

Identification of Gastrointestinal Protozoa in Long-Tailed Macaque (*Macaca fascicularis*) in Sabang

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ABSTRACT

This study aimed to determine the types of gastrointestinal protozoa that infested long-tailed macaque (*Macaca fascicularis*) in Sabang Municipality. This study could be used as a basis of consideration for controlling zoonotic diseases caused by gastrointestinal protozoa. This research was conducted at the Laboratory of Parasitology, Faculty of Veterinary Medicine, Universitas Syiah Kuala. This study used 60 samples of *M. fascicularis* faeces collected from two sub-districts, 30 samples of Sukakarya District and 30 samples from Sukajaya District. Samples were examined using the floatation, formol ether, and modified acid-fast ziehl-neelsen method. The results showed that the highest infestation of protozoa in long-tailed macaque from Sukakarya was *Cyclospora cayetanensis* species (73%), followed by the genus *Eimeria* sp. (53%), *Balantidium* sp. (46%) and *Cryptosporidium parvum* species (43%). Long-tailed macaque from Sukajaya was found to be highly infested by *Balantidium* (53%), followed by *Eimeria* sp. (46%), *Cyclospora cayetanensis* (30%) and *Cryptosporidium parvum* (13%). The results exhibited that there were 4 genera or species of gastrointestinal protozoa that attack long-tailed macaque in Sabang City, namely *Eimeria* sp., *Balantidium* sp., *Cyclospora cayetanensis* and *Cryptosporidium parvum* species.

Keywords: *Gastrointestinal protozoa, Cryptosporidium parvum, Macaca fascicularis, Sabang, Cyclospora cayetanensis*

1. INTRODUCTION

Sabang island is located in the north of Sumatra Island and is part of Aceh Province. Sabang has great potential in developing as one of world destinations of tourism. This is due to the natural beauty that can spoil tourists who come to this city [1]. Not only for its natural beauty, Sabang City is also known as habitat for long-tailed macaque (*Macaca fascicularis*), and it becomes one of the main attractions in tourist areas. The long-tailed macaque is a primate that has a very close relationship with humans. The population of long-tailed macaque is spreaded across regions of Indonesia [2]. These primates are often used as pets and as experimental animals. Long-tailed macaques often interact directly with humans, and as a result they have potential to transmit zoonotic diseases to humans [3].

Parasitic disease, caused by worms or protozoa, can attack humans or animals, including primate species. Parasitic disease in primates plays an essential role in the primate group's population, both in-situ and ex-situ [4]. Protozoa are unicellular organisms that can only be seen microscopically. Gastrointestinal protozoan infestation occurred by ingesting of food or drink that has been contaminated by oocysts or by direct transmission. Gastrointestinal protozoa can cause several symptoms, such as abdominal discomfort, diarrhea, vomiting, and fever [5].

The most common gastrointestinal protozoa in primates are *Balantidium* sp, *Eimeria* sp, *Entamoeba* sp, *Enteromonas* sp, *Blastocystis* sp, *Giardia* sp, and *Pseudolimax* sp and *Trichomonas* sp [6]. Irene *et al.* [7] have conducted research on the Sumatran orangutan (*Pongo abelii*) and several protozoa were found including

Entamoeba sp, *Entamoeba histolyca*, *Blastocystis hominis*, *Enteromonas hominis*, and *Balantidium coli*. Rahmi *et al.* [4] have conducted research on gastrointestinal parasites in long-tailed monkeys (*Macaca fascicularis*) in Weh island, Sabang Nature Park, and found that from 25 fecal samples, 12% were positive with *Eimeria*. sp. In this study, we would like to identify the types of gastrointestinal protozoa infested long-tailed monkeys, and this results could provide information about protozoal infection occurred in long-tailed monkeys in Sabang.

2. MATERIALS AND METHODS

This research was conducted at the Laboratory of Parasitology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh. The research sample used was 60 fresh feces from *M. fascicularis* obtained from Sukakarya dan Sukajaya subdistricts, and then added with 10% formalin. This research was a descriptive study using qualitative methods, namely Floation, Formol-ether, and Ziel-Neelshen method. These methods were intended to identify the types of gastrointestinal protozoa infecting *M. fascicularis*. The results obtained will be presented in tabular form and microscopic images.

