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RESEARCH PAPER

Prevalence and Associated Risk Factors of *Entamoeba histolytica, E. dispar* and *E. moshkovskii* Infection among Orang Asli Communities in Slim River, Perak

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Abstract

Entamoeba infection is still widespread in Malaysia's rural area particularly among Orang Asli communities which usually associated with poverty and lack of sanitation. Due to scarce information on these *Entamoeba* infections in Slim River, Perak we conducted this study to investigate the prevalence and associated risk factors towards this infection. A total of 55 stool samples from voluntary participants with and without symptoms of amoebiasis were collected and examined using PCR technique. PCR analysis showed 16.4% stool samples were detected positive for *Entamoeba* spp., discriminated as 7.3% that were positive for *E. histolytica* and 9.1% for *E. dispar*. No *E. moshkovskii* was detected at all. Factors such as indiscriminate defecation, improper sewage disposal and not washing hand after playing with soil or gardening showed significant association with *E. histolytica* infection*;* while gastrointestinal symptom such as vomiting was associated with *E. dispar* infection. In conclusion, the study reveals there is still an occurrence of *Entamoeba* spp. among Orang Asli communities in Slim River, Perak. This implies that good personal and hygiene practices should be enhanced through awareness strategy to control *Entamoeba* infections in Orang Asli communities in Malaysia.

Keywords Amoebiasis; *Entamoeba*; Orang Asli; epidemiology; *E. histolytica*; *E. dispar*

INTRODUCTION

A report by Lozano et al. (2012) stated that amoebiasis was caused by *Entamoeba histolytica* is a life-threatening public health issue which approximately infected 0.05 billion people worldwide and caused up to 0.1 million deaths per annum. However, this occurrence is misjudged due to the inability of microscopy examination to differentiate the species. The disease is usually found in areas with poor sanitary conditions and low socioeconomic status such as South and Central America, Africa, and Asia. According to Fotedar et al. (2007a), 90% of the infected individuals are asymtomatic carrier, while the other 10% manifest intestinal and extraintestinal amoebiasis. Humans are the only reservoir for *E. histolytica* and generally acquired through oral-genital or oral-anal contact (Garcia et al., 2016).

Laboratory diagnosis of *E. histolytica* is still mainly depending on the microscopic examination using unstained and stained as gold standard reference method. However, this method poses many problems because the morphologies of pathogenic and the other two common non-pathogenic species (*E. dispar* and *E. moshkovskii*) under the microscope are indistinguishable, that subsequently lead to the misdiagnosis (Stanley, 2003; Garcia et al.,

2016). Likewise, stool culture with isoenzyme analysis can replace microscopic examination as a gold standard. However, this technique is time-consuming, laborious, and insensitive due to false-negative results (van Hal et al., 2007). As an alternative, the commercial ELISA antigen-based method is currently used to detect *Entamoeba* spp. due to its simplicity and rapid. Still, they varied in their diagnostic sensitivities and specificities (Fotedar et al., 2007a; Saidin et al., 2019).

In the past, the prevalence of amoebiasis varies based on geographical region and diagnostic method being used in the studies (Norhayati et al., 2003). However, reliable data were only obtained when researchers started to use molecular tools which were able to differentiate between species at the DNA level (Burch et al., 1991; Tannich et al., 1992; Clark and Diamond, 1993). A few studies reported the use of polymerase chain reaction (PCR) methods in detecting *E. histolytica* infection. A study among children in Gaza, Palestine conducted by Al-Hindi et al. (2005) reported higher prevalence rates of *E. histolytica* (69.6%) than *E. dispar* (22.8%), while 18.8% and 2.1% of samples from hospitals and primary schools in South Africa were detected positive for *E. histolytica* and *E. dispar*, respectively (Samie et al., 2006). In addition, few research in Mexico and Venezuela have reported the infection of *E. histolytica* is more common than *E. dispar* (Ramos et al., 2005; Rivero et al., 2009). Studies from different region of Malaysia, showed higher prevalence rates of *E. histolytica* (13.2-76%) and *E. dispar* (5.6-13.2%), when using PCR assay on microscopy positive stool samples from Orang Asli community in Perak, Selangor, Pahang and Negeri Sembilan and Johor (Noor Azian et al., 2007; Ngui et al., 2012; Lau et al., 2013).

