Zoonotic potential of gastrointestinal parasite in long-tailed Macaque Macaca fascicularis at Baluran National Park, Situbondo, East Java, Indonesia

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RESEARCH PAPER

Zoonotic potential of gastrointestinal parasite in long-tailed Macaque *Macaca fascicularis* at Baluran National Park, Situbondo, East Java, Indonesia

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ABSTRACT

Baluran National Park (BNP) is one of the highest number of tourist visit among Indonesian national park. In the past decades, excessive feeding has induced change in macaque behaviour which increased the number of recorded human-macaque interaction. The close contact between macaque and humans can increase the risk of disease transmissions. This study aimed to identify gastro intestinal (GI) parasite in the long-tailed macaque. To provide identification, we adopted morphologic methods. We collected 100 faeces from unidentified individuals of Long-tailed macaque in BNP. Fecal samples were tested using direct smear and modified sugar floatation techniques. Microscopic examination showed 89% (89/100) samples were found to be positive of GI parasite. The prevalence of protozoa infection was higher (89%) than helminth (83%). The most prevalent GI parasite is *Trichostrongylus* sp (66%) following with *Entamoeba* sp. (53%), *Strongyloides* sp. (32%), *Blastocystis* sp. (32%), *Trichuris* sp. (17%), *Giardia* sp. (10%) and *Enterobius* sp. (3%). All of GI parasite that successfully identified have zoonotic concern. In conclusion, GI parasites found in faeces of long-tailed macaque at Baluran National Park potentially a zoonotic transmission.

Keywords : GI Parasite; Long tailed Macaque; Baluran National Park; Zoonosis

INTRODUCTION

Baluran National Park (BNP) is one of the natural habitats for long-tailed macaque. Since a decade ago, close interaction between humans and long-tailed macaque has been reported there. This problem arises due to excessive feeding by tourists inducing changes in the behavior of long-tailed macaque who were initially afraid of humans to become bold and unafraid (Friishansen *et al*., 2015). This causes the distance between the long-tailed macaque and human getting closer which increased the risk of disease transmission.

Pedersen *et al*. (2005) showed that 68% of pathogens in non-human primates were reported able to infect many hosts. One of the pathogens that often infects Long-tailed macaque is parasites. Transmission of zoonotic parasites often occurs when human and wildlife populations coexist and share resources in the high-density area (Daszak and Cunnigham, 2003). Non-human primates, including Long-tailed macaques, have been identified as potential hosts and reservoirs of gastrointestinal (GI) parasites diseases found in humans such as Microfilariasis, Giardiasis, Cryptosporidiosis, Schistosomiasis, hookworms, Taeniasis, Ascariasis, and Amoebiasis. They were reported to be prevalent across populations in Bali Island (Lane-DeGraaf *et al*., 2011; Lane-DeGraaf *et al*., 2014). Seven different taxa of GI parasites also were reported by Zanzani *et al*. (2016) from Long-tailed macaque from China i.e. *Trichuris* sp., *Oesopagustomum* sp., *Entamoeba* sp., *Endolimax nana*, *Giardia* sp., *Blastocystis* sp., and *Cryptosporidium* sp. All GI parasites describe could be transmitted via a fecal-oral route or penetration through the skin.

In the human case, GI parasite disease still becomes neglected disease especially in developing countries (Beaumier *et al*., 2013). Whereas, amoebic colitis and amoebic liver disease were caused by Entamoeba histolytic is responsible for 100.000 deaths annually (Stanley, 2003). In young children, *Cryptosporidium* sp. has been the top four causes of moderate-to-severe diarrhea (Shirley *et al*., 2012; Kotloff *et al*., 2013; Checkley *et al*., 2015; Sow *et al*., 2016). Approximately 200 million people in Asia, Africa and Latin America reported have symptomatic giardiasis with moderate to severe diarrhea (Feng and Xiao, 2011). *Blastocystis* sp. is one of the causative agents for GI disorders such as Irritation Bowel Syndrome, diarrhea, and other GI disorders (Stensvold *et al*., 2016). Strongyloides fuelleborni reported naturally in non-human primates occasionally found also in humans in Africa and Southeast Asia (Thanchomnang *et al*., 2017). Due to parasitic similarities and the possibility of zoonotic disease, this study was conducted to identify the presence and variability of GI parasite infection in long-tailed macaque at BNP.

MATERIALS AND METHODS

Ethical Approval

This research was approved by the 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, Airlangga University.

Study Site

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 (Figure 1).

