Detection of *Entamoeba histolytica and Entamoeba* spp. among Psychiatric Patients by Polymerase Chain Reaction (PCR) Using Genus-Specific and Species-Specific Primers

การตรวจหาเชื้อ *Entamoeba Historytica* และ *Entamoeba* spp. ในผู้ป่วยจิตเวช โดยวิธี Polymerase Chain Reaction (PCR) และใช้ Species-Specific Primers

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Abstract

In this study, a total 269 stool samples collected from psychiatric patients at Hospital Bahagia Ulu Kinta (HBUK) in Perak, Malaysia were examined for *Entamoeba* spp. using PCR and genus-specific primers that amplify DNA of *E. histolytica, E. dispar, E. coli, E. hartmanni, E. moshkovskii, E. chattoni* and *E. polecki.* PCR results showed that 48 (17.84%) samples were positive for *Entamoeba* spp. Further examination of the *Entamoeba*-positive samples by PCR assay using species-specific primer for E. *histolytica*, results showed that 7 (2.60%) samples were positive for *E. histolytica.* In addition, a total of 221 (82.16%) samples were negative for *Entamoeba* spp. and *E. histolytica.*

Keywords: PCR, *Entamoeba* spp., *Entamoeba histolytica*, Genus-specific primer, Epidemiology

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Introduction

Amebiasis is an important health problem worldwide. It has been estimated that about 500,000 individuals are infected with *Entamoeba* species, with invasive amebiasis causing up to 100,000 deaths annually. Amebiasis is the third most common cause of death due to parasitic infection after malaria and schistosomiasis (1)

Infections with *Entamoeba* spp. can result in either a harmless colonization of the intestine or invasion of the colon wall and damage of other host tissues, such as the lung, liver, and brain. Most of the *Entamoeba* species are commensal parasites in intestinal lumen and do not cause human diseases. Only *E. histolytica* is known as a causative agent of amebic dysentery and invasive extraintestinal amebiasis.

Although this infection is more prevalent in developing countries, outbreaks are typically associated with crowding and unsanitary conditions (1). Infections with *E. histolytica* and/or *Entamoeba* spp. are also not uncommon in institutionalized psychiatric patients and in patients with mental retardation. The transmission of amebiasis in these populations has been attributed not only to the direct fecal-oral route but also to poor hygiene behaviors, such as pica, that are often associated with the patients' mental conditions (2, 3, 4, 5, 6, 7).

Although information on amebiasis among institutionalized psychiatric patients is readily available, so far there is no reports on the rates of *Entamoeba* spp. or *E. histolytica* infection among these group of patients in Malaysia. We, therefore for the first time investigated the presence of *Entamoeba* spp. and *E. histolytica* among the psychiatric patients admitted at Hospital Bahagia Ulu Kinta (HBUK), Perak, Malaysia.

Materials and methods

DNA samples

A total of 269 stool samples were collected individually from psychiatric patients at HBUK, Perak, Malaysia. All specimens were first examined by microscopy and subsequently subjected for DNA extraction by using a QIAamp stool DNA extraction kit (QIAGEN, Hilden, Germany). The extracted DNA was then stored at 20˚C until further use. Genomic DNA of Entamoeba histolytica (HM-1: IMSS) were kindly provided by Dr. Graham Clark, London School of Hygiene and Tropical Medicine, London, England.