Accurate diagnosis through the determination of true prevalence is very crucial for better management and prevention of amoebiasis, especially among the vulnerable groups. To date, there is no epidemiological study has been carried out in the Southern region of Perak, specifically in Slim River district. Thus, we conducted a multiplex single-round PCR assay for the detection of the three morphologically identical *Entamoeba* spp. and determined its associated risk factors among Orang Asli communities in Slim River, Perak.

MATERIALS AND METHODS

Study population and sample size

This community-based study was carried out among Orang Asli communities in three villages, namely Kampung Orang Asli Pos Bersih, Kampung Orang Asli Ulu Rasau and Kampung Orang Asli Gesau in Slim River, Perak – between September 2018 to Mac 2019. These settlements were selected using a purposive sampling strategy from the available villages lists provided by the Department of Orang Asli Development (JAKOA).

The minimum sample size required in the present study was calculated based on Kish et al. (1968). At a 95% confidence level, absolute precision (d) = 0.05, and expected prevalence of *Entamoeba* spp. at 3.2% (Tengku and Norhayati et al., 2011), the expected number of participants for this study was 48 individuals. All villagers aged ≥2 years were invited to participate.

Ethical consideration

The study protocol was evaluated and approved by the Research Ethics Committee of Universiti Pendidikan Sultan Idris (Ref. No.: 2019-0003-01). Meanwhile, permission for the fieldwork was obtained from JAKOA [(Ref. No.: JAKOA/PP.30.052Jld12 (36)].

Questionnaire survey and stool sampling

Meeting and discussion were made with the head of the villagers or Batin and Orang Asli communities before the commencement of the study to handed detailed explanations of the aims and procedures. Upon agreement to participate, their consent was obtained in written form, or parents were approached for consent on behalf of their children (less than 12 years old). General socio-demographic, environmental factors, and clinical symptoms data were collected by interviewing all the subjects using a pre-designed questionnaire.

Following the administration of the questionnaire, a wide mouth screw-capped container pre-labelled with the individual's name and code was distributed to each participant for the collection of an early morning stool sample. A clear explanation of the procedure of stool collection and how to avoid possible contamination in the course of collection at home was provided to the participants. They were instructed to collect a thumb-sized stool sample using a provided scoop into the container, which then placed into a zip-locked plastic bag. Parents were instructed to monitor their children during the sample collection to ensure their stool samples were placed into the correct container.

Genomic DNA extraction

DNA from stool specimens (200-500 mg) were extracted using the QIAamp Fast DNA Stool Mini Extraction Kit (QIAGEN, Germany) according to the manufacturer's instructions. The extracted DNA was then kept at -20 °C until further use.

Detection and discrimination of *Entamoeba* spp.

Entamoeba specific primer, EntaF (5′-ATGCACGAGAGCGAAAGCAT-3′) and EhR-(5′-GATCTAGAAACAATGCTTCTCT-3′), EdR-(5′-CACCACTTACTATCCCTACC-3′) and EmR-(5′-TGACCGGAGCCAGAGACA T-3′)-specific primer described by Hamzah et al., (2006) were use as forward and reverse primer pair, to amplify a 166 base pair (bp), 752 bp and 580 bp region of *E. histolytica*, *E. dispar* and *E. moshkovskii* small subunit rRNA gene sequence, respectively. The reaction mixture contained 12.5 µL NexPro e-PCR 2x Master Mix (Genes Laboratories, Korea), 1 µL of 10 pmole of each primer and 10 µL of extracted DNA samples. Nuclease free water was added to a final volume of 25 µL. The PCR conditions were started with an initial denaturation step at 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 58 °C for 1 min, and 72°C for 1 min, with a final extension step at 72 °C for 7 min. Amplified products were visualized with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Korea) after electrophoresis on 1.5% agarose gels. DNA isolated from axenically grown *E. histolytica* HM-1:IMSS, synthesized *E. dipar* and *E. moshkovsii* DNA were used as the positive control. To confirm the species descriptions obtained by PCR, all of the targeted size amplicon were sequenced using respective primer pair and subjected to homology search using BLAST database. GenBank accession number of X56991, Z49256 and AF149906 for *E. histolytica*, *E. dispar* and *E. moshkovskii*, respectively, were used as reference sequences in the analyses. The phylogenetic analysis was performed using MEGA X (Kumar et al., 2018).

