Seroepidemiology of Giardiasis in Mexico

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INTRODUCTION

Giardia duodenalis (synonymous with G. lamblia and G. intestinalis) is a common intestinal flagellated protozoan and a cause of diarrhea in humans and animals worldwide.1,2 Most infections are asymptomatic but in some cases, acquisition of infection is followed by acute diarrhea after an incubation period of 12–19 days.3 In developed countries, frequent outbreaks of giardiasis are caused by contaminated water.4 In developing countries, recurrent exposure and endemic infections are common and are often associated with asymptomatic excretion of G. duodenalis cysts. High rates of endemicity have been reported in Bangladesh,6 Nigeria,7 Peru,8 and Guatemala.9 In Mexico, the reported prevalence varies from 3% to 50%.10–12 Host risk factors include sex and age. Children 1–5 years of age have a particularly high prevalence (4–42%).13,14

Standard diagnostic methods of infection rely on identification of trophozoites in duodenal aspirates or cysts in stool.15,16 However, antigen detection by enzyme-linked immunosorbent assay (ELISA) has shown more sensitivity than detection of cysts by microscopy (95.0% versus 83.2%).17–19 In addition, detection of serum antibodies against G. duodenalis by ELISA is a useful method in epidemiologic studies for determining seroprevalence in large communities.13,20,21 Elevated levels of parasite-specific antibodies have been reported in different groups in United States.22,23 In Mexico, Mioti and others reported that 79% of lactating women had IgA antibodies against Giardia spp. in breast milk and 77% had serum IgG against Giardia spp.24

A community-based study was conducted among a randomly selected sub-population of a national survey in Mexico. Serum samples were collected from persons by a sampling frame based on data from the national population census and included people of all ages and from all development regions of the country.25 Serosurveys of infectious diseases such as infection with Helicobacter pylori, brucellosis, Lyme borreliosis, toxoplasmosis, cysticercosis, amebiasis, and Chagas disease have already been reported using these serum samples.27–33 The aim of this study was to determine the national prevalence of antibodies against G. duodenalis, one of the most important parasites that causes diarrhea in Mexico and to evaluate the association of several demographic and socioeconomic factors with G. duodenalis seroprevalence.

MATERIAL AND METHODS

Seroepidemiologic national survey. The National Serum Bank of the Ministry of Health in Mexico was established to store sera obtained from the 1987 National Serologic Survey of Mexico. The survey included serum samples from persons of either sex 1–98 years of age in each of the 32 states of Mexico from all socioeconomic levels and geographic zones; 40% were from rural areas. Each person completed a questionnaire that included the following socioeconomic and demographic data: age, sex, birth date, place of residence, occupation; household characteristics (type of material on the floor and walls of the house, number of rooms excluding bathroom, living room, and kitchen, number of people living in the household, availability of a sewage system, municipal water, and waste disposal, and ownership of a radio, television, refrigerator, telephone, and automobile); education level of the head of the family, availability of medical services; and geographic area. An urban population was defined as those living in an area with ≥ 2,500 inhabitants, and a rural population was defined as those residing in an area with < 2,500 inhabitants.26 Serum samples were collected, aliquoted, and stored at −20°C in the National Serum Bank.25 The project was reviewed and approved by the Ethical Committee of the National Institute of Public Health.

Regional distribution of areas within Mexico. Distribution of development level in Mexico was based on sociodemographic and public health indices according to Kunz and others.24 The regionalization index included education level, household characteristics, rate of infant and maternal mortality,
mortality rate due to transmissible and non-transmissible diseases, number or physicians in the population, and proportion of the population economically active in urban communities compared with rural communities. For the purpose of this study, states were divided into four regions; high, medium high, medium low, and low levels on the basis of development status (Figure 1).

Study sample. The sample size was calculated by Z test, which estimated a representative population from the four regions and a relative error rate of 0.05. This test gave an estimated sample size of 3,643 sera. Aliquots of 20 µL of each selected serum sample were transported to our laboratory and stored at −70°C until tested. Before use, aliquots were thawed on ice for 60 minutes to avoid denaturation of antibodies. Serum samples that contained a high concentration of lipids were centrifuged at 15,600 × g for 10 minutes at 4°C, and supernatants were collected and stored at 4°C until use.

Serologic test. Antigen preparation. Giardia duodenalis strain IMSS:1090:1, which was obtained from an asymptomatic person with a chronic infection, was used. Trophozoites were axenically grown in modified TYI-S-33 medium supplemented with 10% calf serum and bovine bile. A total of 20 × 10^6 trophozoites/mL in phosphate-buffered saline (PBS), pH 7.2, were centrifuged at 7,500 × g for 10 minutes. The cellular pellet was dissolved in the protease inhibitor N-ethylmaleimide (50 mg/mL), pH 8.3, (Sigma, St. Louis, MO). The suspension was sonicated for four cycles. The first three cycles were performed without detergent and the fourth cycle was performed using 10 mM Tris base, pH 8.3, 10% Triton X100. The extract was centrifuged at 15,600 × g for 30 minutes at 4°C. The G. duodenalis antigens (Åg) for ELISA were in the supernatant, which was collected and stored at −70°C until use.