Genus-specific PCR assay

PCR assays for detection of *Entamoeba* species were performed using genus-specific PCR primers based on small-subunit rRNA gene sequences as described by Verweij *et al.*, 2001. The PCR amplifications were performed in a final volume of 40 µl PCR buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTPs, 25 pmol of each genus-specific primer, 1 unit of *Taq* DNA polymerase (Amersham) and 2 µl of DNA samples. Reactions were carried out in a thermal cycler (Px2 Thermal Cycler, Thermo Hybaid, UK) PCR System. Samples were denatured at 94˚C for 5 minutes, and then subjected to 35 cycles of 94˚C for 1 minute, 55˚C for 1 minute, and 72˚C for 1 minute, following by a final extension at 72˚C for 7 minutes. PCR generates approximately 600 bp amplicon (Figure 1)

Species-specific PCR assay

All positive samples from *Entamoeba*

genus-specific PCR assay were further examined using a PCR assay employing species-specific primers for detection of *E. histolytica* as previously described (9). PCR amplifications were performed in a final volume of 50 µl containing 1X PCR buffer, 200 µM of each dNTPs, 0.1 µM of each species-specific primers, 6 mM $MgCl_2$, 0.5 unit of *Taq* polymerase (Amersham) and 10 µl of DNA samples. Amplification of each species-specific DNA fragment started with an initial denaturation at 94˚C for 3 minutes, followed by 30 cycles of 94˚C for 1 minute, 58˚C for 1 minute, and 72˚C for 1 minute, with a final extension at 72˚C for 7 minutes. PCR generates amplicon of a 166-bp for *E. histolytica* (Figure 2). Amplicons from genus-specific and species-specific PCR were separated by electrophoresis in 1.5% agarose gel and visualized in a UV transilluminator following staining with ethidium bromide.

Results

PCR assays performed using *Entamoeba* genus-specific PCR primers showed that a total of 48 (17.84%) samples were positive for *Entamoeba* spp (Figure 1). Using species-specific primers, PCR amplification showed that of the 48 *Entamoeba*-positive samples, only 7 (2.60%) samples were positive for *E. histolytica* (Figure 2). A total of 221 (82.16%) samples were negative for *Entamoeba* spp. and *E. histolytica* by both genus and species-specifc PCR assays. The PCR assays showed high specificity with no cross reaction observed.

Discussion

This is the first report on detection of *E. histolytica* and *Entamoeba* spp. by PCR assay in institutionalized psychiatric patients in Malaysia.