Data analysis

Data analysis were carried out using IBM SPSS Statistics, version 20.0 (IBM Corporation, NY). Prevalence of *E. histolytica*, *E. dispar* and *E. moshkovskii* were determined based on the molecular method. Only those subjects with complete questionnaire data and stool samples were included in the final analyses. *Entamoeba* spp. infection was assigned as a dependent variable while independent variables were demographic, socioeconomic, environmental as well as the clinical symptom. A Chi-squares test (X^2) was used to test the associations between *Entamoeba* spp. infections with the explanatory independent variables. The odds ratios (OR) and 95% confidence intervals were computed with the level of statistical significance was set as $P<0.05$.

RESULTS

General characteristics of the participants

A total of 55 individuals (38% male, 62% female) aged 2 and 74 years from the Semai tribe participated in this study. Approximately 69% of parents have less than 6 years of formal education in Malaysia. The majority of the parents were self-working in rubber plantation. Only 36% of participants had at least primary education, and about 12.7% of the families had more than eight members. Although 78% of the houses have a basic amenities such as a treated water supply and 49% have toilets, at least 22% are still using untreated water for domestic use, and 51% still defecate in the river or bush. Some household/participant (78%) kept dogs, cats, pigs, and poultry as their domestic animals. The details of the participants are presented in Table 1.

Table 1 Demographic and socioeconomic characteristics of the participants (*n*=55).

Characteristics	n (%)
Age groups (years)	
<15	17 (30.9)
15-24	4 (7.3)
25-44	20 (36.3)
45-74	14 (25.5)
Gender	
Male	21 (38)
Female	34 (62)
Socioeconomic status	
Father's education level (< 6 years)	44 (80)
Mother's education level (< 6 years)	46 (83.6)
Occupational status (working)	23 (41.8)
Low monthly household income (< RM500)	50 (91)
Large family (> 8 members)	7 (12.7)
Supplied with piped water	43 (78.2)

Presence of toilet at household	27 (49.1)

Prevalence and distribution of *Entamoeba* spp. infections

PCR products were detected in 16.4% (9/55) stool samples. *E. histolytica* was detected in 7.3% (4/55) of the samples with an approximate amplicon size of 166 bp, while *E. dispar* (amplicon size is 752 bp) was detected in 9.1% (5/55) of samples. All of the five *E. dispar* amplicons were 98-100% homologous to previously published sequences of *E. dispar* (accession number Z49256), and four *E. histolytica* sequences obtained were highly similar (99–100%) to the *E. histolytica* sequences in GenBank (accession number X56991). No evidence of the presence of *E. moshkovskii* among participants. The phylogenetic tree analysis of the representative of *E. histolytica* (M36-D-Eh) and *E. dispar* (M07-E-Ed) detected in the current study shared a common clade to reference sequences of *E. E. histolytica* and *E. dispar*, respectively (Figure 1a and b). Thus, the result showed the correct assignment of the *Entamoeba* species and in accordance with the PCR assay.