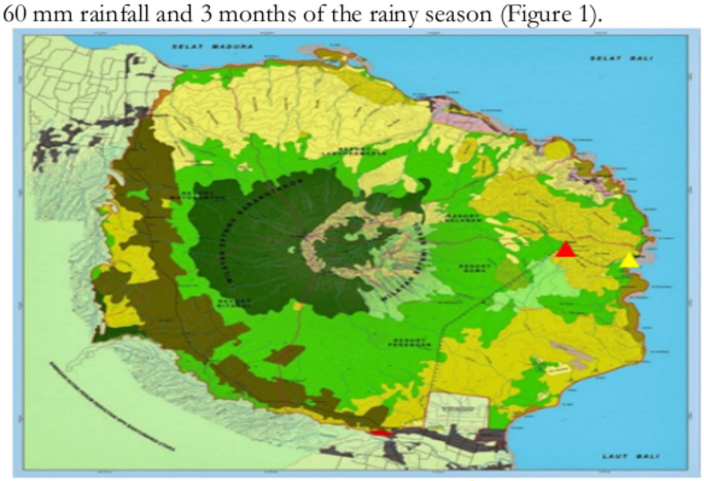

Figure 1. Map of Baluran National Park, ▲= Bama Beach, ▲= Bekol

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Fecal Samples Collection

Fecal samples were taken from 100 unidentified individuals of Long-tailed macaque in BNP. They were collected in Bekol and Bama zones which most human-macaque conflict. Samples were collected from fresh dung (<8 hours). Faecal samples were collected opportunistically and noninvasive from the soil immediately after defecation. Collection samples conducted during August-September 2019. The storage method divided into 10% formalin solution and non-formalin solution.

Examination of Fecal Samples and Data Analysis

Fecal samples were analyzed with direct smear and modified sugar flotation method at Laboratory of the Department of Veterinary Parasitology, Faculty of Veterinary Medicine, Airlangga University. For direct smear, a pea of the fecal sample placed on glass object and covered then examined under a microscope. Sugar flotation methods used with a specific gravity of 1.27 - 1.3 (Matsubayashi *et al*., 2005). To perform a fecal flotation, we weighed approximately 2-4 grams of homogenized fecal sample and mixed the sample with 12 mL of distilled waters. The fecal solution filtered through a tea strainer, and the filtrate was transferred to a 15 mL centrifuge tube. The sample centrifuged at 2500 rpm for 10 minutes. The supernatant discarded, and the sediment was re-suspended in sugar solution. The suspension mixed and centrifuged at 2500 rpm for 10 minutes. After centrifugation, we took supernatant and examined in the glass slide at 100X and 400x magnification to identify the GI parasites. These parasites were identified based on morphological features such as size, shape, number of nuclei, and other notable characteristics present (Soulsby, 1982). Data were analyzed descriptively.

RESULTS

A total of 100 fecal samples have been analyzed, where 89 (89%) samples found to be positive of GI parasite. Prevalence of protozoa infection higher (89%) than helminth (83%) (Table 1). A total of 7 different GI parasites successfully identified in the long-tailed macaque at Baluran National Park. These are three protozoa species namely; *Giardia* sp., *Entamoeba* sp., and *Blastocystis* sp.) and four helminths (*Trichostrongylus* sp., *Strongyloides* sp., *Trichuris* sp., and *Enterobius* sp. were recorded during the study.

Among GI parasites the highest prevalence rate of 66% was detected for *Trichostrongylus* sp. followed by *Entamoeba* sp. (53%), *Strongyloides* sp. (32%), *Blastocystis* sp. (32%), *Trichuris* sp. (17%), *Giardia* sp. (10%) and *Enterobius* sp. (3%) (Table 2, Figure 2). This study reported mix infection (double, triple, multiple infections) were higher than a single infection (Table 3). The highest infection status was 30% for triple infection which followed by double infection (25%), single infection (24%), and multiple infections (10%). From the result of this study, three protozoa and four helminths were reported. All of GI parasites were found in this study have a zoonotic potential concern.