2.1 FloationMethod

In this method, three grams of faecal samples were put into the mortar, distilled water was added and then the samples were homogenized. The homogenized samples were then transferred into ¾ volume of a centrifuge tube, and centrifuged at 1500 rpm for 5 minutes. The supernatant was then removed, and the sheater sugar solution was added until the volume is ¾ of the tube. The samples were re-centrifuged at 1500 rpm for 5 minutes. The tube was then placed on the tube rack perpendicularly, sheater sugar solution was added by using a pipette until the surface becomes convex. The samples were then incubated for three minutes. Afterward, the cover glass was carefully attached to the convex liquid surface, and the samples were examined under a microscope with an objective magnification of 40x [8].

2.2 Formol-Ether Sedimentation Examination

This method used 0.5 grams faecal samples and was put into a beaker glass which contained 7 ml of 10% formalin and then homogenized. The faecal sample was transferred into a centrifuge tube and 3 ml of ether was added. The samples were then homogenized and centrifuged for 3-5 minutes at 1500 rpm. After centrifugation, the supernatants and debris were removed. The sediment was dissolved with 10% formaldehyde sufficiently. The samples were placed on a glass object and observed under a microscope [9].

2.3 Modified Fast Acid Ziehl-Neelsen

In this method, 2-3 drops of faeces were placed on object glass and flattened as thin as possible, as if making a blood smear, then dried. The samples were then fixed in methanol for 3 minutes. Carbol fuchsin was added evenly and dried for 15-20 minutes, then washed with running water. Afterward, acid alcohol was added, dried for 15-20 seconds, and re-washed with running water. Then, malachite green or methylene blue was added for 30-60 seconds and washed under running water. The samples were dried and observed under a microscope with a magnification of 40x or 100x [10].

3. RESULTS AND DISCUSSION

The results of 60 samples of *M. fascicularis* faeces in Sabang City (Sukakarya and Sukajaya Districts) showed that four genera or species of gastrointestinal protozoa infected the long-tailed macaque, namely *Eimeria* sp., *Balantidium* sp., *Cyclospora cayetanensis* and *Cryptosporidium parvum* (Table 1).

Table 1. The prevalence of protozoal infections in *Macaca fascicularis* in Sabang City

Sample	Genera/ Species	Case
n = 60	<i>Eimeria</i> sp.	30 (50%)
	<i>Balantidium</i> sp.	30 (50%)
	<i>Cyclospora cayetanensis</i>	31 (51.67%)
	<i>Cryptosporidium parvum</i>	17 (28.33%)

From Table 1, it was shown that the prevalence rate of gastrointestinal protozoa from the four genera or species that infected long-tailed macaque in Sabang City was high. The highest prevalence was from *Cyclospora cayetanensis* (51.67%), followed by *Eimeria* sp. (50%), *Balantidium* sp. (50%) and *Cryptosporidium parvum* (28.33%). The high prevalence of protozoa infestation could be because of these primates live in the wild and never been given anti-protozoal drugs. Also probably due to low awareness and regulations by local authorities and local communities. Lack of food source could also resulted in having a poorly nutritious diet [11]. Most of long-tailed macaque in Sabang Municipality forage by going to the streets and sometimes even to residential areas, causing high opportunity of being trasmitted infectious diseases, including gastrointestinal protozoal infections. Observations in the field showed that several long-tailed macaques in Sabang Municipality had unique behavior, for instance, picking out another individual's anus and then putting his hand in his mouth. This behavior could be one of the factors in the high prevalence of protozoal infestation in long-tailed macaque in Sabang

Municipality. The prevalence of protozoal infestation in each subdistrict is provided in Table 2 and Table 3.

Table 2. The prevalence of protozoal infections in *Macaca fascicularis* faeces in Sukakarya Subdistrict.

Sample	Genera/Species	Case
n = 30	<i>Eimeria</i> sp.	16 (53%)
	<i>Balantidium</i> sp.	14 (46%)
	<i>Cyclospora cayetanensis</i>	22 (73%)
	<i>Cryptosporidium parvum</i>	13 (43%)

Table 3. The prevalence of protozoal infections in *Macaca fascicularis* feces in Sukajaya Subdistrict.