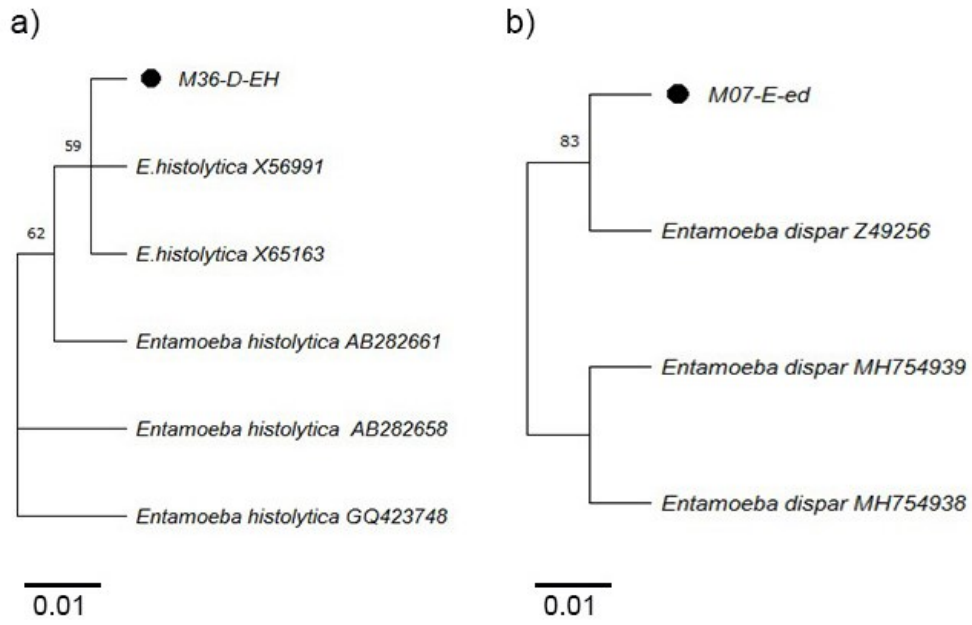

Figure 1 Phylogenetic relationship of a) *E. histolytica* and, b) *E. dispar* obtained in the study and Genbank retrieved. The amplified sequences are indicated by circle. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicate) are shown next to the branches (< 50 bootstrap values was not shown). The rate variation among sites was modeled with gamma distribution (shape parameter = 1).

Risk factor associated with *Entamoeba* spp. infection

Table 2 lists a univariate analysis of demographics and the prevalence of *Entamoeba* spp. infection. Based on the result, age groups and gender of participants were not significantly associated with the prevalence of *E. histolytica* and *E. dispar* infections. Of the four participants positive for *E. histolytica*, 12.5% were from individuals ≤ 15 years old. Whereas, 10.5% participants from people >15 are infected with *E. dispar*. It was observed that the prevalence rate of *E. dispar* increased from 6.7% to 10% with higher education level of the

participants. While, non-educated participants were 1.09 times (95% CI= 0.190, 6.351; p= 0.916) more likely to be infected with pathogenic species of *Entamoeba* as compared to educated participants. However, the difference was proved not to be statistically significant.

Through univariate analysis, we found that the socio-demographic factors such as occupational status, household monthly income, household members, marital status, type of water supply as well as present of toilet at household were independently connected to the infection. The results of the univariate analysis of various environmental factors associated with *Entamoeba* spp. infection rate are shown in Table 3. We observed participants who not washing hand after playing with soil or gardening (OR=3.922; 95% CI= 0.718, 21.42; p =0.000) more likely to be infected with *E. histolytica* as compared to those who washing their hand after gardening or playing with soil. Whereas, participants who defecate indiscriminately in the river or bush (OR= 2.588; 95% CI= 0468, 014.302; p< 0.011) and improper sewage disposal at outdoor (OR= 0.49; 95% CI= 0.371, 0.0.649; p= 0.049) more likely to be infected with *E. histolytica* as compared to *E. dispar*. Unfortunately, *E. dispar* infection was found to be not significantly associated with environment factors. Among other clinical factors, participants who have vomiting are most probably being infected with *E. dispar*. On the other hand, *E. histolytica* does not show any association either with diarrhea or other gastroenteritis symptoms such as nausea, abdominal pain, watery stool, and blood or mucus stool.

DISCUSSIONS

Amoebiasis is a common life-threatening parasitic disease globally, being the third ranked after malaria and schistosomiasis (Ximénez et al., 2011). *Entamoeba* consists of several species of intestinal protozoans that infect humans including *E.histolytica, E.dispar, E.morskovikii, E.coli, E.hartmani, E.polecki, E.bangladeshi, E.gingivalis, Iodamoeba butschlii* and *Endolimax nana* (Ali et al., 2005). Only *E. histolytica* is considered pathogenic since it invades the lumen epithelium causing amoebic dysentery (Sateriale and Huston, 2011). Several methods such as microscopy examination, culture, and zymodeme analysis, antigen-ELISA based format and molecular techniques are being used for detection and discrimination of *Entamoeba* spp. in stool specimens (Fotedar et al., 2007a; Saidin et al., 2019; Carrero et al., 2019). However, it is very challenging to determine the true prevalence of *Entamoeba* infection because much of the data reported are based on microscopy examination, which has poor sensitivity and specificity.