Table 1. Prevalence of GI infection in Long-tailed macaque at Baluran National Park (N=100). Value in parentheses is the total individual of sample

GI parasite	Frequency (%)/N
Protozoa	89 (89)
Helminth	83 (83)
Absence	11 (11)

Table 2. Prevalence of GI parasite in Long Tailed Macaque at Baluran National Park (N=100). Value in parentheses is the total individual of sample

GI parasite	Frequency (%)
Trichostrongylus sp.	66 (66)
Entamoeba sp.	53 (53)
Strongyloides sp.	32 (32)
Blastocystis sp.	32 (32)
Trichuris sp.	17 (17)
Giardia sp.	10 (10)
Enterobius sp.	3 (3)

Table 3. Infection status of GI parasite in Long-tailed macaque at Baluran National Park (N=100). Value in parentheses is the total individual of sample

GI parasite	Frequency (%)
Single infection	24 (24)
Double infection	25 (25)
Triple infection	30 (30)
Multiple infection	10 (10)

DISCUSSION

Gastrointestinal (GI) parasite infection is the most common disease found in Non-Human Primate (NHP) (Strait *et al*., 2012). In this study, the prevalence of GI parasites was high 89% (89/100). These research have higher for GI parasites result from macaque in Sulawesi which ranges from of 42%-73%, working macaque in Malaysia with 52% and long-tailed macaque in Kupang, Indonesia with the prevalence of 86% (Jones *et al*., 2004; Joesoef *et al*., 2018; Choong *et al*., 2019). The prevalence of parasites is considered high if the prevalence rate belongs to 62-96% (Klaus *et al*., 2017). According to Nunn and Altizer (2006), the high prevalence rate of parasites could be found in the long-tailed macaque because of their social behavior and high population in one group. The sample in this study was taken in high-density areas of long-tailed macaque according to Frishansen *et al*. (2015) which in Bekol and Bama Beaches.

The overall result showed infection of protozoa 89% is higher than helminth. Junqiang *et al*. (2017) also reported a similar results with 40.1% and 29.6% for protozoa and helminth infection. The different result was demonstrated by Adhikari *et al*. (2018) that 52.68% hanuman langur and rhesus macaque were positive for helminth and 40.86% for protozoa infections. Zang *et al*. (2019), also reported from rhesus macaque that helminth was the most prevalent with 93.23% than protozoa with 89.96% of infections.

Mix infection (double, triple, and multiple infections) have a higher prevalence than single infection were recorded in this study. This data similar to research conducted by Klaus *et al*. (2017) in proboscis monkey at Borneo, but have a contradictive result with Adhikari *et al*. (2018) in *M. mullata* in Nepal. The co-infection pattern between helminth and protozoa was available in the wild African ape population or new world monkey species (Klaus *et al*., 2017). However, the variation of prevalence and type of infection might be different depending on seasonal parasitic life cycles or various infection rates and shedding intensities of individuals (Eckert *et al*., 2006). These differences may also due to geographic conditions, source of feeds, and feeding behavior of monkeys. Non-

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human primate (NHP) with more time spent on the ground may have a higher infection (Zinner *et al*., 2013; Adhikari *et al*., 2018). From the result of this study, three protozoa and four helminths were reported. All of the GI parasites were found in this study have a zoonotic potential concern because also occur in the human.

The study revealed that *Trichostrongylus* sp. showed the highest prevalence (61%) among other helminths. The research conducted by Dwipayati *et al.* (2014) found a similar prevalence of 73.3% for *Trichostrongylus* sp. from long-tailed macaque sold in Satria market Denpasar. Islam *et al.* (2017) reported *Trichostrongylus* sp. with lower prevalence of 17%. In veterinary, genus Trichostrongylus has an important role, *T. colubriformis* is one of the species that considered to be zoonotic. In overlapping habitats, *T. colubriformis* is a public health concern (Obanda *et al*., 2019).

Strongyloides sp. has a wide range host including non-human primate. The prevalence of Strongyloides sp. in long-tailed macaque at BNP was similar to the long-tailed macaque in Thailand (Thanchomnang *et al*., 2019). However, it was slightly higher than the reported prevalence of *Strongyloides* sp. in Bonnet macaque with 13% (Kumar *et al*., 2018). The number of people infected with this causative agent S*trongyloides* sp. was estimated up to 370 million worldwide (Nutman, 2017). *Strongyloides stercoralis* and *Strongyloides fuelleborni* were described as a potential zoonotic disease (Cogswel, 2007). *Strongyloides fuelleborni* was reported in 3 out of 10 cases of human Strongyloidiasis in Zambia caused by *S. fuelleborni* (Olsen *et al*., 2009). *Strongyloides* sp. could be transmitted through skin penetration or by ingestion (Parr *et al*., 2013). The ability of reproduction inside the intestinal wall generated the high infection rates of helminths (Barutzki and Schaper, 2011).

