

ORIGINAL ARTICLE

Molecular Detection of *Entamoeba* Spp in Long-tailed Macaque (*Macaca Fascicularis*) at Baluran National Park, Indonesia

Nunuk Dyah Retno Lastuti¹, Lucia Tri Suwanti¹, Poedji Hastutiek¹, Dyah Ayu Kurniawati², Heni Puspitasari³

¹ Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Universitas Airlangga, 60286 Surabaya, Indonesia

² Magister Student of Faculty of Veterinary Medicine, Universitas Airlangga, 60286 Surabaya, Indonesia

³ Institute of Tropical Disease, Universitas Airlangga, 60286 Surabaya, Indonesia

ABSTRACT

Introduction: Baluran National Park (BNP) is one of the oldest national parks in Indonesia. Due to its natural beauty, BNP is also the highest number of tourists in Indonesia. *Macaca fascicularis* is one of the species that has a high population abundance in several park locations. Several species of *Entamoeba* have been reported as pathogenic and non-pathogenic to humans and Non-human primates (NHP). The aim of this research was to detect gen 18 SSU rDNA of *Entamoeba* spp in long-tailed macaque at Baluran National Park. **Methods:** A total 100 faecal samples of *Macaca fascicularis* taken from BNP, Indonesia. Samples were purified with sugar flotation methods, and extracted the DNA using DNAzol. For molecular identification of *Entamoeba* spp, using primer pairs *Entamoeba*-specific primers, including Entam1 (5-GTT GAT CCT GCC AGT ATT ATA TG-3) and Entam2 (5-CAC TAT TGG AGC TGG AAT TAC-3), have been designed by Verweij et al. Amplification is carried out 5 min at 95° C; 40 denaturation cycles of 30 s, 95° C; 30 s for annealing at 57° C, and 30 s for extension at 72° C, with a final step of 2 min at 72°C. **Results:** The prevalence of *Entamoeba* spp. based on PCR examination was 53% (53/100). The PCR products from *Entamoeba* spp. isolates from *Macaca fascicularis* produced bands of around 600 bp with 2% agarose gel electrophoresis. **Conclusion:** there was a high prevalence of *Entamoeba* spp in *Macaca fascicularis* in BNP, and *E. coli* were identified with 18 SSU rDNA genes.

Keywords: *Entamoeba* spp., *Macaca fascicularis*, Baluran National Park, Indonesia

Corresponding Author:

Nunuk Dyah Retno Lastuti, Drh
Email: nunukdyah53@gmail.com
Tel: (+62) 31 5041566

incidence, ranging from 10-18%. Based on the results of routine examination of stool specimens of patients visiting the hospital with symptoms of diarrhea, it is known that 39.6% are amoebic dysentery (11).

INTRODUCTION

Entamoeba spp is a parasite that infests Non-human primates (1,2,3). Several species of *Entamoeba* have been reported as pathogenic and non-pathogenic to humans and Non-human primates (NHP) (4,5). Although most of the *Entamoeba* species are known as non-pathogenic, concern should be taken to *Entamoeba* histolytica because this species can induce amoebiasis both human and NHP (6,7). In severe cases *E. histolytica* can cause hemorrhagic dysentery, liver ulcers, extra-intestinal lesions, even death (8,9). Amoebiasis reported as the second cause of death induced by parasitic disease in humans, Around the world approximately 35–50 million symptomatic cases and 100,000 human deaths was reported annually (3,10). In Indonesia, many intestinal amoebiasis is found endemically with a fairly high

Regarding Genus *Entamoeba*, in recent study, six species (*E. histolytica*, *E. dispar*, *E. moshkovskii*, *E. hartmanni*, *E. coli* and *E. polecki*-like organisms) recorded in NHP in Belgium and Netherland (12). The clinical relevance of *Entamoeba* spp. is more difficult to ascertain in NHP. Several species are considered non-pathogenic, while *E. histolytica* and its virulent variant *E. nuttalli* are known to provoke severe and sometimes lethal intestinal and extra-intestinal disorders in monkeys and apes (3,4,13).

There are various methods to detect *Entamoeba* spp. Morphologic identification is the inexpensive methods but low sensitivity and specificity. In the diagnosis of *E. histolytica*, it's difficult to differentiate between *E. dispar* and *E. moshkovskii*. Therefore, researchers suggest using both morphologic and molecular identification (15).

