

PREVALENCE OF *DIENTAMOEBIA FRAGILIS* AMONG AN ORANG ASLI POPULATION IN RURAL MALAYSIA

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Abstract. *Dientamoeba fragilis* is a trichomonad parasite that can infect the gastrointestinal tract of humans causing gastrointestinal disease. Little is known about its epidemiology. We evaluated the prevalence of *D. fragilis* by conducting a cross sectional study of an Orang Asli population in rural Malaysia. We examined stool samples from 150 participants for *D. fragilis* using Wheatley's trichrome stain and collected demographic data from each participant using a structured questionnaire. Five participants (3.3%) had *D. fragilis* in their stool; four of these were aged <15 years; 3 were male and 2 were female. All participants with positive stool sample for *D. fragilis* were symptomatic; 3 had diarrhea and 2 had other gastrointestinal symptoms. *D. fragilis* is present in the study population. Further studies are needed to determine the virulence, pathogenicity and mode of transmission of *D. fragilis* in the study population.

Keywords: prevalence, dientamoebiasis, Orang Asli, Malaysia

INTRODUCTION

Dientamoeba fragilis is a trichomonad protozoan parasite with a worldwide distribution commonly found in the gastrointestinal tract of humans. Since the first description by Jepps and Dobell in 1918, many aspects of *D. fragilis* had been left unresolved. Despite widespread belief to the contrary, numerous reports document that *D. fragilis* is a common

cause of gastrointestinal disease in both developed and developing countries of the world and has the propensity to exist as a chronic infection with associated clinical signs of disease (Stark *et al*, 2010; Barratt *et al*, 2011).

The transmission route of *D. fragilis* has yet to be definitively described. Only a trophic stage of the parasite (characteristically binucleate) has been described, although in contrast to other pathogenic colon-dwelling protozoa transmitted via the fecal-oral route, no cystic stage has been identified (Stark *et al*, 2006). However, both cyst and precystic forms from human clinical samples have been described recently (Munasinghe *et al*, 2013; Stark *et al*, 2014). It was initially suggested

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that *D. fragilis* was transmitted via the ova of nematodes. Reports of a higher than anticipated rate of co-infection between *D. fragilis* and *Enterobius vermicularis* led others to postulate pinworm may be a probable vector of transmission (Roser *et al*, 2013).

Clinical symptoms, such as diarrhea, abdominal pain, anorexia, nausea, vomiting and flatulence, which usually disappear with the elimination of the parasite, are reported to be associated with dientamoebiasis (Grendon *et al*, 1995). This parasite has been successfully isolated from patients with clinical disease in different countries around the world (Stensvold *et al*, 2007; Libman *et al*, 2008). *D. fragilis* has been postulated to be a possible etiological agent in irritable bowel syndrome (Windsor and Macfarlene, 2005).

Little is known about *D. fragilis* in Malaysia. There are no data about the prevalence of *D. fragilis* among the Orang Asli (aboriginals) in Malaysia. We studied the prevalence of *D. fragilis* among an Orang Asli population in rural Malaysia.

MATERIALS AND METHODS

This study was conducted among the Orang Asli population in Negeri Sembilan, Malaysia between June and December 2011. The expected prevalence of dientamoebiasis among the Orang Asli was 2.3% (Ali *et al*, 1990); using a 95% confidence interval and significance set at $p < 0.05$. The minimum sample size required for the study was estimated to be 35 participants. Simple random sampling was used to select study households. The list of households (sampling frame) was obtained from a preliminary census survey conducted by the same research team.

The participants were asked by a

trained field assistant to complete a structured questionnaire about demographic data, socioeconomic status, signs and symptoms and medical treatment. Participants were asked if they had diarrhea or other gastrointestinal symptoms (*eg*, nausea, vomiting, abdominal pain, watery stools and blood or mucus in the stools). Diarrhea was defined as unusually loose stools passed ≥ 3 times/day (WHO, 1988). For children aged < 15 years, the questionnaire was completed by a parent or guardian. Informed consent was obtained from each participant or parent/guardian, prior to participation. All participants provides a single stool sample. Due to limited financial resources and the problem of obtaining multiple stool samples from each participant.