Due to its disadvantages, there is now a wide variety of PCR methods, targeting different genes for recognition and discrimination of the three *Entamoeba* species such as small subunit rRNA, 30 kDa protein gene, DNA highly repetitive sequences, haemolysin gene (HL Y6), cysteine proteinase, serine-rich *E. histolytica* (SREHP) gene, actin gene and tandem repeats in extrachromosomal circular DNA (Zindrou et al., 2001; Freitas et al., 2004). Among them, small subunit rRNA was mostly used to detect *Entamoeba* species in stool samples due to its presence in multiple copies of extrachromosomal plasmids and high genetic variation among *E. histolytica*, *E. dispar,* and *E. moshkovskii* (Bhattacharya et al*.,* 1989; Clark and Diamond, 1991; Cruz-Reyes et al*.,* 1992; Stensvold et al., 2011). Therefore, in this study, PCR based methods targeting small subunit rRNA was used to determine the true prevalence of *Entamoeba* spp. in the samples collected among Orang Asli community in Slim River district, Perak.

Table 2 Socio-demographic features of the participants and its association with *E. histolytica* and *E. dispar* infection (*n*=55).

Variables	*E. histolytica*				*E. dispar*			
	No. of examined	% infected	OD (95%, CI)	*P*-value	No. of examined	% infected	OD (95%, CI)	*P*-value
Age group								
<15	16	12.5	1.451 (0.537, 3.922)	0.339	17	5.9	1	0.580
>15	39	5.1	1		38	10.5	1.600 (0.265, 9.670)	
Gender								
Male	21	4.8	1	0.573	21	9.5	1.033 (0.489, 2.183)	0.930
Female	34	8.8	1.569 (0.278, 8.861)		34	8.8	1	
Marital status								
Single	19	10.5	1.333 (0.491, 3.261)	0.500	19	5.3	1	0.473
Married	36	5.6	1		36	11.1	1.800 (0.300, 10.798)	
Education								
Educated	15	6.7	1	0.916	40	10	1.400 (0.229, 8.542)	0.702
Non-educated	40	7.5	1.098 (0.190, 6.351)		15	6.7	1	
Occupation								
Working	22	9.1	1.216 (0.445, 3.319)	0.672	22	18.2	3.200 (0.548, 18.699)	0.056
Non-working	33	6.1	1		33	3.0	1	
Household monthly income								
<RM500	50	8.0	0.902 (0.824, 0.987)	0.511	50	8.0	1	0.373
>RM500	5	0.0	1		5	20	1.150 (0.736, 1.796)	
Household members								
<8	48	6.3	1	0.444	48	10.4	0.860 (0.769, 0.962)	0.370
>8	7	14.3	1.176 (0.662, 2.090)		7	0.0	1	
Type of water supply								
Safe	43	9.3	0.765 (0.657, 0.890)	0.273	43	9.3	1.100 (0.177, 6.851)	0.918
Unsafe	12	0.0	1		12	8.3	1	
Presence of toilet at household								
Yes	27	11.1	2.118 (0.380, 11.790)	0.282	27	3.7	1	0.172
No	28	3.6	1		28	14.3	2.600 (0.441, 15.312)	

CI, Confident interval; OR, Odd ratio; Reference group marked as OR=1.

Table 3 Univariate analysis of selected environmental factors and subject's clinical symptoms associated with *E. histolytica* and *E. dispar* infection among participants (n=55).

Variables	*E. histolytica*				*E. dispar*			
	No. of examined	% infected	OD (95%, CI)	*P*-value	No. of examined	% infected	OD (95%, CI)	*P*-value
Drinking untreated water								
Yes	45	8.9	0.804 (0.702, 0.921)	0.328	45	8.9	1	0.912
No	10	0.0	1		10	10.0	1.025 (0.649, 1.619)	
Bathing and washing in the river								
Yes	30	10.0	1.882 (0.336, 10.535)	0.394	30	10.0	1.150 (0.377, 3.505)	0.797
No	25	4.0	1		25	8.0	1	
Not washing hand after playing with soil or gardening								
Yes	4	75.0	3.922 (0.718, 21.42)	0.000[a]	4	25.0	1.175 (0.754, 1.831)	0.250
No	51	2.0	1		51	7.8	1	
Close contact with domestic animals								
Yes	43	4.7	1	0.156	43	9.3	1.100 (0.177, 6.851)	0.918
No	12	16.7	1.608 (0.598, 4.324)		12	8.3	1	
Indiscriminate defecation								
Yes	21	19.0	2.588 (0.468, 14.302)	0.011[a]	21	9.5	1.033 (0.489, 2.183)	0.930
No	34	0.0	1		34	8.8	1	
Sewage disposal								
Outdoor	29	13.8	0.49 (0.371, 0.649)	0.049[a]	29	13.8	2.500 (0.424, 14.748)	0.200
Common drainage	26	0.0	1		26	3.8	1	
Eating with hand								
Yes	55	7.3	[b]	[b]	55	9.1	[b]	[b]
No	0	0.0			0	0.0		
Consuming raw vegetables								
Yes	54	7.4	0.980 (0.943, 1.019)	0.777	54	9.3	0.980 (0.942, 1.020)	0.750
No	1	0.0	1		1	0.0	1	
Eating fresh fruits								
Yes	55	7.3	[b]	[b]	55	9.1	[b]	[b]
No	0	0.0			0	0.0		
Diarrhoea								
Yes	24	8.3	1.137 (0.415, 3.118)	0.790	24	8.3	1	0.863
No	31	6.5	1		31	9.7	1.100 (0.360, 3.365)	
Vomiting								
Yes	2	0.0	1	0.687	2	50.0	1.225 (0.789, 1.902)	0.040[a]
No	53	7.5	0.961 (0.909, 1.016)		53	7.5	1	

