Table S1. Detailed Data Extraction from Included Studies on the Prevalence of Entamoeba histolytica Infection in Malaysia

No.	Author & Year	Sampling	Setting	Methodology/Study Design	Main results	Conclusion	Assess-ment score
1.	Thomas & Sinniah, 1982	**Sampling method:** Random sampling. **Inclusion criteria:** - Residents from Bukit Lanjan (Orang Asli), Sungei Penchala (Malays) - Age ranges from two months to 75 years. **Sample size:** - 50 aborigine schoolchildren - 34 aborigine adults - 271 children (Urban general) **Study design:** Cross-sectional	**States:** Selangor (Bukit Lanjan, Sungei Penchala) **High risk groups:** Orang Asli community, children. **Comorbidities:** Gastrointestinal health. **Time period of data collection:** Over 15-month period.	**Objective:** To investigate the prevalence of *Entamoeba histolytica* antibodies in two rural Malaysian populations. **Data collection method:** Blood samples (collected by finger prick onto Nobuto's strip filter paper), stool samples. **Methods of parasite detection:** Serological testing (Indirect Immunofluorescence Technique (IIF); serum samples were tested against *Entamoeba histolytica* antigen prepared from axenic cultures), stool examination (direct smear method,Formalin-ether concentration technique). **Analysis:** Prevalence rates were calculated based on proportion of positive samples, a serum titre of 1:80 or higher was considered clinically significant for invasive amoebiasis.	**Main outcome:** Seroprevalence of *Entamoeba histolytica* antibodies in Orang Asli were 8.3% seropositive at titre ≥ 1:80, in Malays were 6.1% seropositive at titre ≥ 1:80. Stool examination; prevalence of *E.histolytica* cysts excretion was 4.8% in both Orang Asli and Malays. **Other outcomes:** Slightly higher seroprevalence in Orang Asli (8.3%) compared to Malays (6.1%), prevalence increased with age in both populations. **Existence of coinfection:** no coinfection was observed.	The prevalence rates of *E. histolytica* antibodies were low among both Orang Asli and Malays, indicating that these areas are low-endemic zones for invasive amoebiasis. The study highlighted that seropositivity reflects cumulative exposure to the parasite rather than active disease. There was no significant difference between males and females, but prevalence increased with age, which may reflect cumulative lifelong exposure. Stool examinations showed low cyst-passing rates and limited correlation with seropositivity, emphasizing that serological positivity may not indicate current infection but past exposure.	6
2.	Ngui *et al*., 2012	**Sampling method:** Cross-sectional sampling **Inclusion criteria:** Participants from rural villages that gave informed consent and agreed to provide stool samples **Sample size:** 426 human faecal samples **Study design:** Cross-sectional study	**States:** Pahang, Selangor, Johor **High risk group:** Individuals in rural villages that exposed to unsafe water sources, poor environmental sanitation and in close contact with domestic animals that roam freely **Time period of data collection:** During study year (2012)	**Objective:** To determine the prevalence of *Entamoeba* species and the true *E. histolytica, E. dispar* and *E. moshkovskii* infections in human faecal samples using molecular techniques. **Data collection method:** Faecal sample were collected and processed by direct wet smear and formalin ethyl acetate concentration technique followed by iodine staining and examined via microscopy for the presence of Entamoeba species and other intestinal parasites. Microscopically positive samples for Entamoeba species cysts were further characterized using a Nested Polymerase Chain Reaction (Nested-PCR) targeting 16S-like ribosomal RNA gene. **Analysis:** The data entry and analysis were carried out using the SPSS software.	Based on single faecal examination, prevalence of Entamoeba infection was 17.6% (75/426). Comparison by age groups showed that adults (23.9%) had higher infection rates than children (15.3%). The PCR results showed that 52 out of 75 microscopy positive samples successfully generated species-specific amplicons. The infection with E. histolytica (75.0%; 39/52) was the most common, followed by E. dispar (30.8%; 18/52) and E. moshkovskii (5.8%; 3/52). Of these, 33 (63.5%) were shown to contain only E. histolytica, 10 (19.2%) contained E. dispar and 3 (5.8%) contained only E. moshkovskii. Mixed infection with E. histolytica and E. dispar was found in 6 (11.5%) samples.	The present study essentially emphasized the benefit of molecular techniques in discriminating the pathogenic Entamoeba species from the non-pathogenic for accurate diagnosis and better management of amoebiasis.	5

