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Original paper

Prevalence of *Entamoeba histolytica* and *Entamoeba dispar* among residents of an urban slum area in Manila, Philippines as detected by the polymerase chain reaction

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ABSTRACT. This study aimed to determine the prevalence of *Entamoeba histolytica* and *Entamoeba dispar* infections among residents in BASECO compound, Manila, Philippines using polymerase chain reaction (PCR). Formalin-ether concentration technique (FECT)-treated stool samples were examined under the light microscope to determine the presence of *Entamoeba*, helminths and other protozoan parasites. DNA was directly extracted from the FECT-treated samples and was subjected to PCR to determine *E. histolytica* and *E. dispar* infections. In this study, stool samples were collected from 2,232 residents of BASECO compound. Microscopic examination of FECT concentrated samples found 38 samples (1.703%) positive for *E. histolytica/E. dispar*. The *E. histolytica/E. dispar* microscopically positive samples were further analyzed by PCR and found 8 samples (0.358%) infected with *E. histolytica* and 23 samples (1.030%) infected with *E. dispar*. No statistically significant difference was observed in the sex distribution, while statistically significant difference was observed among the age group and area distribution of both the *Entamoeba* species. The results demonstrate PCR using DNA extracted from the formalin-fixed stools as an effective epidemiologic detection method of *E. histolytica* and *E. dispar* infections.

Keywords: *Entamoeba histolytica*, *Entamoeba dispar*, prevalence, polymerase chain reaction, slum area, Philippines

Introduction

Entamoeba histolytica is the known causative agent of human amoebosis, it is responsible for up to 100,000 deaths worldwide each year [1,2]. The infection with this intestinal protozoan parasite is common in developing countries more particularly in places with low socioeconomic conditions, overcrowded areas, poor sanitation and unhygienic practices. Previous epidemiologic studies on the prevalence of *E. histolytica* infection were done using microscopic examination. But since the pathogenic *E. histolytica* and the non-pathogenic *E. dispar* are morphologically indistinguishable, the previous prevalence data are questionable [3]. Several studies aimed to establish clear genetic distinction of the two species into pathogenic species or non-pathogenic species [4]. This led to the use of molecular biology techniques, such as the polymerase chain reaction (PCR) to distinguish the two amoeba species [5–8].

In previous studies, successful extraction of DNA from formalin-ether concentration technique (FECT)-treated stool samples for PCR was

demonstrated which was then used for accurate rapid diagnosis of both *E. histolytica* and *E. dispar* infections in Pampanga, Philippines [6] and the northern Philippines [7]. This paper reported on the application of this method to document the prevalence of *E. histolytica* and *E. dispar* infections among residents in the BASECO compound, a slum area near the Manila Harbor, Philippines.

Materials and Methods

Study population and sample collection

Stool samples were collected from 2,232 residents of BASECO Compound, a slum area found adjacent to Manila Harbor, Philippines. This compound is bordered by the sea on one side and a swamp on the other. It is divided into 18 blocks but for this study, the samples were collected from only 17 blocks as referenced from a similar study of Yason and Rivera [9]. At the time of collection, the residents were informal settlers and they had no sewerage, no drainage system and no sanitary toilets. Most obtain their water from deep wells and public faucets installed in each block. During sample collection, data such as age, sex and stool consistency were noted.

Ethical clearance

The protocol used in this study was approved by the Ethical Committee for Human Studies of the Institute of Tropical Medicine, Nagasaki University, Japan.

Formalin-ether concentration technique (FECT)

Formalin-ether concentration technique (FECT) was done to concentrate helminthic ova and protozoa as described by Beaver et al. [10] and as done by Yason and Rivera [9]. Each of the stool sample collected was homogenized with 5 to 10 ml formalin in a 15-ml tube. It was then centrifuged at 5,000 rpm for 5 min. The aqueous layer was discarded while the pellet was resuspended in 3-ml distilled water and 3-ml ether. The mixture was then mixed vigorously and was centrifuged at 5,000 rpm for 5 min. The pellet obtained was washed four times using 1× phosphate buffered saline (PBS) before transferring to a clean microfuge tube. The concentrated samples were examined under light microscope and stored at 4°C until use. Aside from *Entamoeba*, helminths and other protozoan parasites found were noted.

