

Diversity of *Entamoeba* spp. in Long-tailed Macaque (*Macaca fascicularis*) in Baluran and Alas Purwo National Parks, Indonesia

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Manuscript received: 13 June 2022. Revision accepted: 1 September 2022.

Abstract. Chrismanto D, Lastuti NDR, Suwanti LT, Hastutiek P, Kurniawati DA, Witaningrum AM, Paramita CA, Pratiwi A. 2022. Diversity of *Entamoeba* spp. in Long-tailed Macaque (*Macaca fascicularis*) in Baluran and Alas Purwo National Parks, Indonesia. *Biodiversitas* 23: 4524-4531. *Entamoeba* spp. is one of the most common protozoa in humans and non-human primates. Several species of *Entamoeba* are considered pathogenic, and one of them is *Entamoeba histolytica* has become a major public health concern. Contact between humans and Long-tailed Macaques (*Macaca fascicularis*) in Baluran National Park and Alas Purwo National Park has become more frequent in the last 10 years due to the habit of tourists providing food. This condition increases the potential of zoonoses between humans and Long-tailed Macaques. The aims of this study are to determine the diversity of species of *Entamoeba* spp. and the potential zoonosis from the Long-Tailed Macaque in Baluran and Alas Purwo National Park. In this study, 200 Fecal samples were collected from Long-tailed Macaques from Baluran National Park (100 samples) and Alas Purwo National Park (100 samples), examined both morphological and molecular examination with *Entamoeba* genus-specific primer. Positive samples were continued to be examined for the presence of 7 *Entamoeba* species by PCR amplification of the SSU rRNA gene. The results were found that 53% (53/100) of the samples were identified as *Entamoeba* spp. from Baluran National Park, and four *Entamoeba* species were identified, *Entamoeba coli* 84.9% (45/53), *Entamoeba chattoni* 58.9% (31/53), *Entamoeba hartmanni* 7.5% (4/53), and *Entamoeba moshkovskii* 13.2% (7/53), and among these, 58.49% (31/53) were mixed infections. The results from Alas Purwo National Park showed 82% (82/100) and three species of *Entamoeba* were detected by molecular tests, *E. chattoni* (90%), *E. coli* (85%) and *E. moshkovskii* (8.5%). The conclusion of the current study revealed the prevalence and diversity of *Entamoeba* species in Long-tailed Macaques in Baluran National Park and Alas Purwo National Park, Indonesia. This study is the first report on the diversity of *Entamoeba* species with molecular detection. The risk of zoonosis transmission in the region needs to be closely monitored.

Keywords: *Entamoeba* spp, genetic diversity, Long-tailed Macaque, PCR zoonosis

INTRODUCTION

Entamoeba species that are both pathogenic and non-pathogenic have been reported to infect humans and Non-Human Primates (NHP) (Chang et al. 2019; Zhang et al. 2019). *Entamoeba* is transmitted through ingestion of mature cysts from contaminated food or water (Hemmati et al. 2015). *Entamoeba* can be spread widely and quickly because it has a simple life cycle, a low infection dose, and a short prepatent period (Madden et al. 2019). The genus *Entamoeba* is commonly found due to the fact that transmission of *Entamoeba* spp. can be through the fecal-oral route, namely *Entamoeba* cysts can enter the body when consuming food or water contaminated with *Entamoeba* spp. cysts. There are seven *Entamoeba* species most known, which include *E. histolytica*, *E. dispar*, *E.*

moshkovskii, *E. hartmanni*, *E. coli*, *E. polecki* (Levecke et al. 2010). Most of the *Entamoeba* species are non-pathogenic species, but *Entamoeba histolytica* is one of the *Entamoeba* species that causes amoebiasis and has a concern for human health and conservation of NHP (Chang et al. 2019). *Entamoeba histolytica* causes clinical symptoms of dysentery, liver ulcers, extra-intestinal lesions, and even death (Fu et al. 2022). *Entamoeba histolytica* accounted for at least 100,000 deaths in humans due to amoebiasis. Infection of *Entamoeba histolytica* in humans has a global distribution of more than 50 million cases in the world with up to 100,000 deaths annually and has become a global health concern. This is the second-highest case of death caused by parasitic disease after malaria (Skappak et al. 2014; Dong et al. 2017).

Besides *E. histolytica*, *E. nuttalli* was found as a pathogenic *Entamoeba* species in NHP. Recent studies have shown that *E. nuttalli* is frequently found in the wild and captive Macaque. It has been confirmed in human zoo caretakers who had contact with *E. nuttalli* positive NHPs (Levecke et al. 2010). That suggests the transmission of *Entamoeba nuttalli* from NHPs to humans might be also possible. *Entamoeba moshkovskii* found free-living and the capability to cause disease in humans is still unclear (Heredia et al. 2015). *Entamoeba nuttalli* is phylogenetically close to *E. histolytica* and is a potential pathogen (Tachibana et al. 2007). The pathogenic species of *Entamoeba* found in non-human primates might pose a threat to NHP and pose a risk of transmission to humans. Close taxonomy and close social relations between humans and NHP have an increased risk for *Entamoeba* transmission through urbanization, habitat fragmentation, logging and tourists (Robertson et al. 2019).

Risk of the spread of zoonotic diseases is critical to human and animal health, especially in systems characterized by a high degree of overlap between humans and animals (Carrero et al. 2020). Potential risk factors that influence the prevalence of *Entamoeba* spp. are hygiene, socioeconomic, exposure to human and animal waste, and household wastewater (Atabati et al. 2020). Baluran and Alas Purwo National Park are two of the most visited national parks in Indonesia. There are many other national parks. But we chose Alas Purwo and Baluran National Park because the Long-tailed Macaque they have close contact with tourists. Tourism objects in Alas Purwo National Park are beaches, forests, caves, historical sites, sacred water sources and wildlife. These tourist objects attract tourists to enjoy natural and wildlife panoramas, historical, cultural, spiritual tours, surfing, diving, fishing and cross-country tourism. Long-tailed Macaques are one of the common animals in tourist areas. It is also known as the crab-eating Macaque, which is widely distributed in Southeast Asia. Since 10 years ago, there has been an increase in the interaction between Macaques and humans in Baluran and Alas Purwo National Park. This is due to the habit of tourists feeding Long-tailed Macaques (Hansen et al. 2015). The initially calm Long-tailed Macaques become aggressive. The existence of this interaction has led to increased concerns about the spread of zoonotic diseases.

