rural subjects had anti-E. histolytica IgG antibodies. Multivariate analysis showed that E. histolytica exposure was positively associated with source of drinking water (OR = 2.73; 95% CI: 1.33 - 5.58; P = 0.005), and poor education of the head of the family (OR = 1.53; 95% CI: 1.03 - 2.27; P = 0.03). In contrast, E. histolytica exposure was negatively associated with consumption of unpasteurized cow milk (OR = 0.55; 95% CI: 0.31 - 0.96; P = 0.03), and crowding at home (OR = 0.33; 95% CI: 0.17 - 0.64; P = 0.0009).

Conclusions: The seroprevalence of E. histolytica infection found in adults in rural Durango is high compared with those reported in other Mexican populations. The correlates of E. histolytica seropositivity found in the present study may be useful for the planning of optimal preventive measures against E. histolytica infection.

Keywords: Entamoeba histolytica; Seroepidemiologic studies; Rural population; Risk factors; Mexico

Introduction

The protozoa parasite Entamoeba histolytica is an important cause of morbidity and mortality worldwide [1, 2]. Infections with E. histolytica are common and are one of the major health problems in developing countries [3, 4]. Humans are the host of E. histolytica and there are no other known animal reservoirs of this parasite [5]. The clinical spectrum of E. histolytica infections varies from asymptomatic infection to hemorrhagic colitis and extra-intestinal disease [6]. Most persons infected with E. histolytica are carriers [7]. Infection with E. histolytica is responsible from a considerable number of cases of prolonged diarrhea in travelers [8]. In addition, infection with E. histolytica may lead to the development of live-threatening abscess in liver, brain [9] or lungs [5]. Transmission of E. histolytica occurs in areas with poor sanitation by contamination of drinking water or food with human feces [10]. Water-associated outbreaks of E. histolytica disease have been reported [11]. Transmission of E. histolytica can also be sexual [12]. Very little is known on the seroepidemiology of E. histolytica infection in rural adults in Mexico. Rural communities in Mexico have commonly poor sanitation, and this is an important condition for transmission of E. histolytica among the population. A considerable number of houses in rural Mexico have poor availability of drinkable water and poor disposal of excretes. Therefore, contamination of water and food with E. histolytica is highly feasible to occur in rural communities. The lack of laboratory tests for diagnosis of E. histolytica infection in rural health centers does not allow having reliable statistical information about the magnitude of E. histolytica exposure in rural Mexico. We sought to determine the seroprevalence of E. histolytica IgG antibodies in adults in rural Durango, Mexico. Furthermore, socio-demographic and behavioral characteristics of the rural subjects associated with E. histolytica seropositivity were investigated.

Materials and Methods

Study design and study population

The design of this study was cross-sectional. We analyzed stored serum samples used in a previous survey about the
seroepidemiology of *Toxoplasma gondii* infection in rural populations in Durango, Mexico [13]. Serum samples were collected from December 2006 to August 2007 in three rural communities: San Dimas, Villa Montemorelos, and Santa Clara. Inclusion criteria for enrollment were: 1) inhabitants of rural Durango; 2) aged 18 years and older; 3) any sex; and 4) who accepted to participate in the survey. Exclusion criteria for enrollment were: 1) subjects with insufficient amount of serum; and 2) subjects with incomplete socio-demographic and behavioral data. Selection of subjects was performed randomly. In total, 282 subjects were included in this study, 94 of them were inhabitants of San Dimas; 82 were inhabitants of Villa Montemorelos, and 106 were inhabitants of Santa Clara.

**General socio-demographic and behavioral characteristics of rural adults**

Socio-demographic and behavioral characteristics of the participants were obtained with the aid of a standardized questionnaire. Socio-demographic items included age, birthplace, residence, educational level, socio-economic status, and employment. Housing conditions of the participants were determined by using the Bronfman’s criteria [14]. This tool allowed to assess crowding, type of flooring (ceramic, concrete, soil), availability of drinkable water (within the house, out of the house), and form of elimination of excretes (flush toilet, latrine, or other). In addition, the educational level (years of education) of the head of the family was recorded. Behavioral items included consumption of unpasteurized milk or untreated water, consumption of unwashed raw vegetables or fruits, frequency of eating away from home (in restaurants or fast food outlets), raising farm animals, foreign travel, and contact with soil (gardening or agriculture).

