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# *Entamoeba histolytica* Infections in a King Horseshoe Bat (*Rhinolophus rex*): A First Case Report

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## ABSTRACT

This is the first case report of amebic dysentery due to infection of *Entamoeba histolytica* (*E. histolytica*) in a King Horseshoe bat. A fresh fecal sample was collected from a King Horseshoe Bat (*Rhinolophus rex*) and identified by microscopic examination, *E. histolytica* stool antigen detection kit, two PCR assays and DNA sequence analysis. A daily dose of 10 mg of metronidazole was given orally for 5 days and no amoeba was detected in the fecal sample after the treatment. The results of present study suggest that amoebiasis of bat should be monitored more closely in the future.

**Key words:** *Entamoeba histolytica*, bat, amoebiasis, PCR, examination, diagnosis

## INTRODUCTION

Amoebiasis caused by infection with *Entamoeba histolytica* occurs almost all over the world and is highly endemic especially in the developing countries. It has been found in naturally infected humans and many other mammalian species throughout the world (Stedman *et al*., 2003; Bruhn *et al*., 2005). King Horseshoe bat (*Rhinolophus rex*) is endemic to the east coast of China and belongs to the Rhinolophidae family (Pretzlaff *et al*., 2010).

Traditionally, laboratory detection of *E. histolytica* is relied upon by microscopic examination of fresh stool samples. However, this method is incapable of distinguishing the pathogenic species *E. histolytica* from the nonpathogenic species *E. dispar* and the amphizoic amoeba *E. moshkovskii* by the morphology of cysts and trophozoites (Jiang *et al*., 2008; AL-Megrin, 2010; Franca-Botelho *et al*., 2010). Based on the differences between these parasites in biochemistry and in genetics, a number of methods have been developed in recent years, such as protein and Deoxyribonucleic Acid (DNA) detection techniques which are able to distinguish *E. histolytica* from *E. dispar*. The amplification of amoeba DNA fragments by Polymerase Chain Reaction (PCR) has proven to constitute a sensitive and specific method to detect and differentiate *E. histolytica* or *E. dispar* from the feces (Verweij *et al*., 2004; Solaymani-Mohammadi *et al*., 2006; Shathele and EL-Hassan, 2009). The aim of the present communication is to record the occurrence of *E. histolytica* in a new host, the King Horseshoe bat.

## MATERIALS AND METHODS

The fresh fecal sample used in this study was collected from a King Horseshoe bat from the Qian-Ling Park, GuiYang, China (10 July 2010). The parasite was cultured in Trypsin-yeast-iron (TYI-S-33) medium axenically at 37°C. After 48 h growth, the culture tubes were placed in

ice-cold bath for 5 min and trophozoites were centrifuged, resuspended in phosphate-buffered saline pH 7.2. Trophozoites were harvested and used in the following experiments. The presence of parasites in the fecal sample and culture fluid was determined by microscopic examination (Tanyuksel and Petri, 2003). A commercially available kit (ELISA kit *E. histolytica*-II; Techlab, Inc., Blacksburg, VA.) was used in this study. This kit is based on the monoclonal antibody-peroxidase conjugate specific for *E. histolytica* adhesin. According to the manufacturer's instructions, a positive result was defined as an optical density reading >0.05 after subtraction of the negative control optical density. Trophozoites were centrifuged at 5,000x g for 5 min and washed twice in phosphate-buffered saline. After sodium dodecyl sulfate-proteinase K treatment (2 h at 55°C), the sample was processed further by the conventional phenol-chloroform DNA extraction method (Blin and Stafford, 1976). The PCR was conducted in a volume of 25 μL containing (final concentrations) 20 pmol of each primer specific for *E. histolytica* and *E. dispar* (p11 plus p12 and p13 plus p14, respectively) as previously described by Tachibana *et al.* (1991), Pinheiro *et al.* (2004) and Zongo *et al.* (2008). The PCR product was isolated by electrophoresis on 2% agarose gels containing ethidium bromide and the gels were photographed under ultraviolet light. The product was cloned into a pMD18-T plasmid vector (TaKaRa, Japan) according to the manufacturer's recommendations. The cloned insert was sent to Shanghai Sangon Biological Engineering Technology and Services for sequencing. The obtained DNA sequence was submitted for BLAST searching in Gen-Bank to ensure that the required sequence had been amplified.

