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Original paper

# *Entamoeba histolytica* infections in a slum community in Manila, Philippines as detected by stool ELISA

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**ABSTRACT.** This study aimed to determine the prevalence of *Entamoeba histolytica* in BASECO, an urban slum community situated in Manila Harbor, Manila, Philippines using stool enzyme-linked immunosorbent assay (ELISA). It also aimed to determine if age, sex, and geographic location are contributory factors to the prevalence of *E. histolytica*. Stool samples were collected from 627 urban slum community residents of BASECO. Samples were viewed under light microscopy and the different parasites observed were identified. Stool ELISA was done using *E. histolytica* II antigen detection kits (TECHLAB®). Using *E. histolytica* II kits, *E. histolytica* had a prevalence of 9.09% (5/55) among the microscopically-positive samples for *E. histolytica/E. dispar* indicating a greater prevalence for the nonpathogenic species. No significant difference was observed in the prevalence of infection across all three variables: age, sex and geographic location. The overall prevalence of *E. histolytica* in BASECO, Manila, Philippines is 0.797% (5/627) which is lower than previous studies done on estimating the prevalence of *E. histolytica* using various techniques.

**Keywords:** *Entamoeba histolytica*, stool ELISA, prevalence, Manila, Philippines

## Introduction

The causative agent of amoebosis is the enteric dwelling protozoan parasite *Entamoeba histolytica,* which is a major cause of diarrhea and liver abscess in developing countries [1]. Infection primarily occurs via ingestion of food or water contaminated with cysts; however, transmission may also occur through oral and anal sex. Amoebosis is endemic in developing countries with poor sanitation and low socio-economic status, and occurs sporadically in developed countries involving special groups such as travelers to locations where *E. histolytica* is endemic, homosexual males, and immuno-compromised patients [2]. In 2016, a total of 26,748 deaths occurred in 195 countries due to amoebosis, with 4,567 and 9,673 deaths among children younger than 5 years old and among 70 years or older, respectively [3]. The prevalence rates vary among different countries and localities depending on the different socio-economic conditions and the accuracy of diagnostic methods utilized in the region [4].

In 1993, the species morphologically-identical to *E. histolytica*, *Entamoeba dispar,* was confirmed to be different based on genetic, immunological, and biochemical analyses [5]. It is considered to be nonpathogenic and commensal species of *Entamoeba*. A study reported an increased frequency of this organism, resulting in overestimation of amoebosis,

which has clinical and epidemiological implications [6]. Another study confirmed these findings by observing gross overestimations of *E. histolytica* prevalence rates using light microscopy alone. *E. histolytica* is differentiated from *E. dispar* using polymerase chain reaction (PCR) of a variable region of 16S ribosomal DNA and through ELISA to determine the presence of *Entamoeba*-specific Gal/GalNAc lectin [7].

Various techniques have been developed to properly diagnose *E. histolytica*. Antigen detection using stool ELISA has been utilized by various studies and noted its rapidity and comparable sensitivity to stool culturing techniques [8]. Antigen detection of Gal/GalNAc lectin in stool samples demonstrates good sensitivity and specificity in detecting *E. histolytica* in asymptomatic intestinal infections, which renders it excellent for community surveys for estimating the prevalence of *E. histolytica* [9].

Due to overestimations and inaccuracies of *E. histolytica* infections using light microscopy as a diagnostic tool, prevalence studies using more sensitive and specific diagnostic techniques should be employed to contribute to the overall knowledge of the prevalence of *E. histolytica* infections in the Philippines.

This study aimed to determine the prevalence of *E. histolytica* in BASECO, an urban slum community situated in Manila Harbor, Manila, Philippines. Furthermore, age, sex and geographical location were determined if they were contributory factors to the prevalence of *E. histolytica* infections in the community.

## Materials and Methods

*Study population and sample collection*

Stool samples were collected from 627 slum community residents of BASECO, Manila Harbor, located in the western region of Manila, Philippines. BASECO is divided into 18 blocks, as referenced from a similar study by Yason and Rivera in 2007 [10], with houses made from recycled building materials. The absence of sewerage and canal systems resulted in increased transmission of helminthic and protozoal infections amongst its residents. Written informed consent was obtained from the participants, and alternatively, from parents, if unable to give consent prior to this study. The protocol used in this study was approved by the Ethical Committee for Human Studies of the Institute of Tropical Medicine, Nagasaki University, Japan. The specimens were transported to the laboratory within 6 h after collection for processing and examination.