**Laboratory tests**

Serum samples of the participants were analyzed for anti-*E. histolytica* IgG antibodies by a commercially available enzyme immunoassay “*E. histolytica* IgG (Amebiasis) ELISA” kit (Diagnostic Automation Inc., Calabasas, CA). All assays were performed following the manufacturer’s instructions. Samples were run along with positive and negative controls in each assay. According to the information included in the kit’s insert, the enzyme immunoassay used has a sensitivity of 92% and a specificity of 100%.

**Statistical analysis**

We used the software Epi Info version 7 and SPSS version 15.0 to perform the statistical analysis. For calculation of the sample size, a reference seroprevalence of 4.49% [15] as the expected frequency for the factor under study, 300,000 as the population size from which the sample was selected, 2.5% confidence limits, and a 95% confidence level (CI) were considered. The result of the sample size calculation was 263 subjects. The Pearson’s Chi-squared test and the Fisher exact test (when values were small) were used for initial comparison of frequencies among groups. Socio-demographic characteristics, housing conditions, and behavioral variables with a P value equal to or less than 0.05 obtained in the bivariate analysis were further analyzed by multivariate analysis to determine their association with *E. histolytica* seropositivity. Odds ratios (OR) and 95% CIs were calculated by using logistic regression analysis with the Enter method. The Hosmer-Lemeshow goodness of fit test was used to assess the fitness of our regression model. Statistical significance was set at P value < 0.05.

**Ethical aspects**

Only archival serum samples and data from a previous study [13] were used in the present study. The ethical committee of the Mexican Social Security Institute in Durango City, Mexico approved this previous survey. The purpose and procedures of the study were explained to all participants, and a written informed consent was obtained from all of them.

**Results**

Most participants were female (78.0%), of low socioeconomic status (77.3%), and unemployed (76.6%). Mean age of participants was 42.91 ± 17.53 years old (range 18 - 91 years). One hundred and eighteen (41.8%) of the 282 rural subjects had anti-*E. histolytica* IgG antibodies. A correlation of *E. histolytica* seropositivity and socio-demographic and behavioral characteristics and housing conditions of the rural subjects studied is shown in Table 1. Of the socio-demographic data, housing conditions, and behavioral characteristics assessed, the variables age, community of residence, education, consumption of unpasteurized milk, source of drinking water, crowding at home, and educational level of the head of the family had P values < 0.05 by bivariate analysis. Other socio-demographic data, housing conditions, and behavioral characteristics including occupation, socio-economic status, type of flooring at home, form of elimination of excretes, foreign travel, raising animals, consumption of untreated water, unwashed raw vegetables or fruits, eating away from home and contact with soil had P values > 0.05 by bivariate analysis. Further analysis using logistic regression of the socio-demographic, housing conditions, and behavioral characteristics of rural adults showed that *E. histolytica* exposure was positively associated with source of drinking water (OR = 2.73; 95% CI: 1.33 - 5.58; P = 0.005), and poor education of the head of the family (OR = 1.53; 95% CI: 1.03 - 2.27; P = 0.03) (Table 2). In contrast, logistic regression analysis showed that *E. histolytica* exposure was negatively associated with consumption of unpasteurized cow milk (OR = 0.55; 95% CI: 0.31 - 0.96; P = 0.03), and crowding at home (OR = 0.33; 95% CI: 0.17 - 0.64; P = 0.0009). The result of the Hosmer-Lemeshow test (P = 0.60) suggested a good fit of our regression model.
Table 1. Bivariate Analysis of a Selection of Exposure Variables and Seroprevalence of *E. histolytica* in General Population in Rural Durango

