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## Occurrence and molecular characterization of *Enterocytozoon bieneusi* among long-tailed macaques (*Macaca fascicularis*) in Hainan Province: High genetic diversity and zoonotic potential

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**ABSTRACT Objective:** Microsporidia have been rapidly emerging as pathogens in both immunocompromised and immunocompetent humans. *Enterocytozoon bieneusi* (*E. bieneusi*) is the most common microsporidial species found in human. *E. bieneusi* has also been found in a wide range of animals and is considered to be a potentially important zoonotic pathogen. The epidemiological and genetic characterization of *E. bieneusi* among long-tailed macaques [*Macaca fascicularis* (*M. fascicularis*)] is not fully understood. Here, we conducted the first molecular epidemiological investigation of *E. bieneusi* among *M. fascicularis* in Hainan Province, the southernmost part of China. **Methods:** A total of 193 fecal specimens of *M. fascicularis* were collected from a breeding base housing non-human primates for experimental use in Hainan Province, China. *E. bieneusi* was identified and genotyped by nested PCR analysis of the internal transcribed spacer (ITS) region of the rRNA gene. Phylogenetic analysis was performed by constructing a neighboring-joining tree of the ITS gene sequences using MEGA6. **Results:** A total of 59 (30.6%) of the *M. fascicularis* were PCR-positive for *E. bieneusi*. All 59 samples were sequenced successfully and 16 ITS genotypes were identified. These included nine known genotypes: Type IV ( $n=19$ ), D ( $n=11$ ), CM1 ( $n=8$ ), PigEBITS7 ( $n=4$ ), Pongo2 ( $n=4$ ), Peru 8 ( $n=3$ ), Peru11 ( $n=1$ ), WL21 ( $n=1$ ) and CM2 ( $n=1$ ). Additionally, seven novel genotypes named as HNM-I to HNM-VII (one each) were identified. Importantly, genotypes D, Type IV, Peru8, PigEBITS7, and Peru11, which were the predominant (38/59, 64.4%) genotypes identified among *M. fascicularis* in this study, are also well-known human-pathogenic genotypes. All the genotypes of *E. bieneusi* identified in this study, including the seven novel ones, belonged to zoonotic group 1. **Conclusions:** This is the first report of the identification of *E. bieneusi* in *M. fascicularis* in Hainan Province, China. The findings of numerous known human-pathogenic types and seven novel genotypes (HNM-I to HNM-VII) of *E. bieneusi* all belong to zoonotic group 1 indicate the possibility of transmission of this important pathogenic parasite between *M. fascicularis* and humans.

**Keywords:** *Enterocytozoon bieneusi*; Genotype; ITS region; Macaca; Zoonotic

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