Prevalence and Risk Factors Associated with *Entamoeba histolytica/dispar/moshkovskii* Infection among Three Orang Asli Ethnic Groups in Malaysia

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Abstract

**Background:** *Entamoeba histolytica/Entamoeba dispar/Entamoeba moshkovskii* infection is still prevalent in rural Malaysia especially among Orang Asli communities. Currently, information on prevalence of this infection among different ethnic groups of Orang Asli is unavailable in Malaysia. To contribute to a better comprehension of the epidemiology of this infection, a cross-sectional study aimed at providing the first documented data on the prevalence and risk factors associated with *E. histolytica/E. dispar/E. moshkovskii* infection was carried out among three Orang Asli ethnic groups (Proto-Malay, Negrito, and Senoi) in selected villages in Negeri Sembilan, Perak, and Pahang states, Malaysia.

**Methods/Findings:** Faecal samples were examined by formalin-ether sedimentation and trichrome staining techniques. Of 500 individuals, 8.7% (13/150) of Proto-Malay, 29.5% (41/139) of Negrito, and 18.5% (39/211) of Senoi were positive for *E. histolytica/E. dispar/E. moshkovskii*, respectively. The prevalence of this infection showed an age-dependency relationship, with higher rates observed among those aged less than 15 years in all ethnic groups studied. Multivariate analysis confirmed that not washing hands after playing with soils or gardening and presence of other family members infected with *E. histolytica/E. dispar/E. moshkovskii* were significant risk factors of infection among all ethnic groups. However, eating with hands, the consumption of raw vegetables, and close contact with domestic animals were identified as significant risk factors in Senoi.

**Conclusions:** Essentially, the findings highlighted that *E. histolytica/E. dispar/E. moshkovskii* parasites are still prevalent in Malaysia. Further studies using molecular approaches to distinguish the morphologically identical species of pathogenic, *E. histolytica* from the non-pathogenic, *E. dispar* and *E. moshkovskii* are needed. The establishment of such data will be beneficial for the public health authorities in the planning and implementation of specific prevention and control strategies of this infection in different Orang Asli ethnic groups in Malaysia.

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Introduction

Amoebiasis, an infection by the protozoan parasite *E. histolytica* is globally considered as a leading parasitic cause of human mortality besides malaria and schistosomiasis [1]. It is estimated that *E. histolytica* may infect half a billion people annually, with 100,000 deaths worldwide [2]. In developed countries, amoebiasis tends to be more common in older individuals and occurs mostly among homosexual men or in institutions [3]. However, in tropical regions, the epidemiology of amoebiasis is completely different and is more common among the general population and particularly among patients attending health care centres with diarrhoea [4]. Clinical features of amoebiasis range from asymptomatic colonization to amoebic dysentery and invasive extraintestinal amoebiasis which is manifested most commonly in the form of liver abscess. Out of 10% of the world's population infected by *E. histolytica*, only 1% develops invasive form of the disease [1].

In Malaysia, food and water-borne diseases which are closely associated with environmental and personal hygiene practices are still among the major health problems especially among Orang Asli communities; Malaysian aborigines comprising 0.6% of the total population. The prevalence of *E. histolytica/E. dispar/E. moshkovskii* in Malaysia ranges from 1% to 61% [5]. To the best of
our knowledge, there is a paucity of available documented data describing the prevalence and risk factors for *E. histolytica/E. dispar/E. moshkovskii* in the Proto-Malay and Negrito that can be used for comparison with Senoi ethnic group.

The present study was carried out in three Orang Asli ethnic groups (Proto-Malay, Negrito, and Senoi) in Malaysia. Using a cross-sectional design, the purpose of this study was to assess the prevalence and to identify underlying risk factors associated with *E. histolytica/E. dispar/E. moshkovskii* infection among these ethnic groups.

**Materials and Methods**

**Study Area**

The present study was carried out from June to December 2011 in three different states of Peninsular Malaysia without discriminating age or gender. Specific villages within the locations of Jelebu (2° 55′ N latitude, 102° 4′ E longitude), Gerik (5° 26′ N latitude, 101° 7′ E longitude), and Temerloh (3° 43′ N latitude, 102° 22′ E longitude) in Malaysia were included in this study (Figure 1). These three states have a similar climate which is a tropical rainforest climate, being hot, and humid throughout the year.

Parit Gong village, Jelebu, Negeri Sembilan state is considered a sub-urban area with a population of 496 inhabitants. Most of the residents are primarily involved in farming and rubber tapping. There are also many individuals who are engaged in commercial and professional activities such as teaching and government service [6]. The average temperature in this area is 26.6°C whereby the average rainfall is 142 mm/year [7]. The main sub-ethnic residing in this area is the Temuan and they belong to the Proto-Malay ethnic group.

RPS Air Banun area, Gerik, Perak state is considered a remote area, located in a valley approximately 40 kilometres from the town of Gerik. The occupations of the inhabitants include farmers, rubber tappers, and some do odd jobs such as selling forest products. The area comprises five villages with 10–15 households in each [8]. The average temperature in this area is 26.9°C whereby the average rainfall is 180 mm/year [7]. The main sub-ethnic residing in this area is the Jahai which belongs to the Negrito ethnic group.