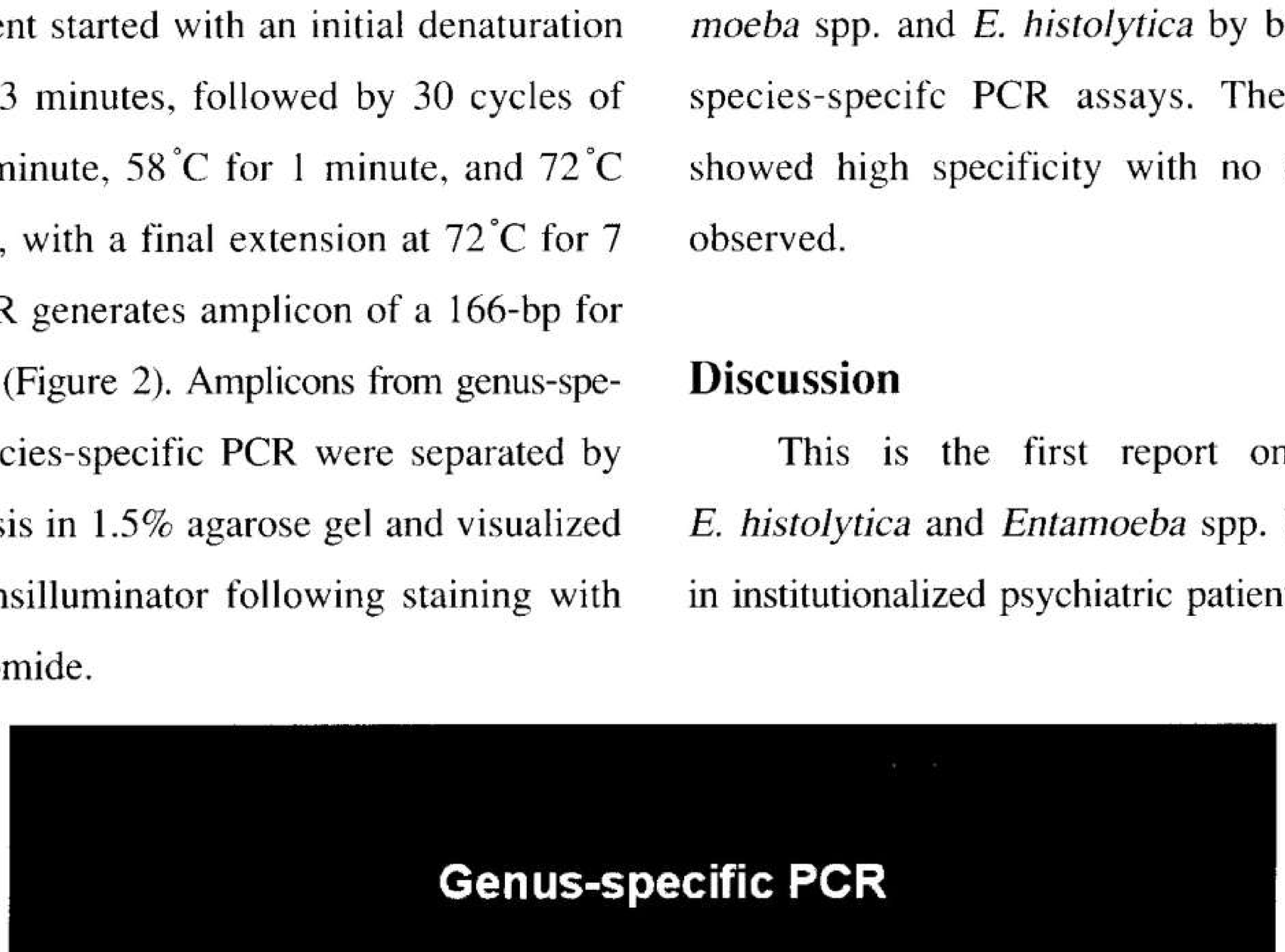

Figure 1 PCR amplification of *Entamoeba* DNA using genus-specific primers. Lane M = molecular marker (100-bp ladders), lanes 1 to 7 = amplified products (600-bp) indicating positive specimens, lane 8 = positive control (*E. histolytica* DNA), lane 9 = negative control (H_2O).