| Characteristic                  | No. of subjects tested | Positive ELISA results | Odds ratio | 95% confidence interval | P value |
|--------------------------------|------------------------|------------------------|------------|-------------------------|---------|
|                                |                        | No. | % |                        |          |
| **Gender**                     |                        |     |   |                        |          |
| Male                           | 62                     | 27  | 43.5 | 1.1                    | 0.61 - 1.93 | 0.75  |
| Female                         | 220                    | 91  | 41.4 | 1.0                    |          |       |
| **Age groups (years)**         |                        |     |   |                        |          |
| 30 or less                     | 75                     | 23  | 30.7 | 1.0                    |          |       |
| 31 - 50                        | 120                    | 47  | 39.2 | 1.5                    | 0.78 - 2.68 | 0.22  |
| > 50                           | 87                     | 48  | 55.2 | 2.8                    | 1.45 - 5.31 | 0.001 |
| **Community**                  |                        |     |   |                        |          |
| One                            | 94                     | 14  | 14.9 | 1.0                    |          |       |
| Two                            | 82                     | 52  | 63.4 | 9.9                    | 4.80 - 20.43 | < 0.0001 |
| Three                          | 106                    | 52  | 49.1 | 5.5                    | 2.77 - 10.90 | < 0.0001 |
| **Educational level**          |                        |     |   |                        |          |
| No education                   | 27                     | 17  | 63.0 | 2.6                    | 1.14 - 5.88 | 0.01  |
| Education                      | 255                    | 101 | 40.4 | 1.0                    |          |       |
| **Occupation**                 |                        |     |   |                        |          |
| Employeda                      | 66                     | 29  | 43.9 | 1.1                    | 0.64 - 1.95 | 0.69  |
| Unemployedb                    | 216                    | 89  | 41.2 | 1.0                    |          |       |
| **Socio-economic level**       |                        |     |   |                        |          |
| Low                            | 218                    | 97  | 44.5 | 1.6                    | 0.91 - 2.95 | 0.09  |
| Medium                         | 64                     | 21  | 32.8 | 1.0                    |          |       |
| **Traveled abroad**            |                        |     |   |                        |          |
| Yes                            | 44                     | 21  | 47.7 | 1.3                    | 0.69 - 2.53 | 0.38  |
| No                             | 238                    | 97  | 40.8 | 1.0                    |          |       |
| **Unpasteurized cow milk consumption** |                |     |   |                        |          |
| Yes                            | 182                    | 68  | 37.4 | 0.6                    | 0.36 - 0.97 | 0.03  |
| No                             | 100                    | 50  | 50  | 1.0                    |          |       |
| **Unwashed raw vegetables**    |                        |     |   |                        |          |
| Yes                            | 51                     | 19  | 37.3 | 0.8                    | 0.42 - 1.47 | 0.46  |
| No                             | 231                    | 99  | 42.9 | 1.0                    |          |       |
| **Unwashed raw fruits**        |                        |     |   |                        |          |
| Yes                            | 66                     | 25  | 37.9 | 0.8                    | 0.45 - 1.41 | 0.45  |
| No                             | 216                    | 93  | 43.1 | 1.0                    |          |       |
| **Untreated water**            |                        |     |   |                        |          |
| Yes                            | 139                    | 59  | 42.4 | 1.1                    | 0.65 - 1.68 | 0.83  |
| No                             | 143                    | 59  | 41.3 | 1.0                    |          |       |
| **Soil contact**               |                        |     |   |                        |          |
| Yes                            | 250                    | 108 | 43.2 | 1.7                    | 0.76 - 3.68 | 0.19  |
| No                             | 32                     | 10  | 31.3 | 1.0                    |          |       |
| **Source of drinking water**   |                        |     |   |                        |          |
| Home                           | 172                    | 93  | 54.1 | 4.0                    | 2.33 - 6.85 | < 0.0001 |
| Out of home                    | 110                    | 25  | 22.7 | 1.0                    |          |       |
The seroepidemiology of *E. histolytica* infection in rural Mexico has been scantily studied. Although amebiasis has been recognized as a major health problem in Mexico for many years [16, 17], very little is known about the seroprevalence of *E. histolytica* infection and risk factors associated with this infection in Mexican populations. Therefore, this study was performed to know the frequency of *E. histolytica* exposure among adults living in rural areas in the northern Mexican state of Durango. We found an overall 41.8% seroprevalence of *E. histolytica* infection in adult people of the three rural communities studied. This *E. histolytica* seroprevalence is higher than other *E. histolytica* seroprevalences in Mexican populations reported so far. In a previous study in people living in northern Mexican states, researchers found a low (< 5%) seroprevalence of *E. histolytica* infection in adult people of the three rural communities studied. This *E. histolytica* seroprevalence is higher than other *E. histolytica* seroprevalences in Mexican populations reported so far. In a previous study in people living in northern Mexican states, researchers found a low (< 5%) seroprevalence of *E. histolytica* in all states surveyed [18]. Nearly 25 years have passed between these studies and difference in the seroprevalences might suggest an increase in *E. histolytica* exposure. However, different laboratory methods were used among the studies; in the previous study, a homemade ELISA was used, whereas we used a commercially available ELISA. The sensitivity and specificity of the homemade ELISA were 95% and 90.7%, respectively [18]. According to the manufacturer of the commercially available ELISA used in the present study, the assay has a sensitivity and specificity of 92% and 100%, respectively. The seroprevalence found in adults in rural Durango is also higher than the mean 8.41% seroprevalence in 32 federal entities and ≤ 8% in northern states reported in a national survey [17]. However, the presence of antibodies against *E. histolytica* in the national survey was detected by an indirect hemagglutination test, which is also a different method from the one we used. The seroprevalence of *E. histolytica* found in our study is also higher than the 4.49% seroprevalence reported in a second national seroepidemiology survey of *E. histolytica* infection by using an ELISA [15]. The seroprevalence found in rural Durango is also higher than the 13.8% prevalence of *E. histolytica* infection in a rural community in the central Mexican state of Morelos obtained by polymerase chain reaction in