Genomic DNA extraction and PCR

Genomic DNA was extracted from FECT-concentrated *Entamoeba* cysts by the method described by Tachibana et al. [11] and used by Rivera et al. [6,7]. The pellet obtained from FECT was washed thrice with PBS and was then resuspended in TE buffer (100 mM Tris, pH 8.0, and 25 mM EDTA). About 50 µl of the suspension was subjected to freezing at -80°C for 10 min. and thawing at approximately 75°C for 2 min. This was done six times. After the last treatment, 200 µl of 1% Triton X-100 was added to the solution and was then heated in 98°C water bath for 10 min. The solution was mixed with 25 µl of Proteinase K (10 mg/ml) and 175 µl 4% lysis buffer and was then incubated at 55°C for 2 h then at 60°C for 1 h. After incubation, 1:1 volume of phenol-chloroform was added. The mixture was placed in the shaker for 30 min., then centrifuged at 12,500 rpm for 10 min. The aqueous phase was obtained and 1:1 volume of phenol-chloroform was added. The mixture was placed in the shaker for 15 min and was then centrifuged at 12,500 rpm for 5 min. Two (2) ml of 95% ethanol and 0.1 ml 3 M sodium acetate was mixed with the aqueous phase. The mixture was then incubated for 2 h at -80°C or an alternative was to incubate it overnight at -20°C. It was then centrifuged at 12,500 rpm for 10 min. and the supernatant was decanted. One (1) ml of 70% ethanol was added, the mixture was centrifuged at 12,500 rpm for 5 min. and the supernatant was decanted. The pellet obtained was then air-dried and was resuspended in 20-50 µl TE buffer. PCR was then carried out using primers specific for *E. histolytica*, p11 plus p12, and for *E. dispar*, p13 plus p14, as previously described [5].

Statistical analysis

Data was analysed using Epi Info 3.5.4 and Mantel-Haenszel procedure was used to compute for the odds ratio [12].

Results

Microscopic examinations

The prevalence of the parasites found in the samples is shown in Table 1. The collected stool samples were analysed in the study in which 38 were found to be positive for *E. histolytica/E. dispar,* resulting in a prevalence of 1.703%. *E. coli* and *Endolimax nana* gave the highest prevalence rates of 16.846% and 16.308%, respectively, among

Table 1. Prevalence of intestinal protozoans and helminths in the study population based on microscopy

Species	Frequency	Prevalence (%)
Protozoa		
E. histolytica/E. dispar	38	1.703
E. hartmanni	4	0.179
E. coli	376	16.846
E. nana	364	16.308
G. lamblia	353	15.815
I. butschlii	2	0.0896
Blastocystis sp.	271	12.142
T. hominis	58	2.599
Helminths		
A. lumbricoides	1205	53.987
E. vermicularis	5	0.224
Hookworm	139	6.228
S. stercoralis	4	0.179
T. trichiura	1375	61.604

all the protozoa found. Helminth eggs of *Ascaris lumbricoides* and *Trichuris trichiura,* on the other hand, were found in a large number of samples with the former having a prevalence rate of 53.987% and the latter, 61.604%.

A number of individuals infected with *E. histolytica/E. dispar* were found to be infected with other parasites as well (Table 2). *E. histolytica/E. dispar* was determined to be associated with all of the protozoa listed and with two helminths, *A. lumbricoides* and *T. trichiura.*

PCR

The 38 *E. histolytica/E. dispar* microscopically-positive samples were further analysed using PCR. Eight (8) were identified as *E. histolytica* while 23 were identified as *E. dispar*. Eight (8) were determined negative for either *E. histolytica* or *E. dispar* and one positive for both species. The PCR positive *E. histolytica* were found to be associated with five protozoa, *E. coli*, *E. nana*, *Giardia lamblia*, *Blastocystis* sp. and *Trichomonas hominis*, and to only one helminth, *T. trichiura*. PCR-positive *E. dispar,* on the other hand, were found to be associated with the same parasites as *E. histolytica* with the exception of *T. hominis.*

Age, sex and block distribution of PCR positive E. histolytica and E. dispar samples

Age-specific prevalence of PCR positive *E. histolytica* and *E. dispar* samples is shown in Table

Table 2. Prevalence of mixed infections of intestinal protozoans and helminths in the study population based on microscopy and polymerase chain reaction

Species	*E. histolytica/E. dispar* *(38)*	*E. dispar* *(23)*	*E. histolytica* *(8)*
Protozoa			
E. histolytica/E .dispar	–	–	–
E. hartmanni	1	1	0
E. coli	32	21	7
E. nana	23	15	5
G. lamblia	11	4	4
I. butschlii	1	0	0
B. hominis	16	7	4
T. hominis	5	2	2
Helminths			
A. lumbricoides	29	16	6
E. vermicularis	0	0	0
Hookworm	1	0	1
S. stercoralis	0	0	0
T. trichiura	34	20	8