*Preparation and microscopic examination of specimens*

Thumb-sized stool samples were subjected to formol-ethyl acetate concentration technique to increase the sensitivity for detection of low-intensity helminth and protozoal infections [11]. The samples were then viewed under light microscopy and the different parasites observed were identified and noted.

*Stool ELISA*

Stool samples were also subjected to *E. histolytica* II antigen detection kits (TECHLAB®). Optical densities (OD) were read at 450 nm using a Spectronic ELISA reader. A sample was considered positive for *E. histolytica* if the difference of the OD between the sample and a negative control was > = 0.050.

*Statistical analysis*

Statistical analysis was performed using STATA to determine the odds ratio (that measures the strength of association between two variables) and confidence interval (95%) for the prevalence of *E. histolytica* infections using stool ELISA across different age group, sex, and geographic distribution based on blocks in the BASECO compound. The differences were considered significant if *P*-value was < 0.05.

## Results

*Characteristics of study population*

A total of 627 stool samples were examined from 493 males and 134 females. The individuals sampled primarily consisted of children younger than 10 years of age making up 77.51% of the sample population. Microscopic examination revealed that 55 individuals were infected with *E. histolytica/E. dispar*, with a prevalence of 8.77%. Meanwhile, *Trichuris trichiura* was the most prevalent amongst helminth infections in the sampled population (69.70%), and *Endolimax nana* amongst protozoal infections (17.07%). Table 1 summarizes the different parasites identified alongside their prevalence. Rare protozoal and helminthic infections would include *Iodamoeba*

Table 1. Prevalence of parasitic infections in the study population based on microscopy

| Parasite | Parasite count (n=627) | Prevalence (%) |
|---|---|---|
| *Trichuris trichiura* | 437 | 69.70 |
| *Ascaris lumbricoides* | 329 | 52.47 |
| *Endolimax nana* | 107 | 17.07 |
| *Entamoeba coli* | 89 | 14.19 |
| *Giardia lamblia* | 72 | 11.48 |
| *E. histolytica/E. dispar* | 55 | 8.77 |
| Hookworm | 44 | 7.02 |
| *Blastocystis* sp. | 29 | 4.63 |
| *Entamoeba hartmanni* | 3 | 0.48 |
| *Hymenolepis nana* | 1 | 0.16 |
| *Enterobius vermicularis* | 1 | 0.16 |
| *Iodamoeba butschlii* | 1 | 0.16 |
| *Strongyloides stercoralis* | 1 | 0.16 |

*butschlii*, *Hymenolepis nana*, *Enterobius vermicularis*, and *Strongyloides stercoralis* with a prevalence of only 0.16% per parasite.

*Prevalence and age, sex, and geographic distribution of E. histolytica infections in the study area*

Using *E. histolytica* II kits, *E. histolytica* had a prevalence of 9.09% (5/55) among the microscopically-positive samples for *E. histolytica/ E. dispar* indicating a greater prevalence for the nonpathogenic species. Interestingly, all stool ELISA-positive samples were also positive using light microscopy. The overall prevalence of *E. histolytica* was estimated to be 0.797% (5/627).

The age-specific prevalence for *E. histolytica* is presented in table 2. There were no observed statistically significant differences between the prevalence rates of *E. histolytica* across different age groups. Individuals aged 0–10 years old had prevalence rate of 0.62%. Those aged between 31 and 40 years old had a prevalence rate for *E. histolytica* of 2.56%, while those aged 41–50 had an age-specific prevalence of 6.67%. Furthermore, it was noted that the difference between the prevalence of *E. histolytica* for males is 0.61% and females, 1.49% (Tab. 3).

This cross-sectional study on the prevalence of *E. histolytica* was implemented in an urban slum community in Manila, Philippines. The community is arbitrarily divided into three regions: the southwestern area (Blocks 4, 5, 6, 7, 8, 11, 13), the southern area (Blocks 14 and 18), and the eastern

Table 2. Age distribution of *Entamoeba histolytica* positive samples as determined by stool ELISA

| Age | Sample size | *E. histolytica* positive | Prevalence (%) | Odds Ratio | CI (95%) |
|---|---|---|---|---|---|
| 0–10 | 486 | 3 | 0.62 | 1.00 | – |
| 11–20 | 36 | 0 | 0 | 0 | 0–17.77 |
| 21–30 | 40 | 0 | 0 | 0 | 0–15.96 |
| 31–40 | 39 | 1 | 2.56 | 4.26 | 0.791–54.302 |
| 41–50 | 15 | 1 | 6.67 | 11.5 | 0.204–151.589 |
| 51–60 | 8 | 0 | 0 | 0 | 0–86.729 |
| 61–70 | 2 | 0 | 0 | 0 | 0–408.833 |
| 71 | 1 | 0 | 0 | 0 | – |

