Comparison and diagnosis of Entamoeba in stool sample from rural community of Nepal

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Objective

To find out the prevalence of *Entamoeba* species in rural community of Nepal.

The purpose of the study is to evaluate Nested PCR, microscopic examination and Elisa assay for detection and differentiation of *Entamoeba* species.

Introduction

Nepal being a developing country has many health problems, which escalate in intensity at different times of the year or in epidemic form. Amebiasis is one of the infectious diseases that is highly seen in rural area of Nepal caused by *Entamoeba* species [1,2]. Recent reports show that open defecation, drinking untreated water, unsanitary habits and lack of basic health knowledge cause higher mortality and morbidity in our country.

E. histolytica is an anaerobic pathogenic parasitic. However, *E. dispar and E.moshkovskii* exits as non-pathogenic. Likewise, *E. histolytica*, *E. dispar* and *E. moshkovskii* are morphologically identical but genetically distinct species [3].

Methods

A total of 270 faecal sample were collected from south eastern terai region of Nepal after the informed consent form. The samples were processed by direct wet smear and formalin ethyl acetate concentration technique [4]. Eventually, microscopic examination were performed for the detection of *Entamoeba* species along with other intestinal parasites. Furthermore, enzyme immunoassay were executed to detect antigens of *E. histolytica* through ELISA. Additionally, microscopically positive samples for *Entamoeba* species cysts were further characterized using a Nested- PCR targeting 16S-like ribosomal RNA gene [5]. The PCR generate amplicons which was subjected to 2% agarose gels electrophoresis and visualized under UV transilluminator.

Results

8.52% of the total collected samples were microscopically positive for *Entamoeba* cysts either singly or in combination with other intestinal parasites. Likewise, among 270 stool sample, viral diarrheal was most significant form of diarrhoea found in 76.67% of patients. Among different organisms, *As. Lumbricoids* and *E. histolytica*, *G. lambia* and *H. nana* were identified in most of the patients accounting for 11.11%, 8.52%, 2.59% and 1.11% respectively. However, *Lumbricoids*, *G. lambia*, *Tenia solium* and *E. histolytica* were present in an individual patient while two patient was found with both As. Lumbricoids and G. lambia. Among several symptoms, diarrhoea seems to be the common symptoms infecting all of the patients which is followed by fever and vomiting which accounts for 55.1% and 46.2% correspondingly. Whereas, nausea appears to be the least common symptoms infecting only 14.4% of patients.

Subsequently, 56 cases were PCR positive, 51 cases were ELISA positive whereas 47 were found to be positive by microscopy.

Conclusions

Molecular techniques are indeed promising tools for epidemiological studies, particularly in discriminating the pathogenic from the non-pathogenic species of the *Entamoeba* species. This study reports a new nested multiplex PCR strategy for detection and differentiation of *E. histolytica*, *E. dispar* and *E. moshkovskii* which is highly rapid, specific and sensitive which is useful for proper diagnosis, immunological assay and drug testing.

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