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3.	Lau *et al.*, 2013	**Sampling method:** Voluntary sampling **Inclusion criteria:** Orang Asli from 7 selected villages who gave oral consent. **Sample size:** 334 faecal samples **Study design:** Cross-sectional study	**States:** Perak, Pahang and Selangor **High risk groups:** Orang Asli communities living without access to toilets which then led the villagers to defecate near rivers that cause contamination in water sources. **Time period of data collection:** May 2010 to October 2011	**Objective:** To compare the results of *Entamoeba* species identification using the real-time assay against the established nested PCR method. **Data collection method:** Fresh faecal sample were preserved immediately in 5% potassium dichromate and stored at 4° **Methods of parasite detection:** Direct wet smear and formalin ethyl acetate concentration methods followed by iodine staining and were microscopically examined for Entamoeba species and other intestinal parasites. Microscopically positive samples were then subject to nested PCR and real-time PCR. **Analysis:** Cohen's kappa test for concordance and McNemar's test for discordance.	The overall prevalence of *Entamoeba* infection was 19.5% (65/334). SK Posh Piah recorded the highest *Entamoeba* prevalence (63.3%) while Kampung Kemensah had the lowest prevalence (3.7%) of *Entamoeba*. Real-time PCR assay found *E. histolytica* and *E. dispar* mixed infection to be most common in the study (39.3%), followed by *E. histolytica* (37.5%), *E. dispar* (19.6%), and *E. moshkovskii* (1.8%). There was one (1.8%) mixed infection of *E. histolytica* and *E. moshkovskii* identified by real-time PCR.	This study is the first in Malaysia to report the use of real-time PCR in identifying and differentiating the three Entamoeba infections. It is also proven to be more effective compared to the conventional nested PCR molecular method.	5
4.	Sinniah *et al*., 2012	**Sampling method:** Voluntary sampling **Inclusion criteria:** Orang Asli individuals from three villages **Sample size:** 77 (24 males and 53 females) **Study design:** Cross-sectional study	**States:** Perak **High risk groups:** Orang Asli communities especially children and adolescents **Time period of data collection:** During study year (2011)	**Objective:** To determine the prevalence of intestinal parasites among children and adult Orang aslis (Aborigines) from different locations in Perak **Data collection method:** A total of 179 stool containers were distributed in three Orang Asli settlements with 77 returned. Additional nail clippings samples obtained from most respondents who submitted stool samples and soil samples collected using a spoon from the top 2-4 cm layer of the ground around participants' homes. **Methods of parasite detection:** Samples were analysed using the direct smear and formal ether sedimentation technique. Stool samples that were suspected for *Cryptosporodium* were stained using the Modified Acid-fast stain. **Analysis:** Descriptive statistics used to calculate the prevalence of infections by gender, age group and location.	Prevalence of the 77 stool samples examined, 39 (50.6%) were positive for at least a single parasite. *Entamoeba histolytica* were the least common (1.3%). **Existent of coinfection:** 24.7% were infected with a single parasite species, 18.2% and 6.5% had three concurrent parasitic infections. 1.3% had a quadruple infection, harboring four different parasite species simultaneously.	The study found that intestinal parasitic infections remain common among the Orang Asli, with a prevalence of 50.6%, especially among children and adolescents. Despite improvements in housing and access to clean water, poor hygiene practices and environmental conditions continue to drive infections. The high rate of coinfections and variation between communities highlight the need for targeted health education, regular deworming, and improved sanitation to reduce transmission and improve community health.	6