Table 3. Sex distribution of *Entamoeba histolytica* positive samples as determined by stool ELISA

| | Population | *E. histolytica* | Prevalence (%) | Odds Ratio | CI (95%) |
|---|---|---|---|---|---|
| Male | 493 | 3 | 0.61 | 1.00* | |
| Female | 134 | 2 | 1.49 | 2.47 | 0.204–21.79 |

Table 4. Geographic distribution of *Entamoeba histolytica* positive samples as determined by stool ELISA

| Block | Sample size | *E. histolytica* positive | Prevalence (%) | Odds Ratio | CI (95%) |
|---|---|---|---|---|---|
| Southwestern Area | | | | | |
| 4 | 6 | 0 | 0 | | |
| 5 | 93 | 1 | 1.08 | | |
| 6 | 74 | 1 | 1.35 | | |
| 7 | 109 | 0 | 0 | | |
| 8 | 20 | 0 | 0 | | |
| 11 | 19 | 0 | 0 | | |
| 13 | 4 | 0 | 0 | | |
| Total | 325 | 2 | 0.62 | 1.00 | – |
| Southern Area | | | | | |
| 14 | 37 | 0 | 0 | | |
| 18 | 22 | 0 | 0 | | |
| Total | 59 | 0 | 0 | 0 | – |
| Eastern Area | | | | | |
| 15 | 59 | 0 | 0 | | |
| 16 | 20 | 2 | 10 | | |
| 17 | 164 | 1 | 0.61 | | |
| Total | 243 | 3 | 1.23 | 2.019 | 0.229–4.312 |

area (Blocks 15, 16, and 17), with each area containing households separated in different blocks. No sampling was done in households in the northern area of BASECO. *E. histolytica* was commonly found in Block 16 with a prevalence of 10.00%. Blocks 4, 7, 8, 11, 13, 14, 15, and 18 did not have any reported cases of *E. histolytica* (Tab. 4).

## Discussion

This study estimated the prevalence of *E. histolytica* infections using stool ELISA in an urban slum community in the Philippines to be 0.797%, which was found to be slightly higher using polymerase chain reaction (PCR) with prevalence estimate reported in a previous study by Rivera et al. [12] in the same community (0.358%) and lower than in Northern Philippines (0.961%) [13]. The comparability of prevalence estimates between studies using stool ELISA and PCR is valid since there is a strong correlation between prevalence estimates between the two techniques [9]. A study in Turkey estimated the prevalence of *E. histolytica* using stool ELISA to be 7.72% [14]. On the other hand, a prevalence survey in a rural community in South Africa demonstrated 4.1% *E. histolytica* positivity using PCR [15].

This present study demonstrates the comparable sensitivity of the antigen detection approach compared to other field studies. This is the first application of stool ELISA antigen detection techniques on estimating *E. histolytica* prevalence in the Philippines particularly in an urban slum community. The age-, sex-, and geography-specific prevalence were also estimated across different groups. Ultimately, no significant difference was observed in the prevalence of infection across all three variables. The lack of significant differences across age groups may be attributed to an uneven distribution of samples with a greater number of samples from younger populations. However, a decrease in infection should be expected with age

due to decreased exposure to modes of parasitic infection. This hypothesis is confirmed by high prevalence estimates among schoolchildren [16].

It is interesting to note that there is an increased prevalence of *E. histolytica* infections in the eastern area of BASECO while the southern part of the community reported no cases of infection. Risk factors leading to the observed increased prevalence include poor sanitation, population density, and a lack of potable water. The eastern region is landlocked, with a swamp on one side and landfill on the other, contrary to other two regions, which are bordered by the sea on one side.

In conclusion, the overall prevalence of *E. histolytica* in BASECO, Manila, Philippines is 0.797%. This estimate is lower than previous studies done on estimating the prevalence of *E. histolytica* using various techniques. However, further assessment of *E. histolytica* infection with greater community sample sizes should elucidate further on the epidemiology of *E. histolytica* in the Philippines. Selection of a more uniformly distributed population in terms of its demographics is the key.

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