Nausea								
Yes	3	0.0	1	0.618	3	33.3	1.200 (0.771, 1.867)	0.133
No	52	7.7	0.941 (0.879, 1.008)		52	7.7	1	
Abdominal pain								
Yes	18	16.7	2.824 (0.512, 15.558)	0.061	18	5.6	1	0.525
No	37	2.7	1		37	10.8	1.700 (0.282, 10.234)	
Watery stool								
Yes	17	5.9	1	0.791	17	11.8	1.167 (0.558, 2.441)	0.645
No	38	7.9	1.255 (0.219, 7.187)		38	7.9	1	
Blood or mucus stool								
Yes	13	7.7	1.020 (0.568, 1.832)	0.947	13	7.7	1	0.841
No	42	7.1	1		42	9.5	1.200 (0.194, 7.414)	

CI, Confident interval; OR, Odd ratio; Reference group marked as OR=1.

[a] significant association ($P<0.05$)

[b] Cornfield 95% CI for odds ratio is not accurate due to low number

Our results showed the overall prevalence rate of *Entamoeba* spp. infection was 16.4%. *E. dispar* infection diagnosed by multiplex single round PCR proved to be higher compared with *E. histolytica*-that is, 9.1% of the examined stool samples. Conversely, *E. histolytica* was diagnosed in only 7.3%. This prevalence is significantly higher than that documented in the previous study conducted in Malaysia. As an example, these results were in accordance with a study among 500 Orang Asli stool samples across Peninsular Malaysia. High prevalence of *E. dispar* (13.4%), as compared to *E. histolytica* (3.2%) and *E. moshkovskii* (1%) was recorded using single-round PCR assay (Anuar et al., 2012). In 2016, *E. histolytica* and *E. dispar* were detected in Temuan and Mah Meri Orang Asli communities at 4.7% and 2.5%, respectively (Chin et al., 2016). A study conducted in Iran reported among 3,825 stool samples from symptomatic patients, 3.5%, 91.4%, and 3.5% were PCR-positive for *E. histolytica*, *E. dispar* and *E. moshkovskii*, respectively (Mojarad et al., 2010). In Australia, among microscopy positive stool samples at St. Vincent's Hospital, PCR analysis showed 70.8%, and 50% were positive for the non-pathogenic *E. dispar* and *E. moshkovskii* respectively (Fotedar et al., 2007b).

As regards to the risk factors, the study showed that the prevalence of *E. histolytica* and *E. dispar* infections was not significantly associated with age groups or gender. This study is in line with Anuar et al. (2012), Lopez et al. (2015), and Rivera et al. (1198), who found that no significant association between gender and the three *Entamoeba* species. By contrast, Calegar et al. (2016) found a positive correlation between the prevalence of *E. dispar* infection with the increased of ages among the studies population in the northeast region of Brazil. On the other hand, other studies reported a significant association of *E. histolytica* infection in younger children (Shetty et al., 1990; Waqar et al., 2003; Sayyari et al., 2005; Tasawar et al., 2010). Despite this, our findings showed that the prevalence rate of *E. histolytica* and *E. dispar* infection was not significantly associated with a low educational level, working status, and the absence of toilet at household. We also did not find any association between an *E. histolytica* infection with diarrhea and other gastroenteritis symptoms. From our observation, most of the positive *E. histolytica* participants are asymptomatic with no signs and symptoms of diarrhea and other gastroenteritis, but with the presence of *E. histolytica* trophozoites or cysts in their stool. This is in contrast to several reports from Pakistan and South Africa that showed *E. histolytica* commonly produces clinical symptoms in patients (Samie et al., 2006; Yakoob et al., 2012).