Information about *Entamoeba* spp. Long-tailed Macaques in Baluran and Alas Purwo National Park are very limited. *Entamoeba* identification in NHP in Indonesia has been mostly done through morphological identification (Joesoef et al. 2018; Putra et al. 2020). *Entamoeba* spp. was detected by molecular technique (Lastuti et al. 2021) and conducted molecular identification using some primer species in NHP in Sulawesi (Tuda et al. 2016). Traditional parasitology methods using microscopic observation cannot be used to detect *Entamoeba*, which is pathogenic and non-pathogenic due to morphological similarities such as that of *E. histolytica*, *E. dispar*, and *E. moshkovskii*, this method is considered less sensitive (Levecke et al. 2015; Calegar et al. 2016). A common diagnostic method used to identify *Entamoeba* is by examining feces, with this

method can be seen the formation of trophozoites and cysts from *Entamoeba* species, but this method has the disadvantage that it is very difficult to differentiate in order to identify the *Entamoeba* species that cause infection. Modern diagnostic methods that can be applied to identify *Entamoeba* species are molecular methods, namely polymerase chain reaction (PCR). Molecular methods have detected considerable diversity within the genus and could detect distinctions between species (Ngui et al. 2012; Jirku et al. 2016). For the detection of *Entamoeba* species, we used the 18SSUrRNA gene as a marker. The 18S SSU rRNA gene is often used in phylogenetic studies and is an important marker in species determination. In addition to the gene encoding 18S SSU rRNA to determine species, several proteins that act as virulence factors also determine the character of *Entamoeba* species. Several proteins play a role in pathogenicity of *Entamoeba* spp. These include the hemolysin gene (HLY6), cysteine proteinase, and actin gene, which have been targeted to differentiate *Entamoeba* species (Cui et al. 2019).

The objective of this study was to determine the prevalence and diversity of *Entamoeba* species in Long-Tailed Macaque in Baluran and Alas Purwo National Park. This study is the first report on the diversity of *Entamoeba* species with molecular detection. The accurate identification of *Entamoeba* species is important to detect zoonotic diseases caused by the protozoan *Entamoeba* spp.

MATERIALS AND METHODS

Ethical approval

The protocol of this research was reviewed by IACUC with ethical clearance No. 2.KE.001.01.2019 under the guidance of the Ethical Clearance Commission Faculty of Veterinary Medicine, Universitas Airlangga.

Study area

Baluran National Park (BNP) is located in the District of Banyuputih, East Java, Indonesia. The area spans 25,000 ha and at 7°55'17.76 S and 114°23'15.27 E coordinate. Alas Purwo National Park (APNP), Banyuwangi is located at coordinates 8°26'45"-8°47'00" South Latitude and 114°20'16"-114°36'00" East Longitude, which has an area of 43,420 ha consisting of from the core zone, jungle zone, rehabilitation zone, utilization zone, and traditional zone. BNP has a range of habitats, from savannah, mangroves, monsoon evergreen forest, swamp forest, coastal forest, sub-montane forest (primary), coral reefs, and sea-grass beds. The climate is dominated by 9 months dry season with less than 60 mm rainfall and 3 months of rainy season. APNP has a range of habitats from the mangrove forest, savanna, lowland monsoon forest and coral-fringed beaches. APNP has separate wet and dry seasons. Wet seasons occur between October to April with Rain mainly falling in the afternoon and dry season, i.e., March through October.

Table 1. Primers used in the characterization of various *Entamoeba* species

Species	Sequence of primers (5'-3')	Amplicon size (bp)	Anneling (°C)
<i>Entamoeba</i>	F-GTTGATCCTGCCAGTATTATATG R-CACTATTGGAGCTGGAATTAC	550	55
<i>E. histolytica</i>	F-ATGCACGAGAGCGAAAGCAT R-GATCTAGAAACAATGCTTCTCT	166	58
<i>E. dispar</i>	F-ATGCACGAGAGCGAAAGCAT R-CACCACTTACTATCCCTACC	752	58
<i>E. moshkovskii</i>	EmF1: CTCTTACGCGGAGTGCG EmR1: TCGTTAGTTTCATTACCT	260	55
<i>E. coli</i>	F-GAATGTCAAAGCTAATACTTGACG R-GATTTCTACAATTCTCTTGGCATA	160	60
<i>E. chattoni</i>	F-AGGATTTGTTTTATAACAAGTTC R-TAAATAACCTTTCTCCTTTTCTATC	215	52
<i>E. hartmanni</i>	EhartR1 5'CCGTAGATCTCCTATTCACCTT-3' EhartR2 5'ACAACACATTTCATGTCGCA-3'	180	55
<i>E. nuttalli</i>	EnF: TTTATACATTTTGAAGACTTTGCATA EnR: AGGTAATATTGATATACTCAGATTA	2400	60

Fecal specimen collection

A Total of 100 fecal samples of long-tailed Macaque were taken from Bama beach and Bekol savanna in Baluran National Park, and 100 fecal samples from Sadengan, Triangulasi, Bedul, Rowobendo, Pancur in Alas Purwo National Park. These two sites of tourism recorded high human-Macaque interaction. Fecal samples were collected randomly and non-invasive from the soil immediately after defecation. Sampling was carried out in November 2020-March 2021. All samples were collected from fresh dung (<8 hours). Approximately 50 gr Long-tailed Macaque feces sample was placed in a plastic bag in an ice box.

Fecal examination

All samples were analyzed with modified sugar flotation methods (Komatsu et al. 2019), we took approximately two to five grams of feces and added 15 mL of distilled water. We dissolved completely and sieved the solution. We centrifuged the supernatant at 2000 rpm for 5 minutes. After centrifugation, we discarded the supernatant and added sugar solution (1.2g specific gravity), then mixed. We repeated centrifugation at 2000 rpm for 10 minutes. The supernatant was pipetted and placed on a slide for microscopic examination to identify *Entamoeba* spp. based on the morphology and size of the parasite (Lastuti et al. 2021). Microscopic examination counting positive if the cyst or trophozooid form of *Entamoeba* spp. found.