Mere use of primers p11 plus p12 in PCR and sequencing is not enough to conclude that the isolate was indeed *E. histolytica* (Inyang-Etoh *et al.*, 2007; Takano *et al.*, 2007). In this report, we use a different molecular marker to identify whether the isolate was indeed *E. histolytica* or not. The real-time PCR assay for the detection of *E. histolytica* was carried out according to a previously described (Roy *et al.*, 2005; Rasti *et al.*, 2006). The performance of the assay was evaluated using a range of control samples including *E. histolytica* (No. GY10), *E. dispar* (No. TR327), *E. moshkovskii* (No. DY12) and *E. nuttalli* (No. ZY27) (These control samples were isolated and maintained by our research center). Amplification results were analyzed using i-Cycler software, version 3.0 for Windows. Amplification was also confirmed in all reactions by gel electrophoresis. A sample was considered positive if the signal Cycle Threshold (CT) value exceeded a preset threshold. The PCR product was isolated by electrophoresis on 2% agarose gels containing ethidium bromide and the gels were photographed under ultraviolet light. The product was cloned into a pMD18-T plasmid vector (TaKaRa, Japan) according to the manufacturer's recommendations. The cloned insert was sent to Shanghai Sangon Biological Engineering Technology and Services for sequencing. The obtained DNA sequence was submitted for BLAST searching in Gen-Bank to ensure that the required sequence had been amplified.

Metronidazole was the drug of choice for enteric *E. histolytica* infections at a dose 5 mg, two times daily orally for 5 days. Fecal samples were examined three times after the treatment on days 5, 10 and 30.

## RESULTS

Abundant amoebic cysts were detected in the fresh fecal sample. They were spherical, about 12.0 μm in diameter. Some precysts as the pecilo oblate type were bigger than the metacysts. Lots of amoeba trophozoites and cysts were found in the smear of the culture fluid. The trophozoites had a thick clear layer of ectoplasm and granular endoplasm. They moved rapidly in a straight line with a single clear pseudopod at the anterior end. The endoplasm of the trophozoites contained a profuse fine particle and a vesicular nucleus. The spherical cysts averaged 12.21 μm (n = 100) with a range

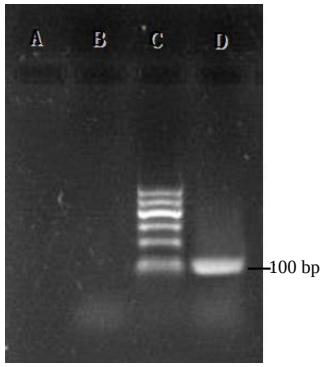


Fig. 1: Agarose gel electrophoresis of PCR products for using primers p11/p12 and p13/p14. Lane A, negative control; Lane B, amplifications for primers p13/14; Lane C, DNA molecular mass standards (DNA Marker |); Lane D, amplifications for primers p11/12

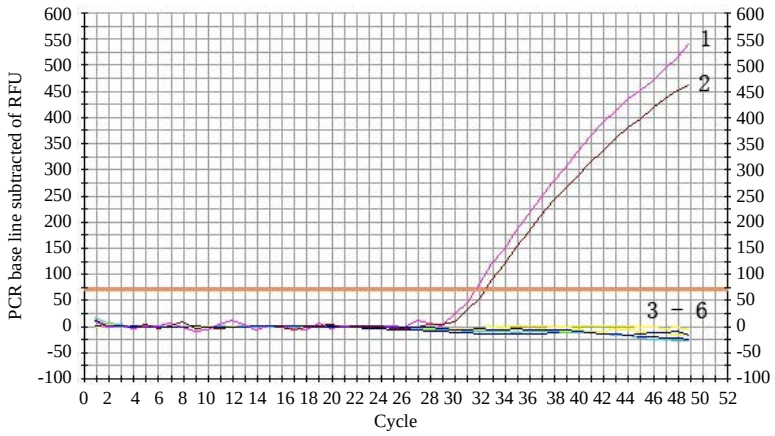


Fig. 2: The Results of real-time PCR specificity detection. 1: DNA from fresh fecal sample of bat after cultrued; 2: DNA from control sample of *E. histolytica*, No.GY10; 3: Control group; 4: DNA from control sample of *E. dispar* (No. TR327); 5: DNA from control sample of *E. nuttalli*, No. ZY27; 6: DNA from control sample of *E. moshkovskii*, No. DY12