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5.	Ngui *et al*., 2020	**Sampling method:** Convenience sampling method **Sample size:** 411 humans and 93 dogs	**States:** Selangor (Gurney, Pangsun, Kemensah,Ulu Tamu, Tun Razak and Kuala Kubu Bharu)	**Objective:** To determine the prevalence and molecular epidemiology of *Entamoeba* species in human and dogs in Malaysia. **Data collection and parasite detection method:** Fecal samples were collected from thr participants. The detection of Entamoeba species via microscopy. Positive fecal samples with Entamoeba spe cies cysts/trophozoite were further analyzed with polymerase chain reaction (PCR) for species identification.	The microscopy data showed an overall occurrence of *Entamoeba* species of 26.3% (108/411) in humans. In humans, the most common species was a single infection of *E. dispar* (26.5%; 13/49), followed by *E. histolytica* and *E. moshkovskii,* (20.4% for each species respectively).	Risk factor analysis showed that members of the community who used untreated water were more prone to be infected with *Entamoeba*. Further study is needed to determine the water quality and to confirm the presence of *Entamoeba* cysts in the water samples collected from the study area.	6
6.	Syazwan *et al*., 2022	**Sampling method:** Cross-sectional sampling. **Inclusion criteria:** Participants from selected villages with or without symptoms of amoebiasis, provided fresh stool samples, age range from 2-74 years. **Exclusion criteria:** Stool samples without complete questionnaire data **Sample size:** 55 eligible participants. **Study design:** Cross-sectional study.	**States:** Perak (Kampung Orang Asli Pos Bersih, Kampung Orang Asli Ulu Rasau, Kampung Orang Asli Gesau) **Comorbidities:** Gastrointestinal symptoms; vomiting, diarrhoea, abdominal pain, nausea, watery stool and blood in stool. **Time period of data collection:** September 2018 to March 2019.	**Objective:** To determine the prevalence of pathogenic *Entamoeba histolytica* and non-pathogenic *Entamoeba dispar* and *Entamoeba moshkovskii* infections using a molecular approach among Orang Asli communities in the southern region of Perak **Data collection method:** Stool samples, questionnaire. **Methods of parasite detection:** DNA extraction, single round multiplex PCR. **Analysis:** Prevalence rates calculated in percentages, Pearson's Chi Square test, univariate analysis, odds ratios (OR).	**Main outcome:** 16.4% combining *E. histolytica* and *E, dispar* infection, for *Entamoeba histolytica* the prevalence is 7.3% (4 out of 55 samples. **Existence of coinfection:** no coinfection was observed.	*E. dispar* was more prevalent than *E. histolytica*. Indiscriminate defecation, improper sewage disposal, not washing hands after playing with soil or gardening, and having gastrointestinal symptoms such as vomiting were associated with *E. histolytica* and *E. dispar* infections. The findings warrant the implementation of specific prevention and control strategies, including health education and improving environmental health conditions, to curb the transmission of Entamoeba spp. infection in these communities.	5
7.	Rajeswari *et al*., 1994	**Sampling method:** Random sampling (group come from different social classes) **Sample size:** 456 schoolchildren **Study design:** Cross-sectional study.	**States:** Gombak, Selangor	**Objective:** To investigate the prevalence of intestinal protozoan and helminth infections and the socio-economic factors contributing to these infections among schoolchildren in Gombak, Selangor, Malaysia **Data collection and parasite detection method:** Fecal samples were collected and examined using the formol ether sedimentation technique. The Beaver's direct smear technique was done on all positive stool sample for helminth infections. The children who were positive for helminth infection were treated with a single dose of	The most common parasites found among schoolchildren were *Trichuris trichiura* (47.1%) followed by *Giardia intestinalis* (14.7%), *E. coli* (11.4%), *E. histolytica* (9.9%), *Ascaris lumbricoides* (7.9%) and hookworm (2.9%). The parasitic infections were highest among those using river water (83.3%) and toilets with no latrine in their homes (83.3%).	Intestinal parasitic infections are highly prevalent among schoolchildren in Gombak, Malaysia with highly associated with poor living conditions and low socio-economic status.The need for improved living conditions, better hygiene practices and more effective strategies to control parasitic infections in this community.	5

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				albendazole. After 3 weeks, post-treatment stool samples were collected to determine the effectiveness of the drug.			