Baluran National Park (BNP) is one of the oldest national parks in Indonesia. Due to its natural beauty, BNP is also one of the national parks with the highest number of tourists in Indonesia. Long-tailed macaque (*Macaca fascicularis*) is one of the species that has a high population abundance in several park locations. Since a decade ago, close interaction between human and long-tailed macaques has arisen due to excessive feeding by tourists. These made the distance between the long-tailed macaque and humans getting closer which increased the risk of zoonosis (16). Identifying *Entamoeba* spp in Macaque fascicularis based on molecular identification have not been evaluated in BNP and the epidemiology is still unclear. Thus, the aim of this research was to detect *Entamoeba* spp in long-tailed macaque at Baluran National Park. This research attempted to analyze potential zoonosis.

MATERIALS AND METHODS

Ethical approval

This Research was permitted by Baluran National Park (Approval Letter Number SI.794/T.37/TU/KSA.6/9/2018) and IACUC with the ethical clearance No. 2.KE.001.01.2019 under the guidance of Ethical Clearance Commission Faculty of Veterinary Medicine, Universitas Airlangga.

Study and sites samples

BNP is located in the District of Banyuputih, East Java, Indonesia. It spans over 25,000 ha and located at 7°55'17.76S and 114°23'15.27E. BNP contains a range of habitats, ranging from savannah, mangroves, swamp forest, coastal forest, sub-montane forest (primary), monsoon evergreen forest, sea-grass beds and coral reefs. The climate is dominated by 9 months dry season with less than 60 mm rainfall and 3 months of the rainy season. A Total 100 faecal samples of long-tailed macaque taken from Bama beach and Bekol savanna in Baluran National Park. These two sites of tourism have high human-macaque interaction. Faecal samples collected opportunistically and non invasive from the soil immediately after defecation. All samples were collected from fresh dung (<8 hours). Collection samples were taken during August-September 2019. Samples analyzed with sugar flotation methods and purified based on Matsubayashi et al (17).

PCR and sequencing

We extracted the DNA of faecal samples using DNAzol (Ohio, USA) according to the manufacturer's recommended procedures. For identification of *Entamoeba* spp, diagnostic fragments were amplified using primer pairs *Entamoeba*-specific primers, including Entam1 (5-GTT GAT CCT GCC AGT ATT ATA TG-3) and Entam2 (5-CAC TAT TGG AGC TGG AAT TAC-3), have been designed by Verweij et al. (18). These genus-specific primers can be used to

amplify the 18S SSU rDNA of *E. polecki*, *E. chattoni*, *E. moshkovskii*, *E. dispar*, *E. histolytica*, *E. hartmanni*, *E. coli*, and *E. nuttalli*. In this study, the amplification reaction was performed in a 25µl solution consisting of 12.5 µl of Bioline Mastermix (Bioline, Taiwan), 10 pmol of each primer, 8.5 µl distilled water and 2 µl of the DNA template. Amplification involved 5 min at 95°C, followed by 40 denaturation cycles of 30 s at 95°C, 30 s for annealing at 57°C, and 30 s for extension at 72°C, with a final step of 2 min at 72°C. The positive sample will be appear band in 600bp.

RESULTS

A Total of 100 faecal samples were analyzed microscopically for parasite, and *Entamoeba* spp. was found in 53% (53/100) samples both microscopically and molecular examination. The positive samples showed a band in 600bp (Figure 1). *Entamoeba* spp has been reported in different prevalence around the world. *Entamoeba* is the most common protozoan infections observed in NHP (2,3). This is the first data of *Entamoeba* spp. in wild long tailed Macaque at National Park in Indonesia based on molecular detection. The prevalence of *Entamoeba* spp. in this study based on molecular examination was 53% (53/100). Results from sequencing only found *E. coli* in faecal samples. Based on a report from Levecke at al (12), the prevalence of *Entamoeba* spp. among Old World monkeys ranging from 30% and 100% and among apes ranging from 0% and 100%. We found moderate prevalence of *Entamoeba* infection in Long tailed macaque in Baluran National park. The moderate prevalence of *Entamoeba* spp. in the macaque at BNP have similar results from fromosan rock macaque in Taiwan (19). In another study, *Entamoeba* spp. was reported in Celebes Crested Macaque at Sulawesi with prevalence in 97% (20). In North-West India, *Entamoeba* spp. were found in 79% of wild rhesus macaques samples (21). High prevalence of *Entamoeba* spp. also reported in 94% of long-tailed macaque in China (22). Research from Zanzani et al (23) in long tailed macaque showed prevalence of *Entamoeba* spp. around 85%.