ELISA. IgG antibodies against G. duodenalis antigens were detected by ELISA. Microplates with flat wells (Combiplate 8; Labsystems, Helsinki, Finland) were coated with 100 µL of antigen in carbonate buffer, pH 9.6, at a concentration of 0.5 µg/well and incubated overnight at 4°C. Wells were blocked with 300 µL of 0.5% bovine serum albumin (BSA) in PBS, pH 7.4, for one hour and washed three times with 300 µL of 0.01 M PBS containing 0.05% Tween 20 and 0.01% thimerosal (PBS–TT). Each serum sample was diluted 1:100 in PBS containing 0.5% BSA. A total of 100 µL was added to each well and incubated for 1 hr at 37°C. Plates were washed three times with 300 µL of PBS–TT. A total of 100 µL of goat anti-human IgG conjugated to horseradish peroxidase (Southern Biotech, Birmingham, AL) diluted 1:1,000 in PBS, 0.5% BSA was added and incubated for one hour at 37°C. The plates were washed three times with 300 µL of PBS–TT and incubated with o-phenylenediamine in citrate-phosphate buffer and hydrogen peroxide for 30 minutes at room temperature. The reaction was stopped by adding 50 µL of 4 M sulfuric acid. Optical density was read at 492 nm in a Multiskan analyzer (Labsystems). All samples were tested in duplicate. If discordance exceeded 30%, samples were tested again in duplicate. The optical density cutoff value for a positive sample was 0.3.

Validation and standardization of in-house ELISA. This assay was validated with 60 serum samples obtained from 30 positive human cases of giardiasis and 30 negative cases. The 30 positive persons were patients from the Infectious Diseases Department of the National Medical Center–Instituto Mexicano del Seguro Social in Mexico City and were considered infected on the basis of cysts in at least three fecal samples collected on three consecutive days. Negative samples were obtained from 30 persons who had one of the following parasitosis: amebiasis, cryptosporidiosis, hymenolepiasis, ascariasis, but not giardiasis. Serum samples from these persons were used for validation of the serologic assay. The threshold for positive samples was defined as the mean value plus three SD of the optical density of the 30 uninfected persons. To determine inter-variations and intra-variations in the assay, all serum samples from infected and uninfected persons were tested for five consecutive days. During testing of the survey samples, the corresponding negative controls were included in quadruplicate in every microplate and the mean of the four optical density values was used as a quality control.

Figure 1. Seroprevalence of Giardia duodenalis in Mexico based on the regional classification of economic development.34
The sensitivity, specificity, positive predictive value, and negative predictive value of the in-house ELISA were 97%, 84%, 83%, and 96%, respectively.

Statistical analysis. Seroprevalence of IgG antibodies against *G. duodenalis* was expressed as the ratio of positive persons to the total number tested per 100. On the basis of previous reports on the age specificity of *G. duodenalis* infections, we analyzed the following age groups: group 1 = < 4 years of age, group 2 = 5–9 years of age, group 3 = 10–19 years of age, group 4 = 20–39 years of age, group 5 = 40–59 years of age, group 6 = ≥ 60 years of age. Association between demographic and socioeconomic variables and seropositivity for antibodies against *G. duodenalis* was estimated by odds ratio (ORs), 95% confidence intervals (CIs), and *P* values determined by the chi-square test. The magnitude of antibody response was analyzed using analysis of variance and compared means of antibody levels per age group. *P* < 0.05 was considered significant in all cases. All analyses were performed with SPSS software version 11.0 (SPSS Inc., Chicago, IL).

RESULTS

Serology and risk factors. We estimated a sample size of 3,643 sera. However, 182 (5%) of them were not tested because they were contaminated, dried, or missing. This left a total of 3,461 serum samples for the analysis. The sample was representative of persons 1–98 years age and all regions of Mexico. The presence of IgG antibodies against *G. duodenalis* was detected in 1,914 of 3,461 sera tested. The overall seroprevalence of *G. duodenalis* was 55.3%. Age-specific seroprevalence for representative groups is shown in Table 1. Seroprevalence for *G. duodenalis* increased significantly with age. Seropositivity for *G. duodenalis* in groups 2–6 increased compared with group 1. The odds of seropositivity increased from 2.4-fold in group 2 to 12.65-fold in group 5 (Table 1). Results by specific ages showed that 10% of the infants one year of age had antibodies against *G. duodenalis*; 40% of children ≤ 10 years of age and 70% of adults ≥ 25 years of age were also positive. Seroprevalence in persons 26–99 years of age remained high (Figure 2). The risk of seropositivity against *G. duodenalis* was higher in males (OR = 1.40, 95% CI = 1.22–1.61, *P* < 0.00001). There was no association between seropositivity against *G. duodenalis* with socioeconomic variables such as crowding, education level, or household characteristics. In addition, similar seroprevalence was documented among regions of the country with different level of development (*P* = 0.81) (Figure 1, Table 1).