The purification was performed using a positive sample. *Entamoeba* spp. cysts were purified by the sugar flotation method above. The *Entamoeba* spp. cysts were placed on the surface of the sugar solution aspirate using a pipette approximately 1-2 mL. The supernatant was washed three times using distilled water. The pellet was added to 1-2 mL of PBS and stored at 4°C until use.

DNA extraction and Polymerase Chain Reaction analysis

We screened the positive samples from microscopic examination, purified and extracted them using a commercial gSYNC DNA Extraction Kit (Geneaid, Taiwan) strictly and carefully following the manufacturer's

protocol. The DNA was stored at -20°C for further PCR amplification. Detection of *Entamoeba* spp. was amplified with universal primers targeting the small-subunit ribosomal RNA (SSU rRNA) gene of *Entamoeba*, Entam1, and Entam2. These genus-specific primers can be used to amplify the 18S SSU rDNA of *E. chattoni*, *E. moshkovskii*, *E. dispar*, *E. histolytica*, *E. hartmanni*, *E. coli*, and *E. nuttalli*. The positive samples continued for the identification of *Entamoeba* species with specific primers and amplified based on a previous study (Table 1) (Hamzah et al. 2006; Debenham et al. 2017; Singh et al. 2021). In this study, all the amplification reactions were 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.

RESULTS AND DISCUSSION

We collected 100 fecal samples from Long-tailed Macaque in Bama and Bekol at Baluran National Park (BNP) and 100 fecal samples from Alas Purwo National Park (APNP). The fecal samples were taken from unidentified individuals Long-tailed Macaque. Various nucleated cysts from *Entamoeba* were detected under microscopic examination. In the current study, 53 (53%) fecal samples were positive for *Entamoeba* spp. from BNP and 82 (82%) samples were positive from APNP. All positive samples from the microscopic examination were also confirmed with PCR using universal primers of *Entamoeba* spp. Four *Entamoeba* species were detected from BNP. *Entamoeba coli* was the most dominant species with an overall infection rate of 84.9% (45/53), followed by 58.49% (31/53) of *E. chattoni*, 13.2% (7/53) of *E. moshkovskii*, and 7.54% (4/53) of *E. hartmanni*, as shown in Table 2, among samples, 58.49% (31/53) were mixed infections with 43.39% (23/53) of double infections, 13.2% (7/53) of triple infections and 1.8% (1/53) of quadruple infections. Others, 32.07% (17/53) had a single infection, the detailed data shown in Table 3. All samples showed negative for *E. histolytica*, *E. nuttalli*, and *E. dispar*. Three

Entamoeba species were detected in APNP. *Entamoeba chattoni* was the most dominant species with an overall infection rate of 90% (74/82), followed by 85% (70/82) of *E. coli*, and 8.5% (7/82) of *E. moshkovskii* (Tables 4-5).

The PCR product with 1.5% gel electrophoresis of *E. coli*, *E. chattoni*, *E. moshkovskii*, and *E. nuttalli* from samples of Baluran and Alas Purwo National Park is shown in Figures 1-4.

Table 2. Result of study areas and results for *Entamoeba* spp. in Long-tailed Macaque from Baluran National Park, Indonesia

Location	No. of examined Macaque	Positive no. (%)	PCR						
			<i>E. coli</i>	<i>E. chattoni</i>	<i>E. moshkovskii</i>	<i>E. hartmanni</i>	<i>E. dispar</i>	<i>E. histolytica</i>	<i>E. nuttalli</i>
Bama	50	21 (42)	15 (71.4)	5 (23.8)	1 (4.76)	1 (4.76)	-	-	-
Bekol	50	32 (64)	30 (93.75)	26 (81.25)	6 (18.75)	3 (9.3)	-	-	-
Total	100	53 (53)	45 (84.9)	31 (58.49)	7 (13.2)	4 (7.54)	-	-	-

Table 3. Detailed composition of *Entamoeba* species infection in Long-tailed Macaque in Baluran National Park, Indonesia

Entamoeba species	Number of positive samples (%)		Total
	Bama	Bekol	
Single infection			
<i>E. coli</i>	10	5	15
<i>E. chattoni</i>	1	1	2
Double infection			
<i>E. coli</i> , <i>E. chattoni</i>	3	18	21
<i>E. coli</i> , <i>E. hartmanni</i>	1	1	2
Triple infection			
<i>E. coli</i> , <i>E. chattoni</i> , <i>E. hartmanni</i>	0	1	1
<i>E. coli</i> , <i>E. chattoni</i> , <i>E. moshkovskii</i>	1	5	6
Quadruple infection			
<i>E. coli</i> , <i>E. chattoni</i> , <i>E. moshkovskii</i> , <i>E. hartmanni</i>	0	1	1

Table 4. Result of study areas and results for *Entamoeba* spp. in Long-tailed Macaque from Alas Purwo National Park, Indonesia

Location	No. of examined Macaque	Positive no. %	PCR						
			<i>E. coli</i>	<i>E. chattoni</i>	<i>E. moshkovskii</i>	<i>E. hartmanni</i>	<i>E. dispar</i>	<i>E. histolytica</i>	<i>E. nuttalli</i>
Sadengan	20	15 (75)	12 (80)	16	2	-	-	-	-
Triangulasi	20	16 (80)	14 (87.5)	15	2	-	-	-	-
Bedul	20	16 (80)	15 (93.75)	14	1	-	-	-	-
Rowobendo	20	17 (85)	14 (82.35)	13	1	-	-	-	-
Pancur	20	18 (90)	15 (83.33)	16	1	-	-	-	-
Total	100	82 (82)	70 (85.36)	74 (90)	7 (8.5)	-	-	-	-

Table 5. Prevalence of *Entamoeba* sp. Infection in Alas Purwo National Park, Indonesia