of 8-16 μm. The cysts contained one to four nuclei as observed with the iodine stain. The *E. histolytica*/*E. dispar* microscopically positive sample was identified as *E. histolytica* using PCR-specific primers P11/P12, demonstrated by amplification of the species-specific fragment (100 bp) (Fig. 1). Conversely, no amplification was observed for nonpathogenic *E. dispar* using PCR-specific primers P13/P14. Sequencing the PCR amplification fragment of the species specific primers P11/P12 produced a 100-nucleotide base sequence. This determined sequence was the part sequence of *E. histolytica* peroxiredoxin gene after BLAST searching in Gen-Bank. Simultaneously, the sample was positive in the immunoenzymatic assay for the presence of *E. histolytica*-specific galactose adhesion.

The amplification plot of the *E. histolytica*-specific real-time-PCR assay is shown in Fig. 2. The real-time-PCR assay was specific for *E. histolytica* detection, as it was negative when DNA was

introduced from other *Entamoeba* species, including *E. dispar*, *E. nuttalli* and *E. moshkovskii*. Sequencing the PCR amplification fragment of the species specific primers produced a 134-nucleotide base sequence. The obtained DNA sequence was submitted for BLAST searching in Gen-Bank and the results showed that this determined sequence was the part sequence of *E. histolytica* peroxiredoxin gene. It indicated that this case has been diagnosed as *E. histolytica* naturally infecting in bat. No amoeba was detected in the fecal sample on days 5, 10 and 30 after the treatment.

## DISCUSSION

Microscopic diagnosis is an important method for detecting the intestinal protozoan parasites. However, this method is incapable of distinguishing the pathogenic species *E. histolytica* from the nonpathogenic species *E. dispar* and the amphizoic amoeba *E. moshkovskii* by the morphology of cysts and trophozoites. The amplification of amoeba DNA fragments by Polymerase Chain Reaction (PCR) has proved to constitute a sensitive and specific method to detect and differentiate *E. histolytica* or *E. dispar* from the feces (Verweij *et al*., 2004; Solaymani-Mohammadi *et al*., 2006; Rafiei *et al*., 2009). Mere use of primers p11 plus p12 in PCR and sequencing is not enough to conclude that the isolate was indeed *E. histolytica*. Tachibana *et al*. (2007) proposed that an *Entamoeba* sp. strain from rhesus monkey is *E. nuttalli*. The primers p11 plus p12 used in this study can amplify the monkey isolates (Takano *et al*., 2007; Tachibana *et al*., 2007; Haghighi *et al*., 2008). So, a different marker (a 134-bp fragment inside the 16S-like small-subunit rRNA gene of *E. histolytica* (Gene Bank accession number X64142) be used to clearly identify the species of the *Entamoeba* isolate in this study. In this study, through observing the morphology, measuring amoeba trophozoites and cysts, using *E. histolytica* stool antigen detection kits, two PCR assays and DNA sequence analysis, the isolated strain was diagnosed as *E. histolytica*.

## CONCLUSION

The results of present study suggest that amoebiasis of bat should be monitored more closely in the future.

## REFERENCES

Al-Megrin, W.A.I., 2010. Intestinal parasites infection among immunocompromised patients in Riyadh, Saudi Arabia. Pak. J. Biol. Sci., 13: 390-394.

Blin, N. and D.W. Stafford, 1976. A general method for isolation of high molecular weight DNA from eukaryotes. Nucleic Acid Res., 3: 2303-2308.

Bruhn, H., T. Jacobs, B. Urban and M. Leippe, 2005. An exceptionally short α-actinin-like protein from the protozoan parasite *Entamoeba histolytica*. J. Biological Sci., 5: 76-81.

Franca-Botelho, A.C., J.L. Franca, E.L. Franca, A.C. Honorio-Franca, H.G.N.O. Busatti and M.A. Gomes, 2010. Relationship between oxidative stress production and virulence capacity of *Entamoeba* strains. Res. J. Parasitol., 5: 139-147.

Haghighi, A., S. Rasti, E.N. Mojarad, B. Kazemi and M. Bandehpour *et al*., 2008. *Entamoeba dispar*: Genetic diversity of Iranian isolates based on serine-rich *Entamoeba dispar* protein gene. Pak. J. Biol. Sci., 11: 2613-2618.