Antibody response. The magnitude of the IgG response against *G. duodenalis* was analyzed including positive and negative serum samples. The mean optical density value obtained by ELISA was compared among the six age groups (Figure 3). The mean titer of IgG antibody against *G. duodenalis* also increased with age up to those 40–59 years of age. Similar results were obtained when only seropositive samples were analyzed.

### DISCUSSION

In this seroepidemiologic study, we documented a seroprevalence of 55.3% for *G. duodenalis* in Mexico. Similar rates of seropositivity were found in the four regions with different economic development, which suggests that giardiasis is endemic throughout Mexico. The data obtained in this study indicate that seroconversion occurs at an early age because 34% of children 5–9 years of age were seropositive. It was also evident that in Mexico the seroprevalence increases with age, reaching the highest seroprevalence (72%) during adulthood. The high seroprevalence against *G. duodenalis* in children is consistent with data reported in several studies. A seroprevalence of 51% was found in children 3–8 years of age living on the Indian reservation in Arizona. In addition, in rural areas of Panama. A seroprevalence of 51% was documented in eight-year-old children.

Mean titers of IgG antibodies against *G. duodenalis* also increased with age, with a mean ± SD optical density of 0.241 ± 0.042 in children 1–4 years of age and a value of 0.379 ± 0.042 in adults 40–59 years of age (*P* < 0.0001). In countries where giardiasis is endemic, infection is acquired at an early age, but re-infection could be frequent later in life. This finding may explain the high IgG antibody levels documented in this study in the adult population. This result is supported by studies from Peru that reported frequent asymptomatic re-infections after treatment.

Several studies have evaluated the association between sociodemographic factors and *G. duodenalis* infection; however, the risk factors that are consistently found are age and water supplies. When the association between socioeconomic and demographic factors with *G. duodenalis* seropositivity was analyzed, only age and sex were significant risk factors. Seroprevalence was higher in males (58.6%) than in females (50.2%). In addition, when we selected randomized samples to have the same number of serum samples from men and women, the results were the same. However, this difference, which has been also documented in other studies, until now remains unexplained. Nevertheless, one possibility is that males have a higher rate of exposure to the parasite because of work or other activities. The similar high seroprevalence in the four development regions reflects the endemic character of the infection in Mexico. However, the lack of an association between seropositivity against *G. duodenalis* and geographic regions with specific development levels might be caused by the multiplicity of either the variables

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. (%) seropositive</th>
<th>OR‡</th>
<th>95% CI*</th>
<th><em>P</em>†</th>
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<tbody>
<tr>
<td>Age, years</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4</td>
<td>165 (17.5)</td>
<td>1.00</td>
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<tr>
<td>5–9</td>
<td>534 (34.0)</td>
<td>2.42</td>
<td>1.53–3.86</td>
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<tr>
<td>10–19</td>
<td>949 (51.1)</td>
<td>4.90</td>
<td>3.16–7.64</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>20–39</td>
<td>1,018 (63.2)</td>
<td>8.04</td>
<td>5.19–12.53</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>40–59</td>
<td>514 (72.9)</td>
<td>12.65</td>
<td>7.93–20.28</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>≥ 60</td>
<td>281 (71.2)</td>
<td>11.58</td>
<td>7.01–19.22</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>Sex‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1,381 (50.25)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2,078 (58.61)</td>
<td>1.40</td>
<td>1.22–1.61</td>
<td>&lt; 0.00001</td>
</tr>
<tr>
<td>Level of development</td>
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<td></td>
</tr>
<tr>
<td>High</td>
<td>921 (55.04)</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medium high</td>
<td>1,017 (54.37)</td>
<td>0.95</td>
<td>(0.77–1.18)</td>
<td>0.65</td>
</tr>
<tr>
<td>Medium low</td>
<td>821 (55.54)</td>
<td>0.91</td>
<td>(0.75–1.11)</td>
<td>0.34</td>
</tr>
<tr>
<td>Low</td>
<td>702 (56.69)</td>
<td>0.94</td>
<td>(0.76–1.15)</td>
<td>0.50</td>
</tr>
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</table>

* OR = odds ratio; CI = confidence interval.
† Estimated by chi-square test for linear trend.
‡ No data were available for two cases.
used to construct these indexes or the route of transmission of this disease.

The quantification of antibodies by ELISA has been demonstrated to be useful for determining seroprevalence against *G. duodenalis*. Results of several studies are more consistent than those obtained by stool examination.6,13,15,16,20–24,37,40 This study is one of the largest community-based serosurveys of *G. duodenalis* in Mexico. Few studies have analyzed sera from one country with personal information that was representative of sex, all age groups, and socioeconomic factors. Therefore, data obtained in this study provide important information regarding the global status of giardiasis in Mexico, demonstrate its endemnicity, and indicate that exposure to the parasite occurs at an early age. The main risk factors for seropositivity were age and sex. No other risk factors were found. This study provides data that will be useful in future epidemiologic studies of giardiasis.

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