Entamoeba species	Number of positive samples (%)					Total
	Sadengan	Triangulasi	Bedul	Rowobendo	Pancur	
Single infection						
<i>E. coli</i>	0	1	1	1	1	4
<i>E. chattoni</i>	1	1	2	4	4	12
Double infection						
<i>E. moshkovskii</i> + <i>E. coli</i>	2	2	0	0	0	4
<i>E. coli</i> + <i>E. chattoni</i>	7	7	8	10	11	43
Triple infection						
<i>E. coli</i> + <i>E. chattoni</i> + <i>E. moshkovskii</i>	0	0	0	1	2	3

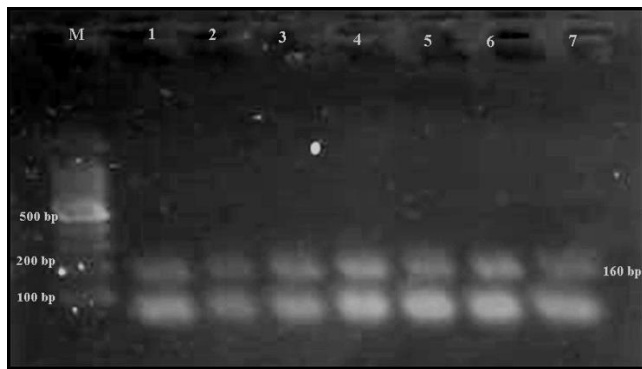


Figure 1. PCR product of *Entamoeba coli* band 160 bp (M: Marker, 1-7: Samples)



Figure 3. PCR product of *Entamoeba moskovskii* band 260 bp (M: Marker, 1-7: Samples)

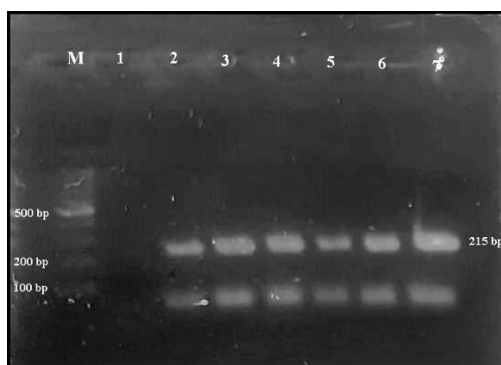


Figure 2. PCR product of *Entamoeba chattoni* band 215 bp (M: Marker, 1-7: Samples)



Figure 4. PCR product of *Entamoeba hartmanii* band 180 bp (M: Marker, 1-7: Samples)

Entamoeba species are the most prevalent intestinal parasites in NHP from various countries (Deere et al. 2019; Zhang et al. 2019; Pu et al. 2020). Our study showed a moderate prevalence of 53% (53/100) and diversity (*E. chattoni*, *E. moshkovskii*, *E. coli*, and *E. dispar*) of *Entamoeba* spp. in Long-tailed Macaques from Baluran National Park, Indonesia. Identification of samples from Alas Purwo National Park showed a high prevalence of 82% (82/100) and three species of *Entamoeba* were detected by molecular tests, *E. chattoni*, *E. coli* and *E. moshkovskii*. The most dominant species of *Entamoeba* spp. in this study is *E. chattoni*, followed by *E. coli* which is the predominant species in Macaques (Feng et al. 2013; Guan et al. 2016) and *E. moshkovskii*. This study demonstrated that Long-tailed Macaques are susceptible to *Entamoeba* infection. Detection and identification of *Entamoeba* spp. on NHP have been reported in Asia, Europe, Africa, and North America, with the highest prevalence in China (Dong et al. 2017; Chang et al. 2019; Zhang et al. 2019; Pu et al. 2020;). The highest prevalence was reported in long-tailed Macaques, with a prevalence of 100% (Feng et al. 2011). Prevalence of *Entamoeba* spp. was reported 57.7% in Formosan Rock Monkey (Chang et al. 2019). However, this study had a lower prevalence than *Entamoeba* spp. was detected in 79% of wild rhesus Macaques (Feng et al. 2011). Approximately 65% and 88.6%, the prevalence of *Entamoeba* spp. in chimpanzee and baboon (Deere et al. 2019). Another study showed a

low number of *Entamoeba* spp. in Macaque with only 9.31% infection rate (Pu et al. 2020). *Entamoeba* transmission easily occurs in humans and non-human primates because it is related to the life cycle. The host is infected with the parasite by ingesting the adult cyst form, then the cyst in the intestinal lumen undergoes excystation, and each nucleus undergoes a double division which develops into a metacystic stage. The metacyst stage develops into a trophozoite with a larger shape and is active, requires food and reproduces. This form of trophozoite remains in the intestinal lumen and penetrates the colon mucosa. The trophozoite stage will multiply by double fission or binary fission and develop into a single-nucleated precyst stage. Furthermore, from the precyst stage, the organism rounds to form a cyst, is a dormant stage, is resistant to food, and is responsible for disease transmission (Manna et al. 2020). Cyst division starts from a single-nucleated cyst, then doubles from one nucleus into two, then divides again into four to eight or more nuclei depending on the species. The cyst stage will come out with the host's feces (Madden et al. 2019; Lastuti et al. 2021).

Among these four species, *E. coli* had the highest infection rate, which had similar results with results from other studies closely related to NHP. The frequency of *E. coli* showed dominant (94%) in Macaques in China (Feng et al. 2013). Other studies reported different results related to the most dominant species of *Entamoeba* in NHP. *E.*

chattoni (88.43%) was the most prevalent of *Entamoeba* spp. in Macaque in China, followed by *E. hartmanni* (88.43%), *Entamoeba coli* (69.87%), and *E. dispar* (58.3%) (Zhang et al. 2019). *Entamoeba chattoni* dominant results were also reported in Taiwan and Southwest China (Feng et al. 2013; Chang et al. 2019). *Entamoeba dispar* showed that it was the most frequently found in experimental Macaques (57.44%) (Pu et al. 2020). In this study, *E. moshkovskii* was detected with a low prevalence. Owl faced monkey, Java langur, and gray langur are also new hosts of *E. moshkovskii* which were previously only detected in wastewater and human samples (Levecke et al. 2010). *Entamoeba hartmanni* was only detected in one sample. *Entamoeba hartmanni* was reported found in captive NHP in Belgium (Levecke et al. 2010) and wild Macaque in China (Zhang et al. 2019).