Pasu village, Temerloh, Pahang state is considered a sub-urban area which is within 10 kilometres from the town of Kuala Krau. Out of 625 residents, 65% are farmers and rubber tappers while the remainder are mostly government and private workers [9]. The average temperature in this area is 25.9°C whereby the average rainfall is 161 mm/year [7]. The main sub-ethnic residing in this area is the Jahut and they belong to the Senoi ethnic group.

**Sample Size**

By using formula provided by Kish [10], the expected sample size was calculated according to the following parameters; expected prevalence of *E. histolytica/E. dispar/E. moshkovskii* at 10% and 20% [11,12], confidence interval at 95% and absolute precision (d) = 0.05 [13]. The required minimum sample size needed in this study was 138 individuals and maximum would be 246 individuals for each ethnic group.

**Study Design and Population Surveyed**

Our cross-sectional surveys were carried out between June and December 2011. In each ethnic group, one to three villages were selected from the available village list in collaboration with the JAKOA, and 15–20 households were randomly selected in each village. All household members aged ≥2 years were invited to participate. The number of inhabitants per household was recorded. Unique identifiers were assigned to households and study participants.
Data Analysis

Data was entered in a Microsoft Access and was cross-checked by the technical staff in order to ensure that data were entered correctly. Statistical analysis was performed using the SPSS version 20 (SPSS, Chicago, IL, USA). Only those individuals who had formalin-ether sedimentation and trichrome staining results together with complete questionnaire data were included in the final analyses.

For descriptive analysis, rate (percentage) was used to describe the characteristics of the studied population, including the prevalence of *E. histolytica/E. dispar/E. moshkovskii*. A Chi-squares test ($\chi^2$) was used to test the associations between the variables. In the univariate analysis, the dependent variable was prevalence of *E. histolytica/E. dispar/E. moshkovskii*, while the independent variables were demographic and socioeconomic factors, behaviour risks, environmental sanitation, living condition characteristics, and gastrointestinal symptoms. All variables that were significantly associated with the prevalence of *E. histolytica/E. dispar/E. moshkovskii* in the univariate model were included in a logistic regression analysis to identify the risk factors for *E. histolytica/E. dispar/E. moshkovskii* infection. For each statistically significant factor, an Odds Ratio (OR) and 95% confidence interval (CI) were computed by the univariate and multivariate logistic regression analyses. The level of statistical significance was set as $P<0.05$.

Ethical Consideration and Treatment

Prior to data collection, the study protocol (Reference Number: UKM 1.5.3.5/244/FF-165-2011) was reviewed and approved by the Ethics Committee of Universiti Kebangsaan Malaysia Medical Centre (UKMMC) and permission for field work was obtained from Department of Orang Asli Development (JAKOA). Village meetings were held and village authorities and villagers were handed detailed explanations of the aims, procedures, potential risks, and benefits of the study. During the meeting, they were also
informed that their identity and personal information would be kept strictly confidential, and they could withdraw from the study at any point of time without citing reasons for doing so. If they agreed to participate, their consent was obtained in written form (signature or thumbprint for those who were illiterate) or parents were approached for consent on behalf of their children.

Since molecular methods were not applied in this study to differentiate the pathogenic (*E. histolytica*) and non-pathogenic (*E. dispar* and *E. moshkovskii*) species, all subjects that were initially diagnosed as microscopically positive were not given any treatment at the moment. However, all the positive cases will be treated according to the Ministry of Health, Malaysia once the species have been identified.

**Results**

**Study Cohort and Socioeconomic Profile**

From 795 enrolled participants, 253 individuals (31.8%) failed to submit their faecal samples and/or were absent during parasitological survey. Thirteen individuals (1.6%) had no PVA-fixed faecal sample and 29 individuals (3.7%) were absent during the household-based interviews, and hence their socioeconomic status could not be determined. Overall, 500 individuals (62.9%) were present during the cross-sectional study and respond to our questionnaire (Figure 2).

Among this cohort, 150 individuals (30%) were from Proto-Malay ethnic group, 139 (27.8%) from Negrito ethnic group, and 211 (42.2%) from Senoi ethnic group. The Proto-Malays comprised of 66 males (44%) and 84 females (56%) aged between 2 and 70 years old with a median age of 24 years [interquartile range (IQR) 10–39]. One hundred and thirty nine participants from the Negritos [66 males (47.5%); 73 females (52.5%)] aged between 2 and 74 years old with a median age of 13 years [interquartile range (IQR) 7–30], and 211 respondents among the Senois [87 males (41.2%); 124 females (58.8%)] aged between 2 and 70 years old with a median age of 20 years [interquartile range (IQR) 10–34] participated in this study. General characteristics of each ethnic group, including their socioeconomic profile are presented in Table 1.

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**Figure 2. Study participants’ compliance of survey in three ethnic groups of Orang Asli.**

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Prevalence of *E. histolytica/E. dispar/E. moshkovskii*

The prevalence and distribution of *E. histolytica/E. dispar/E. moshkovskii* are shown in Table 2. The overall prevalence of this infection was 18.0%. It is evident that, of 211 subjects studied from the Senoi ethnic group, 18.5% were positive for *E. histolytica/E. dispar/E. moshkovskii*. However, subjects from the Negrito ethnic group had a relatively high prevalence of this infection which was 29.5%, whereby only 8.7% of the Proto-Malay ethnic group reported positive for *E. histolytica/E. dispar/E. moshkovskii*. Overall prevalence of *E. histolytica/E. dispar/E. moshkovskii* infection showed an age-dependency relationship, with significantly higher prevalence seen among participants aged less than 15 years old compared to those aged more than 15 years ($\chi^2 = 4.237, P = 0.040$). However, there was no significant difference of the *E. histolytica/E. dispar/E. moshkovskii* infection between males and females ($\chi^2 = 2.433, P = 0.119$). With regards to ethnic group, the prevalence of *E. histolytica/E. dispar/E. moshkovskii* infection was highest among those aged less than 15 years among the Proto-Malays (11.9%) and Senois (21.1%) while in Negritos, the prevalence was highest among the 45–74 age groups (37.5%).