### Discussion

The results of the multivariate regression analysis are shown in Table 2. The analysis was performed to identify factors associated with *E. histolytica* seropositivity. The variables considered were age, community, education of the head of family, consumption of raw cow milk, water at home, crowding, and sewage disposal methods. The odds ratio and 95% confidence interval were calculated for each variable. The results show that consumption of raw cow milk and water at home were significantly associated with a decreased seropositivity, while education of the head of family and sewage disposal methods were associated with an increased seropositivity. The analysis suggests that these factors may play a role in the transmission and prevalence of *E. histolytica* infection in rural Durango.

### Table 1. Bivariate Analysis of a Selection of Exposure Variables and Seroprevalence of *E. histolytica* in General Population in Rural Durango - (Continued)

| Characteristic          | No. of subjects tested | Positive ELISA results | Odds ratio | 95% confidence interval | P value |
|-------------------------|------------------------|------------------------|------------|-------------------------|---------|
|                        | No. | %      |            |                    |         |
| Sewage disposal         |     |        |            |                    |         |
| Pipes                   | 130 | 60     | 46.2       | 1.0                |         |
| Latrine, other          | 152 | 58     | 38.2       | 0.7                | 0.44 - 1.15 | 0.17 |
| Crowding                |     |        |            |                    |         |
| No                      | 62  | 38     | 61.3       | 1.0                |         |
| Yes                     | 220 | 80     | 36.4       | 0.4                | 0.20 - 0.64 | 0.0004 |
| Education of the head of family |     |        |            |                    |         |
| 7 or more years         | 50  | 15     | 30         | 1.0                |         |
| 4 - 6 years             | 119 | 45     | 37.8       | 1.4                | 0.69 - 2.88 | 0.33 |
| Up to 3 years           | 113 | 58     | 51.3       | 2.5                | 1.21 - 4.99 | 0.01 |
| Floor at home           |     |        |            |                    |         |
| Ceramic                 | 19  | 7      | 36.8       | 1.0                |         |
| Concrete                | 184 | 69     | 37.5       | 1.0                | 0.38 - 2.73 | 0.95 |
| Soil                    | 79  | 42     | 53.2       | 1.9                | 0.69 - 5.45 | 0.20 |

*Employed: agriculture, business, construction worker, factory worker, professional, other. Unemployed: housewives, students or none occupation.*

### Table 2. Results of the Multivariate Regression Analysis

| Variable               | P value | Odds ratio | 95% confidence interval |
|------------------------|---------|------------|-------------------------|
| Age                    | 0.25    | 1.24       | 0.85 - 1.82             |
| Community              | 0.13    | 1.37       | 0.90 - 2.08             |
| No education           | 0.37    | 1.54       | 0.58 - 4.04             |
| Consumption of raw cow milk | 0.03    | 0.55       | 0.31 - 0.96             |
| Water at home          | 0.005   | 2.73       | 1.33 - 5.58             |
| Crowding               | 0.0009  | 0.33       | 0.17 - 0.64             |
| Education of the head of family | 0.03    | 1.53       | 1.03 - 2.27             |
E. histolytica stools [19]. However, comparison of the seroprevalence of E. histolytica infection with the prevalence of infection based on polymerase chain reaction in stools should be interpreted with care since a poor correlation between intestinal infection and anti-amebic antibody levels has been reported [19]. It is not clear why subjects in rural Durango have a much higher seroprevalence of E. histolytica exposure than other populations in Mexico. We searched for potential risk factors associated with E. histolytica in rural Durango. Multivariate analysis showed that E. histolytica exposure was positively associated with source of drinking water and poor education of the head of the family. Subjects with water supply within their home had a source of drinking water and poor education of the head of the family in our study. In contrast, the exposure was associated with consumption of unpasteurized cow milk and crowding at home found in the present study suggest that these characteristics did not play any important role in E. histolytica infection among the subjects studied.

Conclusions

We concluded that the seroprevalence of infection with E. histolytica found in rural populations in Durango is higher than E. histolytica seroprevalences reported in other Mexican populations. The correlates of E. histolytica seropositivity found in the present study can be used for an optimal planning of preventive measures against E. histolytica infection.

Conflict of Interest

None.

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