Mix infection between species of *Entamoeba* has commonly appeared in numerous research of NHP. Our current study showed a diversity of *Entamoeba* spp.: *E. coli*, *E. chattoni*, *E. moshkovskii*, and *E. hartmanni*. Mixed infections of *E. dispar* and *E. coli* were determined In China (Feng et al. 2013; Dong et al. 2017). In Tanzania, more diverse co-infections had been reported (*E. hartmanni*, *E. coli*, and *E. dispar*) (Jirku-Pomajbikova et al. 2016) and Belgium (*E. histolytica*, *E. hartmanni*, *E. polecki* *E. coli*, *E. dispar* and *E. moshkovskii*) (Levecke et al. 2010). The high prevalence of parasites in NHP is due to social behaviour and the dense population in the group (Atabati et al. 2020). The study conducted by Dong et al. (2017) detected the prevalence of *Entamoeba coli* (62%) in non-human primates and potentially zoonotic and concluded that it is necessary to consider the maintenance of animals against transmission between animals as reservoirs and humans. The result is different than other studies. The relative prevalence might be due to seasons, geographical conditions, individual shedding intensity, and food sources (Regan et al. 2014; Adhikari and Dakhil 2018). Evidence has shown that *Entamoeba* could be transmissible between non-human primates and humans. Although the most pathogenic *Entamoeba*, *E. histolytica*, and *E. nuttalli* were not found in this research, other species of *Entamoeba* we found have to pay attention. Based on the study reported by Pham-Duc et al. (2011) showed that human and animal waste in the water of the Nhue River is used intensively for agriculture, besides that socioeconomic factors and personal hygiene determine *E. histolytica* infection. The rate of infection by *E. histolytica* differs between countries, and can be influenced by socioeconomic and sanitary conditions as well as population. Diseases caused by *Entamoeba* spp. are highly endemic in all socio-economically poor communities. The use of wastewater and human and animal waste in agriculture and aquaculture is a common problem in China, South and Southeast Asia and various regions of Africa, especially where water scarcity will become more severe (Hansen et al. 2015). The main sources of water for irrigation in Vietnam are freshwater, wastewater and groundwater. A study in Hanoi, North Vietnam, on the epidemiology and etiology of diarrheal diseases in adults engaged in wastewater agriculture and aquaculture has

shown that *E. coli* and *E. histolytica* that cause diarrhea are the most common pathogens (Pham-Duc et al. 2011).

The role of *E. moshkovskii* in human health is still unclear. Some studies still considered that this species is a symbiotic species, such as reported in Colombia, Yemen, Pakistan, and Bangladesh (Yakoob et al. 2012; Al-Areeqi et al. 2017), but recent studies in India and Bengal reported that this species occurred in a patient with gastrointestinal clinical manifestations as the only possible pathogen (Heredia et al. 2015, Parija and Khairnar 2005). Based on isoenzyme analysis, determined eight cases of human infections with *E. chattoni*. These cases included five workers in a primate quarantine farm, one was a zookeeper, and one which had contact with NHP during a visit in India (Parija and Khairnar 2005). Molecular detection of *E. chattoni* like cysts from humans has also been reported, but the transmission from NHP is still unclear (Robertson et al. 2019). *Entamoeba polecki* organisms are very close to *E. histolytica*, which poses a zoonotic risk to animals and humans (Berrilli et al. 2011; Li et al. 2018; Chihi et al. 2019).

Tourists or park employees of Baluran National Park might be at risk of infection due to water, food and sanitation facilities contamination (Calegar et al. 2016; Ben Ayed and Sabbahi 2017; Atabati et al. 2020). The density of the long-tailed Macaques study was recorded in BNP (Hansen et al. 2015). There were 417 individuals of Long-tailed Macaque in the four focus groups (research areas). The estimated household size was 1.75 km²/group, the average density was 59 individuals/km²/group, and the largest group size was 180. The average group size was 104 people/group (Hansen et al. 2015). In fact, the density of long-tailed macaques was particularly normal, but the food supply from tourist feeding makes the long-tailed Macaques in this tourist attraction very active, which may increase the frequency of contact between people and Macaques. Food feeding from tourists, such as vegetables and fruits, contains essential nutrients and minerals, although also common cause some food-borne pathogenic microorganisms. Some research has reported the contamination of vegetables or fruits with pathogenic microorganisms. The contamination of pathogenic parasites in raw vegetables and fruits is now a global public health concern (Li et al. 2020; Tharmaratnam et al. 2020).

The pathogenicity of *Entamoeba* spp. is strengthened due to the entry of bacteria during infection. The bacteria that often accompany *Aerobacter aerogenes* and *Escherichia coli* (Kantor et al. 2018). Both of these germs strengthen the damaged tissue that forms ulcers and inflammation and neutrophil infiltration. In severe infections, *Entamoeba* can invade the lymphatic system and mesenteric veins. Then it goes to the soft tissues, especially in the right lobe of the liver and causes lesions in the hepatic veins and abscesses in the liver, also known as hepatic amoebic abscesses (Tharmaratnam et al. 2020). Amoebic colitis is a sub-acute form with mild to severe symptoms characterized by mild to severe dysentery diarrhea, with abdominal pain, and watery or bloody diarrhea (Kantor et al. 2018). Mortality can reach 40% if serious complications such as fulminant necrotizing colitis,

toxic megacolon, and perianal fistulizing ulcers occur if the diagnosis and treatment is not appropriate. Amoeboma formation is a rare manifestation, possibly occurring in amebic colitis. Symptoms of the disease present with pain and swelling in the right iliac fossa or with symptoms of intestinal obstruction (Skappak et al. 2014). Amoebic liver abscess (hepatic amoebic abscess) is the most common manifestation of extra-intestinal amoebiasis, characterized by right-sided pleural pain, and pleural effusion occurs when the diaphragmatic surface of the liver is involved (Kantor et al. 2018). According to Matsubayashi et al. (2015), most *Entamoeba* parasites are considered harmless and do not cause disease in humans, except in amoebiasis, which is characterized by inflammation, colonic ulceration and the presence of extra-intestinal pathologies such as liver abscesses in humans and animals caused by *E. histolytica*.