In addition, univariate analysis was also carried out based on different ethnic groups of Orang Asli and it was found that the Negrito ethnic group presented a greater risk of the infection than Proto-Malay ethnic group (Negrito versus Proto-Malay: $\chi^2 = 20.602; P < 0.001$) and Senoi ethnic group (Negrito versus Senoi; $\chi^2 = 5.764; P = 0.026$), and presence of other family members infected with *E. histolytica/E. dispar/E. moshkovskii*. Overall prevalence of *E. histolytica/E. dispar/E. moshkovskii* infection was 18.6%. It is evident that, of 211 subjects studied from the Senoi ethnic group, 18.5% were positive for *E. histolytica/E. dispar/E. moshkovskii* infection. However, there was no significant association between *E. histolytica/E. dispar/E. moshkovskii* infection and diarrhoea or other gastrointestinal symptoms such as abdominal discomfort, vomiting, and nausea in all three ethnic groups.

**Risk Factors for *E. histolytica/E. dispar/E. moshkovskii* Infection**

Table 4 shows the results from the logistic regression analysis between *E. histolytica/E. dispar/E. moshkovskii* infection and risk factors, taking into account the random effect of households. The data confirmed not washing hands after playing with soil or gardening and the presence of other family members infected with *E. histolytica/E. dispar/E. moshkovskii* as significant risk factors in all

### Table 1. General characteristics of the Orang Asli communities that participated in this study.

| Characteristics                  | Proto-Malay | Negrito | Senoi |
|----------------------------------|-------------|---------|-------|
| **n (%)**                        |             |         |       |
| **Overall**                      | 150 (30.0)  | 159 (27.8) | 211 (42.2) |
| **Age groups (years)**           |             |         |       |
| <15                              | 59 (39.3)   | 72 (51.8) | 90 (42.7) |
| 15–24                            | 17 (11.3)   | 22 (15.8) | 34 (16.1) |
| 25–44                            | 48 (32.0)   | 29 (20.9) | 54 (25.6) |
| 45–74                            | 26 (17.3)   | 16 (11.5) | 33 (15.6) |
| **Gender**                       |             |         |       |
| Male                             | 66 (44.0)   | 66 (47.5) | 87 (41.2) |
| Female                           | 84 (56.0)   | 73 (52.5) | 124 (58.8) |
| **Socioeconomic status**         |             |         |       |
| Father’s education (<6 years)    | 54 (66.7)   | 68 (77.3) | 100 (76.3) |
| Mother’s education (<6 years)    | 53 (65.4)   | 77 (87.5) | 102 (77.9) |
| Low monthly household income (<RM500) | 17 (11.3)   | 115 (82.7) | 128 (90.7) |
| Working mothers                  | 45 (55.6)   | 33 (37.5) | 75 (57.3) |
| Large family (<8 members)        | 32 (21.3)   | 63 (45.3) | 76 (56.0) |
| Supplied with piped water        | 143 (95.3)  | 76 (54.7) | 138 (65.4) |
| Presence of toilet at household  | 143 (95.3)  | 48 (34.5) | 117 (55.5) |

n = Number examined. RM = Malaysian Ringgit; (US$1 = RM3.17) [16th July 2012].

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ethnic groups. It also further confirmed that eating with hands (OR = 3.65; 95% CI = 1.08, 12.34; P = 0.037), consuming raw vegetables (OR = 2.46; 95% CI = 1.08, 5.62; P = 0.032), and close contact with domestic animals (OR = 3.71; 95% CI = 1.31, 10.52; P = 0.014) were significant risk factors of *E. histolytica/E. dispar/E. moshkovskii* infection among the Senois.