Trichuris sp. was found in NHP species living in natural habitats including colobus monkeys, macaques, baboons, and chimpanzees (Betson *et al*., 2015). This study showed a 17% prevalence for *Trichuris* sp. Several studies from Indonesia reported the prevalence range of *Thrichuris* sp. in long-tailed macaque from 10% in Satwa Kandi Park west Sumatra and 8.82% in Tinjil Island (Rahmah *et al*., 2013; Chrisnawaty, 2008). In the rural area of Bangladesh, 21% of *M. mullata* was infected by *Trichuris* sp. infection (Islam *et al*., 2017). In Asian species, T*richuris* had a lower prevalence of up to 30% (Hartmann *et al*., 2015). Based on molecular studies, some T*richuris* species seemed to be specific to particular NHPs, but others have the potential to circulate between humans and NHPs as zoonotic agents because they have a close genetical relationship (Betson *et al*., 2015).

Enterobius sp. has the lowest prevalence in this study with 3% (Figure 2a). The low prevalence also reported in *M. radiata* with a prevalence of 5% at Kerala (Arjun *et al*., 2015). *Enterobius vermicularis* is the most common human parasitic helminths, with children are the most susceptible (Fan *et al*., 2019). The majority case of Enterobius infection may symptomless, but some of them may have symptoms such as perianal pruritus, insomnia, restlessness, and irritability (Chang *et al*., 2009).

The prevalence of *Giardia* sp. in this study based on microscopic examination was 10%. The lower prevalence was reported by Du *et al.* (2015) that 2% of NHP in China was infected by *Giardia* sp. Sricharern *et al.* (2016) also reported that 7% of Giardiasis occurred in long-tailed macaque in Thailand. In China, the respective prevalences of human-pathogenic *Cryptosporidium* spp. and *G. intestinalis* infections were in *M. mulatta* from Guizhou 10.9% and 8.3%, respectively (Xiao *et al*., 2012). Data from several studies suggested that the NHPs might be sources of giardiasis for human infections (Du *et al*., 2015).

Entamoeba sp. was reported as the second highest prevalent compared to other parasites (53%). Several studies also reported *Entamoeba* sp. was the highest prevalent GI parasites (Junqiang *et al*., 2017), including in the rhesus macaque (89%) (Zhang *et al*., 2019). There are some species infecting NHP i.e. *E. coli, E. hartmanni, E. histolytica, E. histolytica, E. dispar* and *E. moshkovskii*. In humans, *E. histolytica* has a global distribution of >50million cases worldwide with estimated deaths up to 100.000 each year (Dong *et al*., 2017).

Blastocystis sp. is one of the GI protozoa common found in humans and animals (including non-human primates) and potentially zoonotic. In this study, the prevalence of *Blastocystis* sp. was similar to *Strongyloides* sp. (32%). The microscopic examination found Blastocystis in vacuolar, granular, and cyst stadium. Vaisusuk *et al.* (2017) reported 40% B*lastocystis* sp. prevalence in long-tailed macaque in Thailand with the morphological examination. Jones *et al.* (2004) found 43% of the presence of *Blastocystis* sp. in pet monkey in Sulawesi. *Blastocystis* sp. is one of the causative agents for GI disorders such as Irritation Bowel Syndrome, diarrhea, and other GI disorders. Alfellani *et al.* (2013) reported that *Blastocystis* sp. was found in the feces of an NHP keeper in the zoo. In addition, Yoshikawa *et al.* (2009) identified that *Blastocystis* sp. with the same subtype was found in children and rhesus macaque in the same environment at Khatmandu India.

All of the GI parasites found in this study could be transmitted via fecal-oral or penetration through the skin. Soil contamination with the infective GI parasite stage must be considered by tourists and employees in BNP. Feeding behavior from tourists could cause a concentration habitat of long-tailed macaque. Furthermore, these lit to be frequent re-infection between inter-macaque or human-macaque. Educational for tourist and BNP employees is the best step to protect against zoonotic parasitic infection, for example, maintenance safe distance between human and macaque, use good sanitary and practice waste disposal (Muehlenbein and Ancrenaz, 2009; Williamson and Macfie, 2010).

CONCLUSIONS

Zoonotic GI parasites were found in long-tailed macaque at Baluran National Park, i.e. *Trichostrongylus* sp., *Entamoeba* sp., *Strongyloides* sp., *Blastocystis* sp., *Trichuris* sp., *Giardia* sp. and *Enterobius* sp. The highest prevalence among helminth was *Trichostrongylus* sp. However, for protozoa the highest number of prevalence was *Entamoeba* sp. These zoonotic potential GI parasites in long tailed macaque must be considered by tourist and BNP employee with preventive action.

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