8.	Al-Harazi *et al.*, 2013	**Study design:** Cross-sectional study. **Inclusion criteria:** Schoolchildren aged 6-14 years enrolled at a selected school in Pos Senderut. **Exclusion criteria:** Students who did not consent to participate. **Sample size:** 307 schoolchildren.	**States:** Pos Senderut, Pahang. **Comorbidities:** Intestinal protozoan infections such as Giardiasis, Amoebiasis and Blastocystis are linked to malnutrition and impairment growth in children. **Time period of data collection:** August 2008.	**Objective:** To determine the prevalence of intestinal protozoan infections among Orang Asli schoolchildren in a remote area of Pos Senderut, Pahang. **Data collection method:** Stool samples were collected from each participant in labeled 100 ml screw-cap plastic containers. **Methods of parasite detection:** Stool examination Formalin-ethyl acetate sedimentation technique was used to concentrate stool samples, microscopy; direct wet mount and stain with Lugol's iodine. **Analysis:** SPSS version 13.0, Chi-square test to compare infection prevalence between groups.	85% of the Orang Asli schoolchildren were infected with intestinal protozoa (261 out of 307 participants); *E.coli* (24.4%), *G.lamblia* (21.8%), *B.hominis* (21.2%), *E.histolytica* (15.0%) and *I.butschlii* (2.9%). **Existence of coinfection:** Present; *Blastocystis hominis Entamoeba histolytica* were 3.3 %, *Blastocystis hominis* and *Giardia lamblia* were 2.9%, *Giardia lamblia* and *Entamoeba histolytica* were 2.0%, and triple infection (*B.hominis* + *G.lamblia* + *E.histolytica*) were 1.0%.	The outcome results emphasized that intestinal protozoan infections are still a major public health challenge that needs to be addressed to decrease its burden on the health care system and welfare. The high prevalence was attributable to poor environmental management, poor personal hygiene, and lack of health education. The findings serve as a basis to develop strategies and preventive programs targeting high-risk groups, especially schoolchildren in indigenous communities.	7
9.	Gee *et al*., 2020	**Study design:** Convenience sampling **Sample size:** 116 (schoolchildren of pre-school to standard 6 from Sekolah Kebangsaan RPS Banun)	**States:** Perak	**Objective:** To investigate the prevalence of intestinal protozoan infections among aboriginal school children in RPS Banun, Perak, Malaysia **Data collection and parasite detection method:** 10 grams of fecal sample collection and subdivided into 3 parts: (1) fresh fecal sample, (2) fixed with 10% formalin and (3) fixed with polyvinyl alcohol (PVA) Fresh fecal sample examined immediately for the presence of cysts and ova using direct wet mount technique. PVA-fixed samples were subjected to trichrome staining according to the method recommended by World Health Organization. Fecal samples fixed with 10% formalin were used for formalin-ether sedimentation.	65.5% (76/116) of the schoolchildren were found to be infected with at least one intestinal protozoan species. 51.3% (36/79) has multiple intestinal protozoan infections with the majority participants harboring two protozoan species concurrently (62.9%, 27/39). The overall prevalence of *Entamoeba histolytica, Entamoeba coli, Giardia lamblia, Chilomastix mesnili,* and *Blastocytis hominis* infections were 46.6% (54/116), 34.5% (40/116), 21.6% (25/116), 5.2% (6/116) and 1.7% (2/116), respectively.	Single or multiple intestinal protozoan infections are prevalent among schoolchildren in RPS Banun. Effective and sustainable control measures should be implemented including health education and periodic chemotherapy to reduce the prevalence of protozoan infections in aboriginal community.	5

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10.	Adli & Abd Ghani, 2020	**Study design:** Cross-sectional study **Sample size:** 111 volunteered aboriginal school children aged between 7 to 12 years old.	**States:**Pahang (Pos Titom and Kuala Lipis)	**Objective:** To investigate the prevalence of amebiasis among the Orang Asli school children at Pos Titom, Kuala Lipis, Pahang, Malaysia. **Sample collection method:** Only a single stool specimen was collected from each participant and each faecal sample was subdivided into 3 parts: one part was unfixed (fresh sample), the second part was fixed with 10% formalin and the third with PVA (Polyvinyl Alcohol) for trichrome staining. **Methods of parasite detection:** The stool specimens were collected and screened for *E. histolytica* by the direct fecal smear method followed by formalin ether concentration and trichrome staining techniques.	The overall prevalence of amebiasis in this population was 36.0%. The highest infection rate was observed among the higher primary school children aged between 10 - 12 years (37.5%). Trichrome staining exhibits the highest detection rate for the protozoa at 95%, followed by formalin ether concentration technique (75%) and direct fecal smear (57.5%).	High prevalence of *E. histolytica* infection amongst the Orang Asli school children at Pos Titom is attributed to various factors such as low economic status, lack of knowledge on health care and poor hygiene. Improving socioeconomic status, including enhanced access to quality health care and adequate sanitation has the potential to significantly reduce the prevalence and intensity of infection in this aboriginal community.	5