**Discussion**

As shown by the results of the present study, *E. histolytica/E. dispers/E. moshkovskii* is still prevalent in Orang Asli communities in Malaysia with an overall prevalence of 18.6%. This prevalence rate is in agreement with previous studies conducted among Orang Asli communities (Senoi ethnic group) in Malaysia and reported a prevalence rate between 18.5% and 22.5% [12,17]. On the other hand, previous study carried out in eight locations of Orang Asli communities which consist of Senoi and Proto-Malay ethnic groups reported a low prevalence rate (10.2%) of *E. histolytica/E. dispers/E. moshkovskii* infection [18]. However, the specific prevalence of *E. histolytica/E. dispers/E. moshkovskii* infection according to the location of the study area or different ethnic groups of Orang Asli was not reported in their study. In this present study, the prevalence rate of *E. histolytica/E. dispers/E. moshkovskii* infection according to the specific location of the study area was not reported as the three study locations have a similar climate which is a tropical rainforest climate, hot and humid, and has almost similar average rainfall and temperature throughout the year. Thus, it can be considered that the climate does not have any significant role in the distribution or dynamic transmission of this infection. Therefore, reporting the specific prevalence rate of this infection and its risk factors according to different ethnic groups are more meaningful for the public health authorities in the planning of specific measures to control *E. histolytica/E. dispers/E. moshkovskii* infection. This present study also observed some very encouraging trends. Individuals from the Negrito ethnic group have a greater risk of being infected by *E. histolytica/E. dispers/E. moshkovskii* as compared to those from the Senoi and Proto-Malay ethnic groups. This could be attributed to the housing condition and provision of basic amenities: most of houses in Proto-Malay villages have better housing condition and adequate provision of basic amenities as compared to Negrito and Senoi villages which still in traditional-built houses made up of wood or bamboo. Besides that, their behavioural habits in living beside a river is very crucial to them as most of their daily activities such as bathing, washing clothes, and household items are still carried out in the rivers. Nevertheless, some inhabitants preferred defecating at the site of the stream. Our findings are consistent with a previous study by Dunn [19] among 1,273 individuals from seven ethnic groups and sub-groups, the Negritos harboured more intestinal parasite species than any other ethnic group studied. Negritos who survive on hunting, fishing, and gathering do not significantly modify or simplify their habitat and therefore were subjected to a greater diversity of parasites compared to the other groups of aborigine living in surroundings that have been drastically simplified for settlement and cultivation. This proved that although sanitary behaviour appears to be similar for three ethnic groups, certain environmental and cultural practices and taboos may have interacted with their customary behaviour to produce different sanitary conditions [20]. However, this finding contradicted the reported prevalence of *Giardia intestinalis* infection carried out in the same communities which observed a high prevalence rate of giardiasis among members of the Proto-Malay ethnic group who have better housing condition and basic amenities [21]. It indicates that poor sanitary practices and poor provision of basic

### Table 2. Prevalence of *E. histolytica/E. dispers/E. moshkovskii* among different Orang Asli ethnic groups according to age groups and gender.

| Age groups (years) | Overall | Senoi | Proto-Malay | Negrito | Overall | Senoi | Proto-Malay | Negrito | Overall | Senoi | Proto-Malay | Negrito | Overall | Senoi | Proto-Malay | Negrito |
|-------------------|---------|-------|-------------|---------|---------|-------|-------------|---------|---------|-------|-------------|---------|---------|-------|-------------|---------|
| <15               | 221     | 90    | 66          | 65      | 221     | 90    | 66          | 65      | 221     | 90    | 66          | 65      |
| 15-24             | 73      | 4    | 2           | 71      | 4       | 1     | 70          | 2       | 72      | 4     | 2           | 71      |
| 25-44             | 131     | 48    | 18          | 123     | 48      | 16   | 117         | 18      | 137     | 48    | 16          | 123     |
| 45-74             | 75      | 26    | 6           | 59      | 26      | 3    | 52          | 6       | 39      | 26    | 3           | 59      |
| Total             | 500     | 93    | 18.6        | 150     | 13      | 8.7  | 139         | 29.5    | 211     | 39    | 18.5        |         |

| Gender            | Male    | Female | Total     |
|-------------------|---------|--------|-----------|
| Overall            | 219     | 281    | 500       |
| Senoi             | 94      | 155    | 249       |
| Proto-Malay        | 66      | 84     | 150       |
| Negrito            | 45      | 10     | 55        |

| Age group (years) | Male | Female | Total |
|-------------------|------|--------|-------|
| <15               | 72   | 4      | 76    |
| 15-24             | 17   | 11.9   | 28.2  |
| 25-44             | 7     | 11.8   | 46.2  |
| 45-74             | 2     | 11.9   | 29.2  |

| Gender | Overall | Senoi | Proto-Malay | Negrito |
|--------|---------|-------|-------------|---------|
| Male   | 219     | 94    | 66          | 45      |
| Female | 281     | 99    | 84          | 10      |

| Overall | Senoi | Proto-Malay | Negrito |
|---------|-------|-------------|---------|
| Overall | 93    | 18.6        |         |

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Table 3. Associations between *E. histolytica/E. dispar/E. moshkovskii* infection and risk factors among different Orang Asli ethnic groups.