Table 3. Age distribution of *Entamoeba histolytica* and *Entamoeba dispar* positive samples as determined by the polymerase chain reaction

Age group	Sample size	*E. histolytica* positive	Prevalance (%)	OR 95% CI	*E. dispar* positive	Prevalence (%)	OR (95% CI)
0–4	1112	0	0	0	3	0.270	1
5–14	778	6	0.771	1	10	1.285	4.813 (1.320–17.546)
15–24	73	0	0	0	1	1.370	5.134 (0.527–49.983)
25–34	144	0	0	0	6	4.167	16.072 (3.975–64.993)
35–44	70	2	2.857	3.784 (0.7493–19.11)	2	2.857	10.873 (1.787–66.164)
>45	55	0	0	0	1	1.818	6.846 (0.701–66.904)
Total	2232	8	0.358		23	1.030	

3. *E. histolytica* was found in only two age groups, 5–14 and 35–44 years of age with prevalence rates of 0.771% and 2.857% (OR = 3.784, 95%, CI = 0.7493–19.11), respectively. *E. dispar,* however, was found to be distributed among the age groups with 25–34 years of age having the highest prevalence rate of 4.167% (OR = 16.072, 95%, CI = 3.975–64.993). The prevalence rates of each group showed statistically significant difference among the other age groups for both of the two species.

Sex-specific prevalence rates of *E. histolytica* and *E. dispar* PCR-positive samples are shown in Table 4. It was found for both species that there is no statistically significant difference in the prevalence rate between males for *E. histolytica,* 0.279% and for *E. dispar,* 0.929%, and females for *E. histolytica,* 0.433% and for *E. dispar,* 1.126%.

The study area involves 17 out of the 18 blocks of the BASECO compound, Manila, Philippines. The prevalence rates of *E. histolytica* and *E. dispar* by block are presented in Table 5. Statistically significant difference among the prevalence rates of the different blocks were observed. *E. histolytica* is found to be most common in Block 5 with prevalence rate of 2.670% (OR = 4.808, 95%, CI = 0.556–41.567). *E. dispar,* on the other hand, is most common in Block 8 with prevalence of 3.660% (OR = 1.633, 95%, CI = 0.357–7.470).

Discussion

Intestinal protozoan parasite infection such as *E. histolytica* infection is a major problem in developing countries particularly in places with low socioeconomic conditions, overcrowded areas, poor sanitation and unhygienic practices [3]. This is because this parasite can be transmitted through faecal-oral route, either indirectly by consuming faecal contaminated food or water or by direct person-to-person contact such as diaper-changing or sexual practices [13]. In the Philippines, studies have reported prevalence of *E. histolytica* infections in vulnerable places such as the Smokey Mountain, an urban slum community. With the use of microscopy, 21% prevalence rate [14] and in Metro Manila among asymptomatic street children, 2.9% prevalence rate [15], were identified. However, microscopy proved to be an inaccurate detection

Table 4. Sex distribution of *Entamoeba histolytica* and *Entamoeba dispar* positive samples as determined by the polymerase chain reaction

Sex	Sample size	*E. histolytica* positive	Prevalence (%)	OR (95% CI)	*E. dispar* positive	Prevalence (%)	OR (95% CI)
Male	1077	3	0.279	1	10	0.929	1
Female	1155	5	0.433	1.557	13	1.126	1.215
Total	2232	8	0.358		23	1.030	

Table 5. Distribution of *Entamoeba histolytica* and *Entamoeba dispar* positive samples among different blocks as determined by the polymerase chain reaction

Block	Sample size	*E. histolytica* positive	Prevalence (%)	OR (95% CI)	*E. dispar* positive	Prevalence (%)	OR (95% CI)
1	176	1	0.568	1	4	2.27	1
2	40	0	0	0	0	0	0
3	103	0	0	0	1	0.971	0.422 (0.047–3.824)
4	98	0	0	0	1	1.02	0.443 (0.049–4.023)
5	187	5	2.67	4.808 (0.556–41.567)	3	1.60	0.701 (0.155–3.178)
6	156	2	1.28	2.273 (0.204–25.310)	2	1.28	0.558 (0.101–3.091)
7	111	0	0	0	2	1.80	0.789 (0.142–4.381)
8	82	0	0	0	3	3.66	1.633 (0.357–7.470)
9	96	0	0	0	2	2.08	0.915 (0.165–5.086)
10	39	0	0	0	0	0	0
11	112	0	0	0	1	0.893	0.387 (0.043–3.511)
12	39	0	0	0	0	0	0
13	225	0	0	0	1	0.444	0.192 (0.021–1.733)
14	292	0	0	0	1	0.342	0.148 (0.016–1.333)
15	180	0	0	0	0	0	0
16	146	0	0	0	0	0	0
17	150	0	0	0	2	1.33	0.581 (0.105–3.218)
Total	2232	8	0.358		23	1.03	