Part of each sample was preserved in polyvinyl alcohol (PVA) and then stained with Wheatley's trichrome stain as previously described (Salleh *et al*, 2012). Each slide was examined with oil immersion microscopy at 1,000x magnification. Approximately, 250 fields were examined for each slide. Definitive diagnosis was based on the characteristic morphology of the parasite. Criteria used to identify *D. fragilis* were on staining the nuclear membrane is delicate and does not possess peripheral chromatin, the karyosome contains chromatin granules, often appearing as packets and the cytoplasm appears granular and may be vacuolated and contain food inclusions (Stark *et al*, 2005a) (Fig 1).

Participants with a Wheatley's trichrome stain for *D. fragilis* and who completed the questionnaire were included in the analysis. Statistical significance was set as $p < 0.05$. Statistical analysis was performed using the Statistical Package for Social Sciences for Windows version 20 (IBM, Armonk, NY).

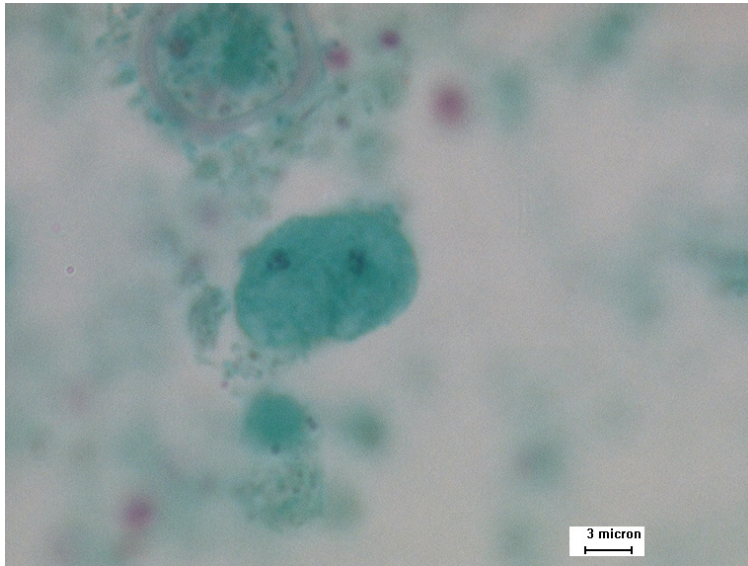


Fig 1—Binucleate trophozoite of *Dientamoeba fragilis* stained with Wheatley's trichrome stain.

Prior to stool and data collections, 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 and permission for field work was obtained from the Department of Orang Asli Development (JAKOA). Before the commencement of the present study, meetings were held with village authorities and villagers explaining the aims, procedures, potential risks and benefits of the study. Participants were informed the identity and personal information would be kept confidential, and they could withdraw from the study at any point without citing a reason for doing so.

RESULTS

One hundred fifty participants were included in the study. The ages ranged from 2 to 70 years old [median: 24 years; interquartile range (IQR) 10-39 years]. There were 66 males. Five participants

(3.3%) were infected with *D. fragilis* (Table 1), 3 males and 2 females ($p=0.464$). Four of the participants with *D. fragilis* infection were aged <15 years. Two of participants with *D. fragilis* infection had a co-infection with one or more parasites: *Entamoeba coli*, *Endolimax nana* and *Chilomastix mesnili*.

No factors associated with *D. fragilis* infection could be determined due to the low prevalence of infection. Three of the infected participants were from one family and 2 were from another family.

All of the *D. fragilis* infected participants were symptomatic. Three had diarrhea and 2 had other gastrointestinal symptoms (Table 2).