| Variables                              | Proto-Malay (n = 150) | Negrito (n = 139) | Senoi (n = 211) |
|-----------------------------------------|------------------------|-------------------|-----------------|
| Age (years)                            |                        |                   |                 |
| <15                                     | 59                     | 72                | 90              |
| ≥15                                     | 91                     | 67                | 121             |
| **% Infected**                          | 11.9                   | 33.3              | 21.1            |
| **OR (95% CI)**                         | 1.34 (0.74, 2.45)      | 1.23 (0.82, 1.86) | 1.15 (0.82, 1.59) |
| **P-value**                             | 0.262                  | 0.304             | 0.396           |
| Gender                                  |                        |                   |                 |
| Female                                  | 84                     | 73                | 124             |
| Male                                     | 66                     | 66                | 87              |
| **% Infected**                          | 11.9                   | 34.2              | 19.4            |
| **OR (95% CI)**                         | 1.23 (0.73, 2.01)      | 1.17 (0.82, 1.68) | 1.09 (0.71, 1.68) |
| **P-value**                             | 0.122                  | 0.366             | 0.013*          |
| Drinking untreated water                |                        |                   |                 |
| Yes                                     | 9                      | 69                | 131             |
| No                                      | 141                    | 70                | 80              |
| **% Infected**                          | 22.2                   | 36.2              | 23.7            |
| **OR (95% CI)**                         | 1.12 (0.89, 1.42)      | 1.41 (0.93, 2.15) | 2.04 (1.07, 3.88) |
| **P-value**                             | 0.004                  | 0.304             | 0.008*          |
| Bathing and washing in the river        |                        |                   |                 |
| Yes                                     | 7                      | 63                | 73              |
| No                                      | 143                    | 76                | 138             |
| **% Infected**                          | 14.3                   | 33.3              | 23.3            |
| **OR (95% CI)**                         | 1.04 (0.88, 1.22)      | 1.17 (0.82, 1.68) | 1.20 (0.89, 1.61) |
| **P-value**                             | 0.588                  | 0.366             | 0.191           |
| Not washing hands after playing with soil or gardening | | | |
| Yes                                     | 27                     | 63                | 73              |
| No                                      | 123                    | 76                | 138             |
| **% Infected**                          | 25.9                   | 33.3              | 23.3            |
| **OR (95% CI)**                         | 1.85 (1.02, 3.34)      | 2.45 (1.20, 5.00) | 1.53 (1.06, 2.23) |
| **P-value**                             | <0.001*                | 0.005*            | 0.007*          |
| Close contact with domestic animals     |                        |                   |                 |
| No                                      | 30                     | 69                | 119             |
| Yes                                     | 120                    | 70                | 92              |
| **% Infected**                          | 13.3                   | 30.4              | 28.6            |
| **OR (95% CI)**                         | 1.17 (0.81, 1.70)      | 1.05 (0.72, 1.51) | 3.35 (1.58, 7.12) |
| **P-value**                             | 0.310                  | 0.810             | <0.001*         |
| Indiscriminate defecation               |                        |                   |                 |
| Yes                                     | 7                      | 91                | 79              |
| No                                      | 143                    | 48                | 132             |
| **% Infected**                          | 14.3                   | 37.4              | 27.8            |
| **OR (95% CI)**                         | 1.04 (0.88, 1.22)      | 2.45 (1.20, 5.00) | 1.53 (1.06, 2.23) |
| **P-value**                             | 0.588                  | 0.005*            | 0.007*          |
| Sewage disposal                         |                        |                   |                 |
| Outdoor                                 | 21                     | 106               | 113             |
| Common drainage                         | 129                    | 33                | 98              |
| **% Infected**                          | 19.0                   | 33.0              | 22.1            |
| **OR (95% CI)**                         | 1.27 (0.88, 1.83)      | 1.88 (0.84, 4.22) | 1.36 (0.87, 2.13) |
| **P-value**                             | 0.068                  | 0.103             | 0.143           |
| Eating with hands                      |                        |                   |                 |
| Yes                                     | 70                     | 105               | 153             |
| No                                      | 80                     | 34                | 58              |
| **% Infected**                          | 15.7                   | 34.3              | 22.9            |
| **OR (95% CI)**                         | 3.70 (1.03, 13.35)     | 2.43 (1.01, 5.83) | 3.06 (1.18, 7.95) |
| **P-value**                             | 0.004*                 | 0.030*            | 0.008*          |
| Consuming raw vegetables                |                        |                   |                 |
| Yes                                     | 63                     | 113               | 62              |
| No                                      | 87                     | 26                | 149             |
| **% Infected**                          | 11.1                   | 33.6              | 32.3            |
| **OR (95% CI)**                         | 1.28 (0.70, 2.34)      | 3.88 (1.02, 10.10) | 1.55 (1.11, 2.16) |
| **P-value**                             | 0.365                  | 0.026*            | 0.001*          |
| Eating fresh fruits                     |                        |                   |                 |
| Yes                                     | 129                    | 122               | 144             |
| No                                      | 21                     | 17                | 67              |
| **% Infected**                          | 9.3                    | 29.5              | 20.8            |
| **OR (95% CI)**                         | 1.90 (0.28, 13.03)     | 1.00 (0.38, 2.67) | 1.46 (0.79, 2.69) |
| **P-value**                             | 0.493                  | 0.993             | 0.197           |
amenities play an important role in the transmission of *E. histolytica*/*E. dispar*/*E. moshkovskii* in the Negrito ethnic group.

Limitations of our study are as follows. First, the prevalence was based on a single faecal sample. Due to the intermittent nature of cyst excretion in the faeces, the prevalence rate is expected to be higher if three faecal samplings were collected and examined. Various studies of single faecal examinations have reported 10%–50% rate of false-negative results in examining the cysts and/or trophozoites [22,23]. However, in our study it was not feasible to collect three faecal samples, due to the reasons stated in the materials and methods. Nonetheless, we have applied standard procedures during faecal collection and examination to overcome this limitation. Second, the method used for faecal analysis, formalin-ether sedimentation and trichrome staining, did not allow a distinction between *E. histolytica*, *E. dispar*, and *E. moshkovskii*. So, these parasites were indicated by *E. histolytica*/*E. dispar*/*E. moshkovskii*. More specialized methods now exist to