method for *E. histolytica* due to its morphological similarity to the non-pathogenic *E. dispar*. Studies establishing clear distinction between the two species were reported and from these findings more accurate and efficient methods for detection were developed. One of the simple and reliable methods developed for distinguishing *E. histolytica* from *E. dispar* is the use of PCR using DNA directly extracted from amoeba cysts obtained from the stool samples using FECT [6]. This method has been used to determine the true prevalence rate of *E. histolytica* in several places in Southeast Asia such as in the Thai/Myanmar border region [16], rural communities in Malaysia [17], northern Philippines [7] and Pampanga, Philippines [6].

This study aimed to determine the prevalence rates of *E. histolytica* and *E. dispar* in BASECO Compound, Manila using the PCR method that was applied in previous studies [6,7]. BASECO compound is an urban slum area near the Manila Harbor composed of depressed communities with no sanitary toilets, no sewerage and no drainage system. It was only in 2008 that this area received regular water supply from Maynilad Water Services Inc. A total of 2,232 stool samples were collected from the residents with ages ranging from <1 year old to 69 years old. Through microscopic examination, several protozoan and helminthic parasites were observed in the samples. *E. histolytica/E. dispar* was detected microscopically in 38 of the samples giving a prevalence rate of 1.703%. The *E. histolytica/E. dispar* microscopically positive samples were analysed using PCR and it was found that out of the 38 samples, 8 were *E. histolytica* and 23 were *E. dispar*. One sample showed mixed infection of the two species while 8 were negative for both species. These eight samples may be another *Entamoeba* species, *E. moshkovskii*, which is morphologically similar to both *E. histolytica* and *E. dispar* [3,18]. This shows the

selectivity and accuracy of the PCR method in the detection of the two species. Results showed that *E. histolytica* PCR-positive samples belonged to only two age groups, 5–14 and 35–44 years old. This may be because these two groups are more exposed to the poor sanitation of the BASECO compound, with the 5–14 years more playful and active while the 35–44 years old whose means of living may be within the compound. *E. dispar,* on the other hand, was present in all age groups, with 5–14 years old having the most number of positive samples but with 25–34 years old having the highest prevalence rate. Age group 25–34 has a higher prevalence rate for *E. dispar* than the exposed 5–14 years old due to its lower population number. It is also interesting to note that the *E. histolytica* PCR-positive samples belonged to three blocks within the compound, Blocks 1, 5 and 6 with Block 5 having the highest number and prevalence rate. This may be due to several factors, such as congestion and water source of each block. *E. dispar* PCR-positive samples were present in almost all blocks with the exception of Blocks 2, 10, 12, 15 and 16. These blocks were also negative for *E. histolytica*.

This study shows the application of the PCR method in detecting *E. histolytica* and *E. dispar* directly from the stool samples. This method can be used for a more accurate epidemiologic data on the prevalence of the pathogenic *E. histolytica* in vulnerable areas in the Philippines and other developing countries which will be essential in planning prevention and control of the infection. It can also be used to obtain epidemiologic studies regarding the non-pathogenic *E. dispar* to determine its association with the pathogenic species, other protozoan and helminthic parasites and its nature in the hosts.

In conclusion, the results showed the prevalence of *E. histolytica* and *E. dispar* in the BASECO compound, Manila. Further studies that include other variables such as means of living, type of settlements and sanitation practices are recommended to better document the epidemiology of the *Entamoeba* infections in this urban slum area.

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References

[1] World Health Organization. 1997. *Entamoeba* taxonomy. *Bulletin of the World Health Organization* 75: 291-294.

[2] Troeger C., Blacker B.F., Khalil I.A., Rao P.C., Cao, S., Zimsen S.R., Albertson S.B., Stanaway J.D., Deshpande A., Abebe Z., Alvis-Guzman N. 2018. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Infectious Diseases* 18: 1211-1228. https://doi.org/10.1016/s1473-3099(18)30362-1

[3] Nozaki T., Bhattacharya A. 2014. Amebiasis: biology and pathogenesis of *Entamoeba*. Springer.