DISCUSSION

Little is known about *D. fragilis* infections. In our study the prevalence of *D. fragilis* infection was 3.3%, similar to another local study (Ali *et al*, 1990) and studies from Honduras (2.25%) (Kaminsky, 1991) and Iran (1.4%) (Ghazanchari *et al*, 2012). Day-to-day variation in the shedding of parasites is common with dientamoebiasis (van Gool *et al*, 2003), leading to possible underestimation of the prevalence of *D. fragilis*. Higher prevalences of *D. fragilis* infection are seen in institutions and crowded settings where hygiene is inadequate. In a semi-communal group of adults in the United States the prevalence of *D. fragilis* was 53% (Millet *et al*, 1983). However, prevalence of *D. fragilis* in Malaysia is unclear, possibly because labora-

Table 1
Distribution and prevalence of *Dientamoeba fragilis* infection among the Orang Asli population by age group and gender.

Variables	Orang Asli population		
	No. infected	No. examined	Prevalence (%)
Age (years)			
<15	4	59	6.8
≥15	1	91	1.1
Gender			
Male	3	66	4.6
Female	2	84	2.4
Total	5	150	3.3

Table 2
Characteristics, symptoms and co-infections among subjects infected with *Dientamoeba fragilis*.

Age (years)	Gender	Stool consistency	Symptoms	Other parasites
42	M	Watery	Diarrhea	-
5	M	Watery	Diarrhea	-
10	F	Watery	Diarrhea	<i>Entamoeba coli</i>
12	M	Soft	Abdominal discomfort, vomiting.	-
4	F	Firm/Normal	Abdominal discomfort, nausea, loss of weight.	<i>Chilomastix mesnili</i> , <i>Endolimax nana</i>

M, male; F, female.

tories may ignore the parasite or are not careful with examining the stool sample before the trophozoite disintegrates or the laboratory does not use a correct staining procedure. Since *D. fragilis* may not be considered a pathogen, laboratories may not report it as a pathogen.

We found no significant difference in the prevalence of *D. fragilis* infection by gender of the participants, consistent with the findings of Grendon *et al* (1995) and Lagace-Wiens *et al* (2006). Findings regarding the age distribution of *D. fragilis* infected participants in our study should

be interpreted with care since we had a low prevalence and small sample size. In our study, 4 out of 5 *D. fragilis* infected participants were aged ≤15 years. de Wit *et al* (2001) also found this parasite more commonly among children aged 5 to 14 years. In contrast, some studies have found *D. fragilis* infection more commonly among adults (Rayan *et al*, 1991; Crotti and D'Annibale, 2007). A possible cause for the apparently higher prevalence of *D. fragilis* infection among children aged ≤15 years is poorer hygiene in this age group and poorer immunity.

It is interesting to note in our study the 5 *D. fragilis* infected participants were clustered in 2 households. A common source of infection or transmission within each family may explain the clustering. Co-infections with other parasites was seen in 2 of the 5 participants infected with *D. fragilis* suggesting a possible hygiene problem. Similar co-infections rates with *D. fragilis* and other parasites were seen in another study (Munasinghe *et al*, 2013). These findings suggest a possible fecal-oral transmission route since the other co-infected species are known to be transmitted by that route. No pinworm co-infections were seen in our study. A study from Australia also found no correlation between *Enterobius vermicularis* infection and *D. fragilis* infection (Stark *et al*, 2005b).

All our participants infected with *D. fragilis* were symptomatic, similar to a study by Sarafraz *et al* (2013). However, significant associations between symptoms and infection could not be determined due to the low prevalence of infection seen in our study and the fact we did not examine for other causes of symptoms, such as bacterial or viral infections. More studies are necessary to determine the role of *D. fragilis* among gastrointestinal disorders and the virulence of this parasite in the studied population.

In conclusion, *D. fragilis* was present in the study population and was seen more frequently among participants aged <15 years. Further larger studies need to be performed to investigate the virulence, pathogenicity and mode of transmission of *D. fragilis* among the Orang Asli of Malaysia.

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