| Table 3. Cont. |
|----------------|
| **Proto-Malay** (n = 150) | **Negrito** (n = 139) | **Senoi** (n = 211) |
| **Variables** | **No. examined** | **% Infected** | **OR (95% CI)** | **P-value** | **No. examined** | **% Infected** | **OR (95% CI)** | **P-value** | **No. examined** | **% Infected** | **OR (95% CI)** | **P-value** |
| **Father’s education** | | | | | | | | | | | | | |
| Non-educated (<6 yrs) | 54 | 13.0 | 1.56 (0.44, 5.52) | 1.053 | 68 | 32.4 | 1.33 (0.54, 3.28) | 1.031 | 100 | 22.0 | 1.67 (0.64, 4.36) | 1.026 |
| Educated (>6 yrs) | 27 | 7.4 | 1.0 | | | 20 | 25.0 | 1.0 | | 31 | 12.9 | 1.0 | |
| **Mother’s education** | | | | | | | | | | | | | |
| Non-educated (<6 yrs) | 53 | 15.1 | 3.38 (0.52, 21.94) | 0.117 | 77 | 33.8 | 4.43 (0.60, 32.87) | 0.097 | 102 | 22.5 | 2.15 (0.70, 6.35) | 0.146 |
| Educated (>6 yrs) | 28 | 3.6 | 1.0 | | | 11 | 9.1 | 1.0 | | 29 | 10.3 | 1.0 | |
| **Mother’s not working** | | | | | | | | | | | | | |
| Yes | 36 | 13.9 | 1.28 (0.60, 2.73) | 0.477 | 55 | 34.5 | 1.38 (0.72, 2.66) | 0.310 | 56 | 28.6 | 1.51 (0.94, 2.44) | 0.052 |
| No | 45 | 8.9 | 1.0 | | | 33 | 24.2 | 1.0 | | 75 | 14.7 | 1.0 | |
| **Household members** | | | | | | | | | | | | | |
| <8 | 118 | 10.2 | 2.94 (0.44, 19.83) | 0.209 | 76 | 31.6 | 1.13 (0.74, 1.72) | 0.554 | 135 | 18.5 | 1.00 (0.63, 1.60) | 0.986 |
| ≥8 | 32 | 3.1 | 1.0 | | | 63 | 27.0 | 1.0 | | 76 | 18.4 | 1.0 | |
| **Household monthly income** | | | | | | | | | | | | | |
| ≤RM500 | 17 | 17.6 | 1.17 (0.86, 1.58) | 0.162 | 115 | 31.3 | 1.59 (0.64, 3.97) | 0.306 | 128 | 22.7 | 1.66 (0.94, 2.90) | 0.052 |
| >RM500 | 133 | 7.5 | 1.0 | | | 24 | 20.8 | 1.0 | | 83 | 12.0 | 1.0 | |
| **Other family members infected with *E. histolytica*/*E. dispar*/*E. moshkovskii*** | | | | | | | | | | | | | |
| Yes | 13 | 46.2 | 1.76 (1.06, 2.92) | <0.001* | 56 | 48.2 | 2.06 (1.32, 3.22) | <0.001* | 55 | 36.4 | 1.64 (1.18, 2.28) | <0.001* |
| No | 137 | 5.1 | 1.0 | | | 83 | 16.9 | 1.0 | | 156 | 12.2 | 1.0 | |
| **Diarrhoea** | | | | | | | | | | | | | |
| Yes | 14 | 14.3 | 1.03 (0.95, 1.12) | 0.433 | 11 | 18.2 | 1.88 (0.43, 8.34) | 0.391 | 22 | 31.8 | 1.11 (0.95, 1.30) | 0.089 |
| No | 136 | 8.1 | 1.0 | | | 128 | 30.5 | 1.0 | | 189 | 16.9 | 1.0 | |
| **Other symptoms of gastroenteritis** | | | | | | | | | | | | | |
| Yes | 72 | 9.7 | 1.51 (0.92, 2.47) | 0.659 | 19 | 15.8 | 2.23 (0.69, 7.25) | 0.159 | 23 | 21.7 | 1.03 (0.90, 0.670) | 0.17 |
| No | 78 | 7.7 | 1.0 | | | 120 | 31.7 | 1.0 | | 188 | 18.1 | 1.0 | |

RM = Malaysian Ringgit; (US$1 = RM3.17) [16th July 2012. Reference group marked as OR = 1 [OR = Odds Ratio]. CI = Confidence interval. *Significant association (P<0.05). doi:10.1371/journal.pone.0048165.t003
It has been noted that those who do not washed their hands after playing with soil or gardening were at two fold higher risk of being infected with *E. histolytica/E. dispar/E. moshkovskii*. Moreover, we found that those eat with hands was 3.65 more likely of being infected. The major role of contaminated hands in the faecal-oral transmission of disease has been well documented in developing countries and washing hands before eating or after defecation has been considered as a secondary barrier. In Vietnam, the transmission routes via contaminated hands play a major role with a more than three folds risk increase if hands are not washed properly [27]. Previous study in Italy and Yemen showed that individuals who do not practice proper hand washing before eating are at two fold higher risk of getting *E. histolytica/E. dispar* infection [28,29]. In addition, not washing hands has been reported to be significantly associated with diarrhoea in Malaysia [30], Myanmar [31], Bangladesh [32], and Indonesia [33].

Our findings suggest that *E. histolytica/E. dispar/E. moshkovskii* is highly infective within a family setting and these findings indicate the possibility of family members as the source of infection (carrier) and transmission occurred within family. Logistic regression analysis showed that individuals living with family members infected with *E. histolytica/E. dispar/E. moshkovskii* were at 2.62 (Senois), 4.92 (Negritos), and 12.32 (Proto-Malays) higher risk of being infected, respectively. Human-to-human contact within family members in all tribes is the possible mode of transmission particularly in situations where the frequency of transmission is high. In El Salvador, higher rates of infection have been observed in contacts of patients with amoebic liver abscess or with amoebic dysentery, or in asymptomatic carriers compared with controls [34]. A study in Mexico also found that 40% of contacts of *E. histolytica* and *E. dispar* carriers were also infected [35]. Under such circumstances, children may be at constant risk of infection as their parents might be the carrier of the cysts and this can be observed in this present study where the prevalence is high in children less than 15 years old. Screening of other family members should be recommended as one of the strategies in controlling *E. histolytica/E. dispar/E. moshkovskii* infection in all ethnic groups of Orang Asli community as an infected family member appears to be an important risk factor for this infection.