11.	Tokijoh *et al.*, 2022	**Study design:** Cross-sectional study **Sample size:** 544 Orang Asli school volunteered from 6 different schools (aboriginal children aged between 7-12 years old)	**States:** Perak	**Objective:** To the investigate the prevalence of *Entamoeba* complex infection comprising *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* and their associated risk factors among the Orang Asli school children in three districts in Perak, Malaysia **Sample collection method:** 544 stool samples were collected. **Methods of parasite detection:** Stool samples were analysed through the nested multiplex PCR to amplify the *Entamoeba* spp. small subunit rRNA (SSU rRNA) gene sequence All PCR products were loaded into a 2% agarose gel and visualized using an ultraviolet transilluminator.	The overall prevalence of *Entamoeba* complex infection was discovered to be 21.3% (116/544) using a nested multiplex PCR assay, with E. dispar and E. moshkovskii were more prevalent than the pathogenic E. histolytica. Approximately 27 (23.3%) and 49 (42.2%) of the 116 PCR positive samples were linked to *E. histolytica* and *E. dispar* infection.	This study showed a high prevalence of *Entamoeba* spp. infections among the Orang Asli school children in Perak, Malaysia. Addressing the identified risk factors coupled with a holistic approach in breaking the transmission of *Entamoeba* complex can help improve their quality of life.	7

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12.	Abd Ghani & Jeyaprakasam, 2021	**Study design:** Cross-sectional study **Sample size:** 139 volunteered aboriginal school children	**States:** Perak (Sungai Raba Village, Gerik)	**Objective:** To investigate the prevalence of amoebiasis among the Orang Asli (aboriginal) school children at Sungai Raba Village, Gerik, Perak, Malaysia. **Sample collection method:** Stool specimens were collected and screened for the parasite **Methods of parasite detection:** Direct fecal smear method followed by formalin-ether concentration technique.	The overall prevalence of amoebiasis in this population was 14.4% (20/139). Males had a slightly higher prevalence rate of 15.1% (10/66) compared to females at 13.7% (10/73). The highest infection rate of 21.3% was observed in school children aged between 10-11 years.	Amoebiasis among the aboriginal school children at Sungai Raba village, Gerik, still indicates a relatively low health standard of this indigenous community. Promoting awareness of good personal hygiene is one of the measures to control this infection.	5
13.	Jamaiah & Rohela., 2005	**Inclusion:** Public participating in the Medical Fair held in conjunction with the Silver Jubilee Week of the University Malaya Medical Center, Kuala Lumpur, Malaysia. **Sample size:** 246	**Location:** University Malaya Medical Center, Kuala Lumpur, Malaysia.	**Objective:** To study the prevalence of intestinal parasitism among the public communities in Kuala Lumpur. **Sample collection and oparasite detection method:** microscopy for protozoan cysts and helminth eggs. Formalin-ether concentration technique for the detection of both protozoan cysts and helminth eggs in low as well as heavy infections.	The overall infection rate was 6.9% (17 out of 246), with Trichuris trichiura being the most common parasite (4.5%), followed by Ascaris lumbricoides (0.8%), Clonorchis sinensis (0.8%), hookworm (0.4%), and Entamoeba histolytica (0.4%).	Overall urban communities advantaged, the infection rate was low (6.9%), high infection rate with intestinal parasites recorded in the age group of 16- 30 years and infection rates among females appeared to be higher than males in this study group. distribution of infection among the various races.	5
14.	Basuni *et al.*, 2012	**Sampling method:** Convenience sampling **Inclusion:** Patients admitted with gastrointestinal disorders at Hospital Universiti Sains Malaysia, Kelantan, Malaysia **Sample size:** 225 faecal samples **Study design:** Cross-sectional study	**State:** Kelantan **Time period of data collection:** The study was conducted from October 2008 to October 2010 (2 years)	**Objective:** To determine the prevalence of selected helminths and protozoan infections among patients admitted with gastrointestinal disorders at Hospital Universiti Sains Malaysia, Kelantan using multiplex real-time PCR **Data collection method:** Faecal samples from patients. **Methods of parasite detection:** Multiplex Real-Time PCR and Microscopic examination (direct faecal smear, zinc sulphate concentration, Kato-Katz thick smear, trichrome stain and modified acid-fast stain) **Analysis:** Statistical analysis using SPSS Statistics 17.0 for descriptive statistics and prevalence comparison.	Among the selected protozoa detected in this study, *Entamoeba histolytica* was the most prevalent by real-time PCR (1.8%), however microscopy detected an equal number of cases with *E. histolytica* and *Giardia lamblia* (0.9%) **Existent of coinfection:** By real-time PCR, 5 cases had coinfections of intestinal helminths and protozoa and 4 that had multiple helminth infection (N=9), while by microscopy, only 2 samples had coinfections (N=2).	Multiplex real-time PCR recorded a prevalence of 26.2% for intestinal parasitic infection, while microscopic examination detected a prevalence of 5.3%. As compared to microscopy, the multiplex real-time PCR detected 5.8 and 4.5 times more positives for the selected helminth and protozoan infections respectively.	6