Transmission of parasites from NHP to humans and vice versa occurs due to interactions between humans and NHP. These interactions can be established in several aspects, such as ecology, work, recreation, and research (Fuentes 2012). NHP can act as a reservoir of potential zoonotic diseases and about 25% of emerging diseases in humans originate from NHP (Burgos-Rodriguez 2011). It is necessary to prevent the potential risk in order to improve public health, such as limiting contact with long-tailed Macaque. This could be done by educating tourists to avoid feeding the long-tailed macaque in National Park area. In addition, improving hygiene and sanitation of feces and washing hands is also significant to reduce the risk of peroral spreading (Issa 2014).

In conclusion prevalence of *Entamoeba* spp. (53%, 53/100) was observed in long-tailed Macaques in Baluran National Park Indonesia. In addition, four species (*E. coli*, *E. chattoni*, *E. moshkovskii*, and *E. hartmanni*) were identified, with *E. coli* (84.9%) being the dominant species. Likewise, the prevalence of *Entamoeba* spp. from Alas Purwo National Park showed 82% (82/100) and three species of *Entamoeba* were detected by molecular tests, *E. chattoni*, *E. coli* and *E. moshkovskii* with *E. chattoni* being the most prevalence species. The diversity of *Entamoeba* species and frequent contact between Long tailed Macaque and humans suggests that the potential of *Entamoeba* transmission should be concerned. In addition, further research is needed on epidemiology, zoonotic transmission, and pathogenesis mechanisms for controlling and monitoring potential zoonoses between animals and humans.

ACKNOWLEDGEMENTS

The author thanks to Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia for supporting facilities and Prof. Makoto Matsubayashi, Department of Infectious Diseases Control, Osaka Prefecture University, Japan as a consultant. Thank you to the Head and staff of Baluran National Park and Alas Purwo National Park, East Java, Indonesia, for providing facilities for sampling.

REFERENCES

- Adhikari P, Dhakal P. 2018. Prevalence of gastrointestinal parasite of rhesus macaque (*Macaca mulatta*, 1780) and hanuman langur (*Semnopithecus Entellus* Dufresne, 1797) in Devghat, Chitwan, Nepal. J Inst Sci Technol 22 (2): 12-18. DOI: 10.3126/jist.v22i2.19590.
- Al-Areeqi MA, Sady H, Al-Mekhlafi HM, Anuar TS, Al-Adhroey AH, Atroosh WM, Dawaki S, Elyana FN, Nasr NA, Ithoi I, Lau YL, Surin J. 2017. First molecular epidemiology of *Entamoeba histolytica*, *E. dispar* and *E. moshkovskii* infections in Yemen: Different species-specific associated risk factors. Trop Med Intl Health 22 (4): 493-504. DOI: 10.1111/tmi.12848.
- Atabati H, Kassiri H, Shamloo E, Akbari M, Atamaleki A. 2020. The association between the lack of safe drinking water and sanitation facilities with intestinal *Entamoeba* spp infection risk: A systematic review and meta-analysis. Plos One 15 (11): e0237102. DOI: 10.1371/journal.pone.0237102.
- Ben-Ayed L, Sabbahi S. 2017. *Entamoeba histolytica*. In: Rose JB, Jiménez-Cisneros B (eds). Water and Sanitation for the 21st Century: Health and Microbiological Aspects of Excreta and Wastewater Management (Global Water Pathogen Project). UNESCO, France.
- Berrilli F, Prisco C, Friedrich KG, Di Cerbo P, Di Cave D, De Liberato C. 2011. *Giardia duodenalis* assemblages and *Entamoeba* species infecting nonhuman primates in an Italian zoological garden: Zoonotic potential and management traits. Parasites Vectors 4: 199. DOI: 10.1186/1756-3305-4-199.
- Burgos-Rodriguez A. 2011. Zoonotic disease of primate. Vet Clin Exotic Anim 14: 557-575. DOI: 10.1016/j.cvex.2011.05.006.
- Calegar DA, Nunes BC, Monteiro KJ, Santos JP, Toma HK, Gomes TF, Lima MM, Bôia MN, Carvalho-Costa FA. 2016. Frequency and molecular characterisation of *Entamoeba histolytica*, *Entamoeba dispar*, *Entamoeba moshkovskii*, and *Entamoeba hartmanni* in the context of water scarcity in northeastern Brazil. Memorias do Instituto Oswaldo Cruz 111 (2): 114-119. DOI: 10.1590/0074-02760150383.
- Carrero JC, Reyes-Lopez M, Serrano-Luna J, Shibayama M, Unzueta J, Leon-Sicaire N, de la Garza M. 2020. Intestinal amoebiasis: 160 years of its first detection and still remains as a health problem in developing countries. Intl J Med Microbiol 310 (1): 151358. DOI: 10.1016/j.ijmm.2019.151358.
- Chang AM, Chen CC, Huffman MA. 2019. *Entamoeba* Spp. in wild formosan rock macaques (*Macaca cyclopis*) in an area with frequent human macaque contact. J Wildl Dis 55 (3): 608-618. DOI: 10.7589/2018-04-113.
- Chihai A, Stensvold CR, Ben-Abda I, Ben-Romdhane R, Aoun K, Siala E, Bouratbine A. 2019. Development and evaluation of molecular tools for detecting and differentiating intestinal amoebae in healthy individuals. Parasitology 146: 821-827. DOI: 10.1017/S0031182018002196.
- Cui Z, Li J, Chen Y, Zhang L. 2019. Molecular epidemiology, evolution, and phylogeny of *Entamoeba* spp. Infect Genet Evol 75: 104018. DOI: 10.1016/j.meegid.2019.104018.
- Debenham JJ, Tysnes K, Khunger S, Robertson LJ. 2017. Occurrence of *Giardia*, *Cryptosporidium*, and *Entamoeba* in wild rhesus macaques (*Macaca mulatta*) living in urban and semi-rural North-West India. Parasites Wildl 6: 29-34. DOI: 10.1016/j.ijppaw.2016.12.002.
- Deere JR, Parsons MB, Lonsdorf EV, Lipende I, Kamenya S, Collins DA, Travis DA, Gillespie TR. 2019. *Entamoeba histolytica* infection in humans, chimpanzees and baboons in the greater Gombe ecosystem, Tanzania. Parasitology 146: 1116-1122. DOI: 10.1017/S0031182018001397.
- Dong H, Li J, Qi M, Wang R, Yu F, Jian F, Ning C, Zhang L. 2017. Prevalence, molecular epidemiology, and zoonotic potential of *Entamoeba* spp. in nonhuman primates in China. Infect Genet Evol 54: 21-220. DOI: 10.1016/j.meegid.2017.07.002.
- Feng M, Yang B, Yang L, Fu Y, Zhuang Y, Liang L, Xu Q, Cheng X, Tachibana H. 2011. High prevalence of *Entamoeba* infections in captive long-tailed macaques in China. Parasitol Res 109: 1093-1097. DOI: 10.1007/s00436-011-2351-2.
- Feng M, Cai J, Min X, Fu Y, Qing Xu, Tachibana H, Cheng X. 2013. Prevalence and genetic diversity of *Entamoeba* species infecting macaques in southwest China. Parasitol Res 112: 1529-1536. DOI: 10.1007/s00436-013-3299-1.
- Fu B, Wang J, Fu X. 2022. A rare case of extraintestinal amebiasis. BMC Infect Dis 22: 364. DOI: 10.1186/s12879-022-07348-9.