Another interesting finding of our study was the significant association observed between close contacts with domestic animal and *E. histolytica/E. dispar/E. moshkovskii* infection among Senoi ethnic group. From our observation, most Senois love to keep dog and cat as their household pets. These animals are left to wander freely in and out of the houses. Moreover, the cysts of *E.histolytica/E. dispar/E. moshkovskii* could be deposited on the surface (fur) of the animals during close contact with infected humans or from environment and then later transmitted to a next person. Study done by Wittnich [36] has reported *E. histolytica* infection in a German shepherd dog.

Epidemiologic studies have shown that consuming raw vegetables has two fold higher risk of acquiring *E. histolytica/E. dispar/E. moshkovskii* and this finding is consistent with a previous study from Brazil [37] and Iran [38]. By contrast, recent reports from Kenya, Yemen, Vietnam, and Tajikistan found no association between *E. histolytica* infection and the consumption of raw vegetables [27,29,39,40]. We observed that tapioca shoots, wild fern shoots and locally planted leaves are the main raw vegetables as salad by the Senois; they usually do not wash these vegetables before eating. The significant association between consuming raw vegetables and *E. histolytica/E. dispar/E. moshkovskii* infection could be directly linked to the contamination with the cysts from the soil garden that vegetables were usually grown. Furthermore, it was frequently seen that vegetables were grown closely to the house where wastewater and human excreta were likely to be used often for irrigation and as fertilizers. Therefore, the current results highlight the potential of unwashed raw vegetables in transmission of *E. histolytica/E. dispar/E. moshkovskii* infection to Orang Asli communities. This study also emphasize the use of standard washing procedure (proper washing and disinfecting) before consumption of vegetables instead of traditional procedure (quick washing without disinfecting).

Finally, we found that there is no significant association between *E. histolytica/E. dispar/E. moshkovskii* infection and gastrointestinal symptoms including diarrhoea. It is well documented that 90% of *E. histolytica/E. dispar/E. moshkovskii* infected individuals are asymptomatic [41]. The possibility of harbouring the non-pathogenic species, *E. dispar* or *E. moshkovskii* cannot be ruled out. Moreover, it is now accepted that *E. dispar* infection is much more common than *E. histolytica* worldwide [42,43].

Table 4. Multivariate analysis of risk factors associated with *E. histolytica/E. dispar/E. moshkovskii* infection among three ethnic groups of Orang Asli.

| Variables | OR  | 95% CI  | P-value |
|-----------|-----|---------|---------|
| (a) Proto-Malay |     |         |         |
| Not washing hands after playing with soil or gardening | 6.21 | 1.52, 25.42 | 0.011  |
| Presence of other family members infected with *E. histolytica/E. dispar/E. moshkovskii* | 12.32 | 2.67, 56.99 | 0.001  |
| (b) Negrito |     |         |         |
| Not washing hands after playing with soil or gardening | 3.89 | 1.46, 10.38 | 0.007  |
| Presence of other family members infected with *E. histolytica/E. dispar/E. moshkovskii* | 4.92 | 2.13, 11.36 | <0.001 |
| (c) Senoi |     |         |         |
| Not washing hands after playing with soil or gardening | 2.98 | 1.34, 6.60 | 0.007  |
| Presence of other family members infected with *E. histolytica/E. dispar/E. moshkovskii* | 2.62 | 1.14, 6.01 | 0.023  |
| Eating with hands | 3.65 | 1.08, 12.34 | 0.037  |
| Consuming raw vegetables | 2.46 | 1.08, 5.62 | 0.032  |
| Close contact with domestic animals | 3.71 | 1.31, 10.52 | 0.014  |

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infections with *E. moshkovskii* has also been reported in Tanzania, Bangladesh, India, Iran, Australia, and Turkey, and in general they are not associated with disease [44,43,46,47,48,49]. However, further studies using molecular approaches are needed to distinguish the morphologically identical species of pathogenic and non-pathogenic species among different Orang Asli ethnic groups.

In conclusion, the present study showed high prevalence of *E. histolytica*/*E. dispar*/*E. moshkovskii* in Orang Asli communities with the highest prevalence was observed among Negrito ethnic group. Poor personal hygiene practices such as not washing hands after playing with soil or gardening and before eating and eating raw vegetables and close contact with domestic animals were the risk factors of *E. histolytica*/*E. dispar*/*E. moshkovskii* infection. Genotyping *E. histolytica*/*E. dispar*/*E. moshkovskii* from humans and animals are highly recommended to identify the species-specific and to understand the actual dynamics of transmission of these protozoa in Senoi ethnic group. Promoting awareness of good personal hygiene is of great importance to control this infection. Screening and giving treatment of the infected family members on the basis of one affected member would appear to be justified since human-to-human transmission is a common mode of acquisition of *E. histolytica*/*E. dispar*/*E. moshkovskii* infection among these communities. It is therefore vital for public health authorities to consider different planning and implementation of specific prevention and control strategies to reduce *E. histolytica*/*E. dispar*/*E. moshkovskii* infection significantly in different Orang Asli ethnic groups in Malaysia.

