

RESEARCH

Open Access

Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase chain reaction (PCR) in rural communities in Malaysia

Romano Ngui, Lorainne Angal, Siti Aminah Fakhurrrazi, Yvonne Lim Ai Lian, Lau Yee Ling, Jamaiah Ibrahim and Rohela Mahmud*

Abstract

Background: In this study, a total of 426 human faecal samples were examined for the presence of *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii* infection via a combination of microscopic examination and nested polymerase chain reaction (PCR) targeting 16S ribosomal RNA of *Entamoeba* species.

Methods: Faecal sample were collected from 426 participants in five rural villages in Peninsular Malaysia. The faecal samples were 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. The data entry and analysis was carried out using the SPSS software (Statistical Package for the Social Sciences) program for Windows version 17 (SPSS, Chicago, IL, USA).

Results: Based on single faecal examination, overall prevalence of *Entamoeba* infection was 17.6% (75/426). Females (19.1%) were more commonly infected compared to males (15.9%). 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.

Conclusions: 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. The presence of *E. moshkovskii* is of great public health concern as it was the first time it has been reported in Malaysia.

Background

The genus *Entamoeba* comprises six species, namely *Entamoeba histolytica*, *E. dispar*, *E. moshkovskii*, *E. coli*, *E. hartmanni* and *E. polecki* that live in the human intestinal lumen. Infections with *Entamoeba* species can result in either a harmless colonization of the intestine or invasion of the colonic wall and damage of other host tissues such as liver, lung and brain. Most of the *Entamoeba* species are

commensal parasites and do not cause human disease. Amoebiasis which is caused by *Entamoeba histolytica* is a global health problem as it is responsible for more than 100,000 deaths per year and is the second leading cause of global death due to protozoa after malaria [1-3]. Major symptoms of amoebiasis are abdominal pain, diarrhea, nausea, vomiting and flatulence. This infection is more preponderant in children compared to adults [4] and is commonly found in tropical and subtropical areas.

It has been reported that 10% of the world's population are infected with *Entamoeba* species, in which pathogenic

* Correspondence: rohela@ummc.edu.my
Department of Parasitology, Faculty of Medicine, University of Malaya, 50603, Kuala Lumpur, Malaysia