Interestingly, this study has identified different environmental factors associated with the *Entamoeba* spp. infection. It was found that those who do not wash their hands after playing with soil or gardening were at 3.92 higher risks of being infected with *E. histolytica*. The finding is relevant since *E. histolytica* is transmitted through faecal-oral transmission routes. In Vietnam, contaminated hands was one of the leading factor for transmission of infection, and the risk can be increased up to three folds if hands are not washed properly (Duc et al., 2011). This behaviour has been reported to be consistent and significantly associated with diarrhoea in Malaysia, Myanmar, Bangladesh, and Indonesia (Han et al., 1989; Knight et al., 1992; Hoque et al., 1999; Gasem et al., 2001). Moreover, we found that those indiscriminate defecation and having improper sewage disposal was 2.59 more likely of being infected compared to those having access to flush latrine. Based on our observation, 51% of the participants did not have proper sanitary facilities at their home, which bring them to defecate discriminately around their houses. Unfavourably, rivers are also preferred sites for defecation, particularly among Orang Asli children (Al-Mekhlafi et al., 2008; Elyana et al., 2016). Thus, it was not surprising that indiscriminate outdoors constituted a significant risk of being infected with *E. histolytica*. The present study also highlights that those were having gastroenteritis symptom such as vomiting

was at 1.22 higher risk of being infected with *E. dispar*. Although *E. dispar* is considered commensal for humans, evidence shows the Brazilian strain of *E. dispar* is pathogenic and can produce amoebic liver abscess under *in vivo* conditions (Clark and Diamond, 1997). Hence, this non-pathogenic species should be considered in the diagnosis of patients presenting with gastrointestinal symptoms, especially when other pathogens such as virus and bacteria are not detected.

There have been no *E. moshkovskii*-positive cases found in the collected samples in the current study. By contrast, a higher prevalence rate of *E. moshkovsii* infection (18.2%) was reported among rural communities in Taiz, Hodeidah, Sana'a and Dhamar province in Yamen (Al-Areeqi et al., 2017). In Australia, *E. moshkovskii* was identified in 61.8% of 89 PCR-positive samples (Fotedar et al., 2007b). Our finding showed fifty samples were found to be negative by PCR. We believe that some of these samples might contain *Entamoeba* spp. But with a low intensity that fell below the PCR detection limit. We also suspected the presence of stool inhibitor substances such as complex polysaccharide, bile salts, lipids, and uric acid that were not completely eliminated prior to PCR (Evangelopoulos et al., 2000).

There are some restrictions in the methodology. This study design has limits our ability to determine more significant associated risk factors due to the small sample size and lack of cooperation from the participants. Moreover, only a single stool sample was collected, instead of the ideal three consecutive samples, due to the cultural belief of some Orang Asli against giving their stool samples, even after general explanations on the benefits of the examination of multiple samples for confirmatory diagnosis. Hence, the prevalence rate of *Entamoeba* spp. is likely to be underestimated due to the intermittent excretory pattern of cysts in the stool. We might expect a higher prevalence of *Entamoeba* spp. infection among Orang Asli communities in these areas. This could be supported by their living beside a river which is very crucial to them as most of their daily activities such as bathing, washing household items and clothes are still carried out in the rivers. Moreover, it is the most preferred defecating site for them. Although they were provided with better housing conditions and adequate provision of basic facilities by the government, villagers still built traditional houses nearer to the modern house. Availability of more stool samples can further enhance the detection of *Entamoeba* spp. especially *E. moshkovskii* as well the other two similar morphology species in future among the population.

CONCLUSION

In conclusion, this study revealed that *E. dispar* was more prevalent than *E. histolytica* in the studied area. Indiscriminate defecation, improper sewage disposal and not washing hand after playing with soil or gardening, and other gastrointestinal symptom such as vomiting were associated with *E. histolytica/ E. dispar*. In addition, more studies are needed to provide data on the epidemiological risk factors of *Entamoeba* spp. infection especially *E. histolytica* to improve health education and environmental sanitary conditions to protect the communities from the infection of this parasite in this area and other part of Malaysia.

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