**Author Contributions**

Conceived and designed the experiments: TSA NM HMA MKAG EO AMY AM. Performed the experiments: TSA SNA FMS NG MB. Analyzed the data: TSA NM HMA. Contributed reagents/materials/analysis tools: NM MKAG EO AMY AM. Wrote the paper: TSA NM.

**References**

1. Walsh JA (1996) Problems in recognition and diagnosis of amoebiasis: estimation of the public health magnitude of morbidity and mortality. Rev Infect Dis 18: 229–239.
2. World Health Organization (1997) World Health Organization/Pan American Health Organization/UNESCO report of a consultation of experts on amoebiasis. Wiley Publ Res 72: 97–100.
3. Hung CC, Ko NY, Ko WC, Lee HC, J DD, et al. (2008) Amoebacidal factors among patrons visiting gay saunas in Taiwan. HIV Med 9: 787–789.
4. Haque R, Mondal D, Duggal P, Kabir M, Roy S, et al. (2006) Entamoeba histolytica infection in children and protection from subsequent amoebiasis. Infect Immun 74: 904–909.
5. Tengku SA, Norhayati M (2011) Public health and clinical importance of amoebiasis in Malaysia: A Review. Trop Biomed 28: 194–222.
6. Department of Orang Asli Development (JAKOA) (2011) Research and Development Division: Information Database, Jelebu District, Negeri Sembilan, Malaysia.
7. Malaysia Meteorological Department (2012) Ministry of Science, Technology and Innovation Database, Malaysia.
8. Department of Orang Asli Development (JAKOA) (2011) Research and Development Division: Information Database, Gerik District, Perak, Malaysia.
9. Department of Orang Asli Development (JAKOA) (2011) Research and Development Division: Information Database, Tenerob District, Pahang, Malaysia.
10. Kish L (1965) Sampling theory of systematic and random sampling in surveys. John Wiley & Sons, Inc. New York, London.
11. Malaysia Meteorological Department (2012) Ministry of Science, Technology and Innovation Database, Malaysia.
12. Hartini Y, Mohamed Kamel AG (2009) Searching for the data: TSA NM HMA. Contributed reagents/materials/analysis tools: AMY AN. Performed the experiments: TSA SNA FMS NG MB. Analyzed the data: TSA SNA FMS NG MB.
13. Lwanga SK, Lemeshow S (1991) Sample size determination in health studies: A practical manual. Oxford: Oxford University Press.
14. Noor Azian MY, Lokman Hakim S, Maslawaty MN (2006) Use of molecular tools to distinguish *Entamoeba histolytica* and *Entamoeba dispar* in northern of Ghana. Trop Med Int Health 11: 1155–1156.
15. Abeba G, Amha K, Mekonnen M, Gerrmemew T (2004) Detection and differentiation of two morphologically identical species of *Entamoeba*. Ethiop J Health Dev 18: 119–124.
16. Markell EK, John DT, Krotoski WA (1999). Medical Parasitology (8th ed). W.B. Saunders Company, Philadelphia.
17. Pham Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J, Dan Cam P, et al. (2011) Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. Parasit Vectors 4: 102.
18. Che Ghani BM, Oothuman P (1991) Patterns of soil-transmitted helminth infection in Orang Asli communities in Senoi ethnic group. Promoting awareness of good personal hygiene is of great importance to control this infection. Screening and giving treatment of the infected family members on the basis of one affected member would appear to be justified since human-to-human transmission is a common mode of acquisition of *E. histolytica*/*E. dispar*/*E. moshkovskii* infection among these communities. It is therefore vital for public health authorities to consider different planning and implementation of specific prevention and control strategies to reduce *E. histolytica*/*E. dispar*/*E. moshkovskii* infection significantly in different Orang Asli ethnic groups in Malaysia.
44. Beck DL, Dogan N, Maio V, Sam NE, Shao J, et al. (2008) High prevalence of *Entamoeba moshkovskii* in a Tanzanian HIV population. Acta Trop 107: 48–49.
45. Haque R, Ali IKM, Clark CG, Petri Jr WA (1998) A case report of *Entamoeba moshkovskii* infection in a Bangladeshi child. Parasitol Int 47: 201–202.
46. Parija SC, Khairnar K (2003) *Entamoeba moshkovskii* and *Entamoeba dispar* associated infection in Pondicherry, India. J Health Popul Nutr 21: 292–295.
47. Solaymani-Mohammadi S, Rezaian M, Bahaei Z, Rajabpour A, Meamar AR, et al. (2006) Comparison of a stool antigen detection kit and PCR for diagnosis of *Entamoeba histolytica* and *Entamoeba dispar* infections in asymptomatic cyst passers in Iran. J Clin Microbiol 44: 2258–2261.
48. Fotedar R, Stark D, Marriott D, Ellis J, Harkness J (2008) *Entamoeba moshkovskii* infections in Sydney, Australia. Eur J Clin Microbiol Infect Dis 27: 133–137.
49. Tanyuksel M, Ulukanligil M, Guclu Z, Araci E, Koro O, et al. (2007) Two cases of rarely recognized infection with *Entamoeba moshkovskii*. Am J Trop Med Hyg 76: 723–724.