- Fuentes A. 2012. Ethnoprimatology and the anthropology of the human-primate interface. *Annu Rev Anthropol* 41: 101-117. DOI: 10.1146/annurev-anthro-092611-145808.
- Guan Y, Feng M, Cai J, Min X, Zhou X, Xu Q, Tan, Cheng X, Tachibana H. 2016. Comparative analysis of genotypic diversity in *Entamoeba nuttalli* isolates from Tibetan macaques and rhesus macaques in China. *Infect Genet Evol* 38: 126-131. DOI: 10.1016/j.meegid.2015.12.014.
- Hamzah Z, Petmitr S, Mungthin M, Leelayoova S, Chavalitsheewinkoon-Petmitr P. 2006. Differential detection of *Entamoeba histolytica*, *Entamoeba dispar*, and *Entamoeba moshkovskii* by a single-round PCR assay. *J Clin Microbiol* 44: 3196-3200. DOI: 10.1128/JCM.00778-06.
- Hansen MF, Hariyawan AW, Supriyanto S, Damanik AR. 2015. The interactions between long-tailed macaques (*Macaca fascicularis*) and tourists in Baluran National Park, Indonesia. *J Indones Nat Hist* 3: 36-41.
- Hemmati A, Hooshmand E, Hosseini MJ. 2015. Identification of *Entamoeba histolytica* by molecular method in surface water of Rasht City, Iran. *Iran J Public Health* 44: 238-243.
- Heredia RD, Fonseca JA, Lopez MC. 2015. *Entamoeba moshkovskii* perspectives of a new agent to be considered in the diagnosis of amebiasis. *Acta Trop* 23 (3): 139-145. DOI: 10.1016/j.actatropica.2012.05.012.
- Issa R. 2014. Non-pathogenic protozoa (review article). *Intl J Pharm Pharm Sci* 6: 30-40.
- Jirku-Pomajbikova K, Cepicka I, Kalousova B, Jirku M, Stewart F, Leveck B, Modry D, Piel AK, Petzelkova KJ. 2016. Molecular identification of *Entamoeba* species in savanna woodland chimpanzees (*Pan troglodytes schweinfurthii*). *Parasitology* 143: 741-748. DOI: 10.1017/S0031182016000263.
- Joeseof JA, Sajuthi D, Wijaya A, Sanam MUE. 2018. Endoparasitic diversity in *Macaca Fascicularis* and its zoonotic potential on different weather in Kupang City. *Jurnal Veteriner* 19 (4): 451-459. DOI: 10.19087/jveteriner.2018.19.4.451.
- Kantor M, Abrantes A, Estevez A, Schiller A, Torrent J, Gascon J, Hernandez R, Ochner C. 2018. *Entamoebahistolytica*: Updates in clinical manifestation, pathogenesis, and vaccine development. *Can J Gastroenterol Hepatol* 2018: 1-6. DOI: 10.1155/2018/4601420.
- Komatsu T, Matsubayashi M, Murakoshi N, Sasai K, Shibahara T. 2019. Retrospective and histopathological studies of *Entamoeba* spp. and other pathogens associated with diarrhea and wasting in pigs in Aichi Prefecture, Japan. *Jpn Agric Res Q* 53 (1): 59-67. DOI: 10.6090/jarq.53.59.
- Lastuti NDR, Suwanti LT, Hastutiek P, Kurniawati DA, Puspitasari H. 2021. Molecular detection of *Entamoeba* spp in Long-tailed Macaque (*Macaca fascicularis*) at Baluran National Park, Indonesia. *Mal J Med Health Sci* 17: 85-88.
- Leveck B, Dorny P, Vercammen F, Visser LG, Van Esbroeck M, Vercruysse J, Verweij JJ. 2015. Transmission of *Entamoeba nuttalli* and *Trichuris trichiura* from nonhuman primates to humans. *Emerg Infect Dis* 21: 1871-1872. DOI: 10.3201/eid2110.141456.
- Leveck B, Dreesen L, Dorny P, Verweij JJ, Vercammen F, Casaert S, Vercruysse J, Geldhof P. 2010. Molecular identification of *Entamoeba* spp. in captive nonhuman primates. *J Clin Microbiol* 48: 2988-2990. DOI: 10.1128/JCM.00013-10.
- Li J, Wang Z, Karim MR. 2020. Detection of human intestinal protozoan parasites in vegetables and fruits: A review. *Parasites Vectors* 13: 380. DOI: 10.1186/s13071-020-04255-3.
- Li WC, Geng JZ, Chen C, Qian L, Zhang T, Liu JL, Luo JX, Gu YF. 2018. First report on the occurrence of intestinal *Entamoeba* spp. in pigs in China. *Acta Trop* 185: 385-390. DOI: 10.1016/j.actatropica.2018.06.020.
- Madden GR, Shirley DA, Townsend G, Moonah S. 2019. Case report: Lower gastrointestinal bleeding due to *Entamoeba histolytica* detected early by multiplex PCR: Case report and review of the laboratory diagnosis of amebiasis. *Am J Trop Med Hyg* 101 (6): 1380-1383. DOI: 10.4269/ajtmh.19-0237.