Inyang-Etoh, P.C., N.G. Etim, M.F. Useh, C.E.J. Udiong and A.W. Essien, 2007. Cryptosporidiosis and infantile diarrhoea in Calabar, Nigeria. J. Medical Sci., 7: 1325-1329.

Jiang, B., G.Y. Yang, X.M. Yu, A.C. Cheng and F.J. Bi, 2008. A case report: *Entamoeba histolytica* infections in the rhesus macaque China. Parasitol. Res., 103: 915-917.

Pinheiro, S.M., R.M. Carneiro, I.S. Aca, J.I. Irmao, M.A. Morais Jr., M.R. Coimbra and L.B. Carvalho Jr., 2004. Determination of the prevalence of *Entamoeba histolytica* and *E. dispar* in the pernambuco state of northeastern Brazil by a polymerase chain reaction. Am. J. Trop. Med. Hyg., 70: 221-224.

Pretzlaff, I., G. Kerth and K.H. Dausmann, 2010. Communally breeding bats use physiological and behavioural adjustments to optimise daily energy expenditure. Naturwissenschaften, 97: 353-363.

Rafiei, A., A. Ajami, M. Hajilooi and A. Etemadi, 2009. Th-1/Th-2 cytokine pattern in human amoebic colitis. Pak. J. Biol. Sci., 12: 1376-1380.

Rasti, S., A. Haghighi, B. Kazemi and M. Rezaian, 2006. Cloning and characterization of serine-rich *Entamoeba histolytica* protein gene from an Iranian *E. histolytica* isolate. Pak. J. Biol. Sci., 9: 654-658.

Roy, S., M. Kabir, D. Mondal, I.K.M. Ali, W.A. Petri Jr. and R. Haque, 2005. Real-time-PCR assay for diagnosis of *Entamoeba histolytica* infection. J. Clin. Microbilol., 43: 2168-2172.

Shathele, M.S. and E.M. EL-Hassan, 2009. Detection of parasitic contaminants in sewage water in Al-Ahsa, Saudi Arabia. Res. J. Parasitol., 4: 63-69.

Solaymani-Mohammadi, S., M. Rezaian, Z. Babaei, A. Rajbpour, A.R. Meamar, A.A. Pourbabai and W.A. Petri Jr., 2006. Comparison of a stool antigen detection kit and PCR for diagnosis of *Entamoeba histolytica* and *Entamoeba dispar* infections in asymptomatic cyst passers in Iran. J. Clin. Microbiol., 244: 2258-2261.

Stedman, N.L., J.S. Munday, R. Esbeck and G.S. Visvesvara, 2003. Gastric amebiasis due to *Entamoeba histolytica* in a Dama Wallaby (*Macropus eugenii*). Vet. Pathol., 40: 340-342.

Tachibana, H., S. Kobayashi, M. Takekoshi and S. Ihara, 1991. Distinguishing pathogenic isolates of *Entamoeba histolytica* by polymerase chain reaction. J. Infect. Dis., 164: 825-826.

Tachibana, H., T. Yanagi, K. Pandey, X.J. Cheng, S. Kobayashi, J.B. Sherchand and H. Kanbara, 2007. An *Entamoeba* sp. strain isolated from rhesus monkey is virulent but genetically different from *Entamoeba histolytica*. Mol. Biochem. Parasitol., 153: 107-114.

Takano, J., T. Narita, H. Tachibana, K. Terao and K. Fujimoto, 2007. Comparison of *Entamoeba histolytica* DNA isolated from a cynomolgus monkey with human isolates. Parasitol. Res., 101: 539-546.

Tanyuksel, M. and W.A. Petri Jr., 2003. Laboratory diagnosis of amebiasis. Clin. Microbiol. Rev., 16: 713-729.

Verweij, J.J., R.A. Blange, K. Templeton, J. Schinkel and E.A.T. Brienen *et al.*, 2004. Simultaneous detection of *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium parvum* in fecal samples by using multiplex real-time PCR. J. Clin. Microbiol., 42: 1220-1223.

Zongo, D., B.G. Kabre, J.N. Poda and D. Dianou, 2008. Schistosomiasis among farmers and fisherman in the west part of Burkina Faso (West Africa). J. Boil. Sci., 8: 482-485.