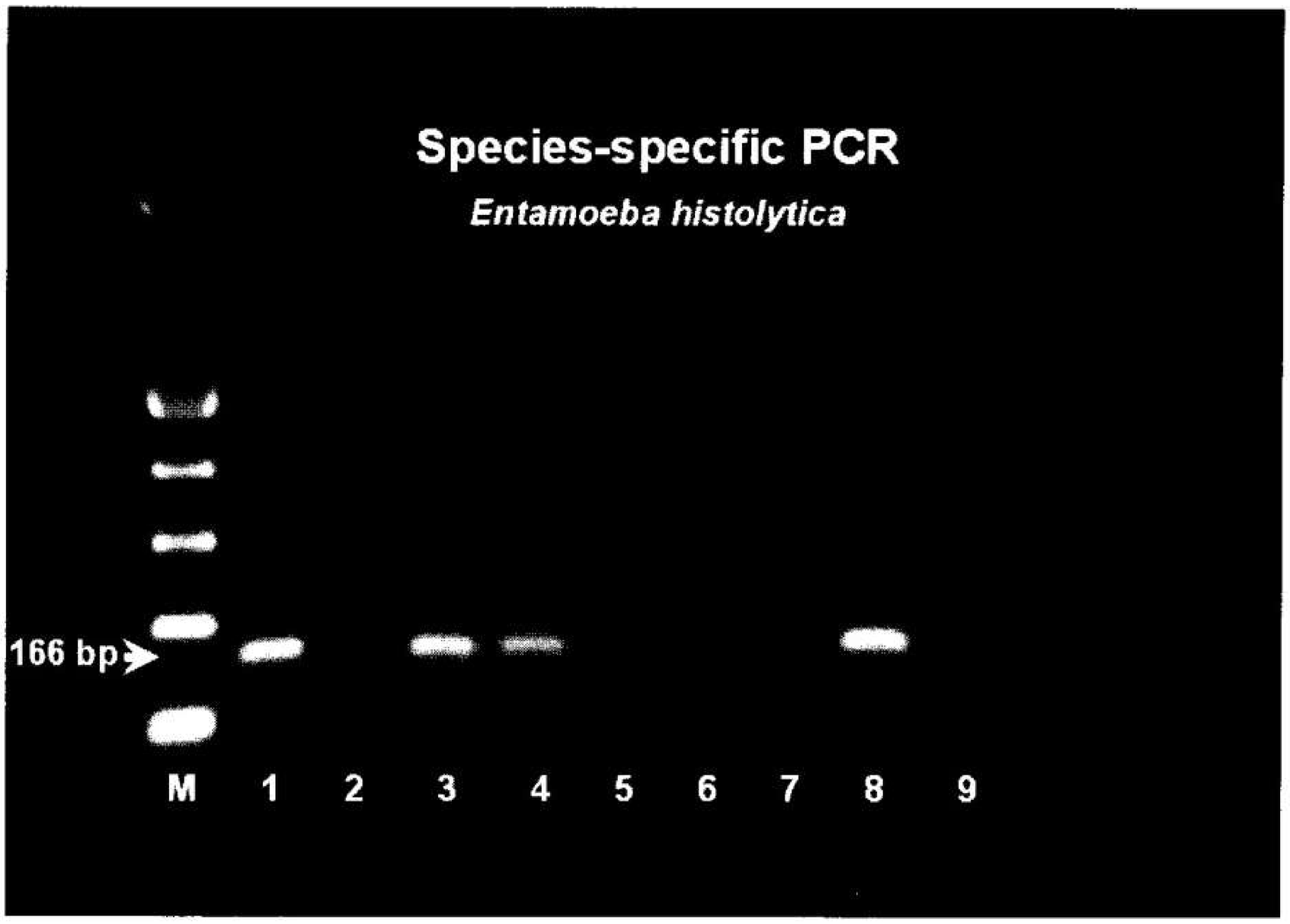

Figure 2 PCR amplification of *E. histolytica* DNA using species-specific primers. Lane M = molecular marker (100-bp ladders), lanes 1 to 7 = amplified products (166-bp) indicating positive specimens, lane 8 = positive control (*E. histolytica* DNA), lane 9 = negative control (H_2O).

presence of *Entamoeba* spp. and *E. histolytica* infections among the psychiatric patients admitted at HBUK, Perak, Malaysia. Findings in this study may indicate the possibility of the circulation and transmission of these parasites among these group of populations.

The rate of *Entamoeba* spp. (17.84%) and *E. histolytica* infections (2.60%) observed in this study were still low compared to the reported rates in other institutionalized psychiatric patients and in patients with mental retardation elsewhere, which ranging from 7% to greater than 30% (2, 3, 7). Since this is the first investigation of *Entamoeba* species among the institutionalized psychiatric patients from Malaysia by using molecular techniques, it is not easy to estimate the sample sizes needed without knowledge of their prevalence.

The low infection rates observed for *E. histolytica* (2.60%) and the higher *Entamoeba*-negative results (82.16%) showed by both PCR assays in this study may possibly due to the small amount of *Entamoeba* DNA that are lower than the detectable level of the assays or they belonged to other *Entamoeba* species. Therefore, detection of the specific species of *Entamoeba* in these samples should be further examined by using a more sensitive method, such as real-time PCR, and employing primers for *E. dispar, E. coli, E. hartmanni, E. moshkovski, E. chattoni* and *E. polecki.*

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