- Manna D, Ehrenkaufer GM, Lozano-Amado D, Singh U. 2020. *Entamoeba* stage conversion: Progress and new insights. *Curr Opin Microbiol* 58: 62-68. DOI: 10.1016/j.mib.2020.09.005.
- Matsubayashi M, Murakoshi N, Komatsu T, Tokoro M, Haritani M, Shibahara T. 2015. Genetic identification of *Entamoeba polecki* subtype 3 from pigs in Japan and characterisation of its pathogenic role in ulcerative colitis. *Infect Genet Evol* 36: 8-14. DOI: 10.1016/j.meegid.2015.08.030.
- Ngui R, Anjal L, Fakhurrazi SA, Lian YLA, Ling LY, Ibrahim J, Mahmud R. 2012. Differentiating *Entamoeba histolytica*, *Entamoeba dispar* and *Entamoeba moshkovskii* using nested polymerase chain reaction (PCR) in rural communities in Malaysia. *Parasites Vectors* 5: 187. DOI: 10.1186%2F1756-3305-5-187.
- Parija SC, Khairnar K. 2005. *Entamoeba moshkovskii* and *Entamoeba dispar*-associated infections in Pondicherry, India. *J Health Popul Nutr* 23: 292-295.
- Pham-Duc P, Nguyen-Viet H, Hattendorf J, Zinsstag J, Dac Cam P, Odermatt P. 2011. Risk factors for *Entamoeba histolytica* infection in an agricultural community in Hanam province, Vietnam. *Parasites Vectors* 4: 102. DOI: 10.1186/1756-3305-4-102.
- Pu LH, Li Z, Zhang YL, Chen J, Yang JF, Zou F. 2020. Prevalence, molecular epidemiology and zoonotic risk of *Entamoeba* spp. from experimental macaques in Yunnan Province, southwestern China. *Parasitol Res* 119 (8): 2733-2740. DOI: 10.1007/s00436-020-06762-9.
- Putra AAWK, Wandia IN, Dwinata IM. 2020. Prevalence and identification of gastrointestinal protozoa in Long-Tail Monkey in Nusa Penida Island. *Buletin Veteriner Udayana* 12 (1): 80-85. DOI: 10.24843/bulvet.2020.v12.i01.p14.
- Regan CS, Yon L, Hossain M, Elsheikha HM. 2014. Prevalence of *Entamoeba* species in captive primates in zoological gardens in the UK. *PeerJ* 2: e492. DOI: 10.7717/peerj.492.
- Robertson L, Clark C, Debenham J, Dubey J, Kváč M, Li J, Ponce-Gordo F, Ryan U, Schares G, Su C, Tsaousis A. 2019. Are molecular tools clarifying or confusing our understanding of the public health threat from zoonotic enteric protozoa in wildlife. *Parasites Wildl* 9: 323-341. DOI: 10.1016/j.jppaw.2019.01.010.
- Singh A, Banerjee T, Khan U, Shukla SK. 2021. Epidemiology of clinically relevant *Entamoeba* spp. (*E. histolytica/dispar/moshkovskii/bangladeshi*): A cross sectional study from North India. *Plos Negl Trop Dis* 15 (9): e0009762. DOI: 10.1371/journal.pntd.0009762.
- Skappak C, Akierman S, Belga S, Novak K, Chadee K, Urbanski SJ, Church D, Beck PL. 2014. Invasive amoebiasis: A review of *Entamoeba* infections highlighted with case reports. *Can J Gastroenterol Hepatol* 28: 355-359. DOI: 10.1155/2014/745130.
- Tachibana H, Yanagi T, Pandey K, Cheng X, Kobayashi S, Sherchand J, Kanbara H. 2007. An *Entamoeba* sp. strain isolated from rhesus monkey is virulent but genetically different from *Entamoeba histolytica*. *Mol Biochem Parasitol* 153 (2): 107-114. DOI: 10.1016/j.molbiopara.2007.02.006.
- Tharmaratnam T, Kumanan T, Iskandar MA, D'Urzo K, Gopee-Ramanan P, Loganathan M, Tabobondung T, Tabobondung TA, Sivagurunathan S, Patel M, Tobbia I. 2020. *Entamoeba histolytica* and amoebic liver abscess in northern Sri Lanka: A public health problem. *Trop Med Health* 48: 2. DOI: 10.1186/s41182-020-0193-2.
- Tuda J, Feng M, Imada M, Kobayashi S, Cheng X, Tachibana H. 2016. Identification of *Entamoeba polecki* with unique 18S rRNA gene sequences from Celebes crested macaques and pigs in Tangkoko Nature Reserve, North Sulawesi, Indonesia. *J Eukaryot Microbiol* 63: 572-577. DOI: 10.1111/jeu.12304.
- Yakoob J, Abbas Z, Beg MA, Naz S, Khan R, Jafri W. 2012. *Entamoeba* species associated with chronic diarrhoea in Pakistan. *Epidemiol Infect* 140 (2): 323-328. DOI: 10.1017/S0950268811000215.
- Zhang Q, Liu K, Wang C, Luo J, Lu J, He H. 2019. Molecular characterization of *Entamoeba* spp. in wild Taihangshan macaques (*Macaca mulatta tcheliensis*) in China. *Acta Parasitol* 64: 228-231. DOI: 10.2478/s11686-019-00026-y.