[4] Wilson I.W., Weedall G.D., Hall N. 2012. Host-parasite interactions in *Entamoeba histolytica* and *Entamoeba dispar*: what have we learned from their genomes? *Parasite Immunology* 34: 90-99. https://doi.org/10.1111/j.1365-3024.2011.01325.x

[5] Tachibana H., Kobayashi S., Takekoshi M., Ihara S. 1991. Distinguishing pathogenic isolates of *Entamoeba histolytica* by polymerase chain reaction. *Journal of Infectious Diseases* 164: 825-826. doi:10.1093/infdis/164.4.825

[6] Rivera W.L., Tachibana H., Silva-Tahat M.R.A., Uemura H., Kanbara H. 1996. Differentiation of *Entamoeba histolytica* and *E. dispar* DNA from cysts present in stool specimens by polymerase chain reaction: its field application in the Philippines. *Parasitology Research* 82: 585-589. doi:10.1007/s004360050169

[7] Rivera W.L., Tachibana H., Kanbara H. 1998. Field study on the distribution of *Entamoeba histolytica* and *Entamoeba dispar* in the Northern Philippines as detected by the polymerase chain reaction. *American Journal of Tropical Medicine and Hygiene* 59: 916-921. doi:10.4269/ajtmh.1998.59.916

[8] Gonin P., Trudel L. 2003. Detection and differentiation of *Entamoeba histolytica* and *Entamoeba dispar* isolates in clinical samples by PCR and enzyme-linked immunosorbent assay. *Journal of Clinical Microbiology* 41: 237-241. https://dx.doi.org/10.1128/JCM.41.1.237-241.2003

[9] Yason J.A.D.L., Rivera W.L. 2007. Genotyping of *Giardia duodenalis* isolates among residents of slum area in Manila, Philippines. *Parasitology Research* 101: 681-687. doi:10.1007/s00436-007-0533-8

[10] Beaver P.C., Jung R.C., Cupp E.W. 1984. Clinical Parasitology. 9th ed., Lea and Febiger, Philadephia, USA.

[11] Tachibana H., Cheng X.-J., Kobayashi S., Fujita Y., Udono T. 2000. *Entamoeba dispar*, but not *Entamoeba histolytica*, detected in a colony of chimpanzees in Japan. *Parasitology Research* 86: 537-541. doi:10.1007/s004360000205

[12] Mannocci A. 2009. The Mantel-Haenszel procedure. 50 years of the statistical method for confounders control. *Italian Journal of Public Health* 6: 338-340. doi:10.2427/5765

[13] Centers for Disease Control and Prevention. 2015. Parasites - Amebiasis - *Entamoeba histolytica* infection. General Information. [on-line publication], reviewed: July 20, 2015.http://www.cdc.gov/parasites/amebiasis/general-info.html

[14] Auer C. 1990. Health status of children living in a squatter area of Manila, Philippines, with particular emphasis on intestinal parasitoses. *Southeast Asian Journal of Tropical Medicine and Public Health* 21: 289-300.

[15] Baldo E.T., Belizario V.Y., De Leon W.U., Kong H.H., Chung D.I. 2004. Infection status of intestinal parasites in children living in residential institutions in Metro Manila, the Philippines. *Korean Journal of Parasitology* 42: 67-70. doi:10.3347/kjp.2004.42.2.67

[16] Intarapuk A., Kalambaheti T., Thammapalerd N., Mahannop P., Kaewsatien P., Bhumiratana A., Nityasuddhi D. 2009. Identification of *E. histolytica* and *E. dispar* by PCR assay of fecal specimens obtained from Thai/Myanmar border region. *Southeast Asian Journal of Tropical Medicine and Public Health* 40: 425-434.

[17] Ngui R., Angal L., Fakhrurrazi S.A., Lian Y.L.A., Ling L.Y., Ibrahim J., Mahmud R. 2012. Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase reaction (PCR) in rural communities in Malaysia. *Parasites and Vectors* 5: 187. doi:10.1186/1756-3305-5-187

[18] Shirley D.A.T., Farr L., Watanabe K., Moonah S., 2018. A review of the global burden, new diagnostics, and current therapeutics for amebiasis. In: *Open forum infectious diseases* 5: 7 (ofy161). US: Oxford University Press.

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