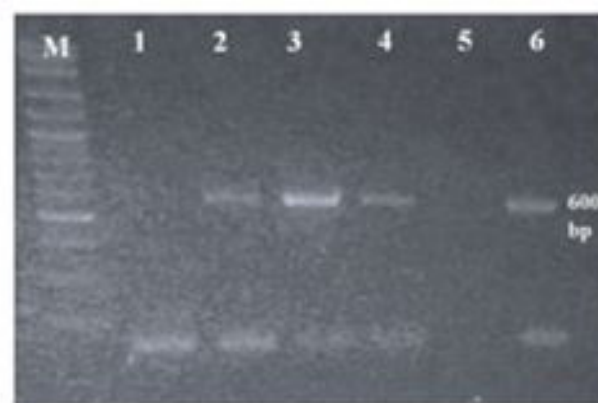


Figure 1 : PCR product of *Entamoeba* spp band 600bp (M:Marker, 1-6: Samples)

According to Nunn and Altizer (24), high prevalence of parasites could be found in the long-tailed macaque because of their social behavior and high population in one group. This is somehow lower compared to other research. The relatively low prevalence might be due to season, shedding intensities of individuals, geographic condition, source of feeds and feeding behaviour of macaque (25,26). The other studies reporting higher prevalence almost come from captive macaque or rural areas. That might be an infection rate higher.

DISCUSSION

Sequencing only identified *Entamoeba coli* in this study. Sample were not identified with *Entamoeba dispar*, *Entamoeba histolytica*, *Entamoeba moshkovskii*, *Entamoeba chattoni*, *Entamoeba nuttalli* and *Entamoeba hartmanni*. Although, that did not mean that other *Entamoeba* species didn't infect macaque because each sequencing result only can identify one species. Research from Debenham et al (21) in wild rhesus macaques showed that *E. chattoni*, *Entamoeba histolytica* nonhuman primate variant, *E. dispar* and *E. moshkovskii* have been identified. Feng et al (1) only found three species of *Entamoeba* i.e. *E. coli*, *E. chattoni*, and *E. dispar* in macaque. Another study from Chang et al (19), has identified *E. coli*, *E. chattoni*, *E. hartmanni*, and *E. nuttalli* in macaque in Taiwan. Few *Entamoeba histolytica* infections had been in the Philippines (4), Belgium (9), Netherlands (12), and China (28). Although only *E. coli* was detected in this study and was universally accepted as nonpathogenic, it was also detected in humans.

Tourists or local residents may be at risk of infection through water and food contamination. Density of Long tailed macaque in BNP was recorded by Hansen et al (16). There are 417 individuals in the four focal groups (in the research area) utilized an estimated home range averaging 1.75km² /group, with an average density of 59 individuals/km² /group and a maximum group size of 180 individuals. The average group size consisted of 104 individuals/group. Actually the density of Long tailed macaque was not too high, but food provisioning made concentration of long tailed macaque in this tourist site high and that may increase the frequency of contact between humans and macaques. The primary potential *Entamoeba* transmission pathways are waterborne, fecal-oral, and contact between humans and NHPs. Waterborne transmission of *Entamoeba* due to contaminated drinking water or poor public water systems has been reported worldwide (27). Actually the report of *Entamoeba* spp infection from NHP to human is still not clear. Although several study reported cases. Sargeant et al. reported eight human cases infections with *E. chattoni* using isoenzyme analysis (29). From that cases, Human records had contact with temple monkeys

during a visit to India. Verveij et al (30) also found *E. chattoni*-like cysts from humans, but it is not clear whether the infections originated from contact with monkeys. An accidental infection of *E. nuttalli* recorded in a caretaker of NHP in a zoo (31) but still it might be concern.

CONCLUSION

In conclusion, there was a high prevalence of *Entamoeba* spp in long-tailed macaque in Baluran National Park, and *E. coli* were identified (53%). The long-tailed macaque might have carried another *Entamoeba* species that had also been detected in humans, thus in next study identification with primer *Entamoeba* specific species will help to determine deeply. That should be attempted to analyze potential mix infection.

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