Sample	Genera/Species	Case
n = 30	<i>Eimeria</i> sp.	14 (46%)
	<i>Balantidium</i> sp.	16 (53%)
	<i>Cyclospora cayetanensis</i>	9 (30%)
	<i>Cryptosporidium parvum</i>	4 (23%)

Based on Table 2, the prevalence of protozoa infested 30 samples of long-tailed macaque in Sukakarya subdistrict was from *Cyclospora cayetanensis* species (73%), followed by the genus *Eimeria* sp. (53%), *Balantidium* sp. (46%) and *Cryptosporidium parvum* (43%). Table 3 showed that 30 samples of long-tailed macaque faeces in Sukajaya subdistrict were highly infested by *Balantidium* sp. (53%), followed by *Eimeria* sp. (46%), *Cyclospora cayetanensis* (30%) and *Cryptosporidium parvum* (13%). The forest geographic condition in Sukakarya and Sukajaya subdistricts is quite similar so the protozoa found in long-tailed macaque in these two districts were also similar.

The results of this study are in line with the results of a research conducted by Ekanayake *et al.* [12]. This group reported that protozoal infections in wild primates in Polonnaruwa, Sri Lanka were caused by *Balantidium coli*, *Entamoeba coli*, *Chilomastix spp.*, *Entamoeba histolyca*, *Entamoeba hartmanni*, and *Cryptosporidium* sp. This result is also aligned with Rahmi *et al.* [4] where this group found 12% of *Eimeria* sp. oocysts in macaque in Pulau Weh Nature Park.

The highest prevalence of protozoal infections in macaque in Sukakarya subdistrict was caused by *Cyclospora cayetanensis* (73%), this could be due to oocysts of *Cyclospora* sp. needs 7-15 days to sporulate with an optimum temperature of 5-25 °C [13]. The forest atmosphere in Sabang City is humid and supported the survival cycle of *Cyclospora* sp. Moreover, this could cause a large amount of *Cyclospora* sp. in the digestive tract of *M. fascicularis*. In addition, the water

contaminated with faeces of *M. fascicularis* can also potentially transmit the disease to other macaques. The long-tailed macaques in Sukakarya District, precisely at Kilometer Zero, are usually consume their food close to the streets, and also expectfoods from tourists. Many of these primates scavenged for leftover foods from tourists in the trash cans, which can also be a factor in the high prevalence of *Cyclospora* sp. infection. Wirawan *et al.* [14] reported that cross-infection between individuals could occur due to interactions when consuming water and food together in one area.

The highest prevalence rate of protozoa infecting long-tailed macaques in Sukajaya subdistrict was *Balantidium* sp. (53%) (Table 3). Selian *et al.* [15] stated that *Balantidium coli* are commonly found in primates. The amount of *Balantidium* sp. infestation could be due to transmission from other infected macaque, a polluted environment, and also contaminated water. The long-tailed macaques in Sukakarya and Sukajaya Districts have been infected bythe same types of protozoa, the only difference is the level of infection caused by each type. The prevalence rate of *Balantidium* sp. infestation in these two subdistricts was similar, which could be attributed to the present of boars in the forest of these districts that could potentially transmit balantidiosis to long-tailed macaque or vice versa. Agustina *et al.* [16] has carried out research related to protozoa in the digestive tract of pigs, and the results indicated that *Balantidium* sp. was found in 61.2% ofpigs sold in traditional markets in Bali.

The prevalence rate of *Eimeria* sp. also showed no difference between two districts, probably due to similar geographic condition of the forest which allows the spread of these protozoa in both subdistricts.

Cyclospora cayetanensis and *Cryptosporidium parvum* infections indicated that these protozoa were found mostly in long-tailed macaque (in Sukakarya subdistrict compared to Sukajaya subdistrict. This condition might be caused by the contaminated environment with oocysts.

Sukakarya subdistrict has more visitors than Sukajaya subdistrict, and tourists often spoiled the macaque by feeding them. This habit could cause oocystic contamination in food, soil and drinking water which resulted in infection among macaque group in Sukakarya District. Oocysts of protozoa found in long-tailed macaque faeces examination in two sub-districts in Sabang Municipality can be seen in Figure 1 below.

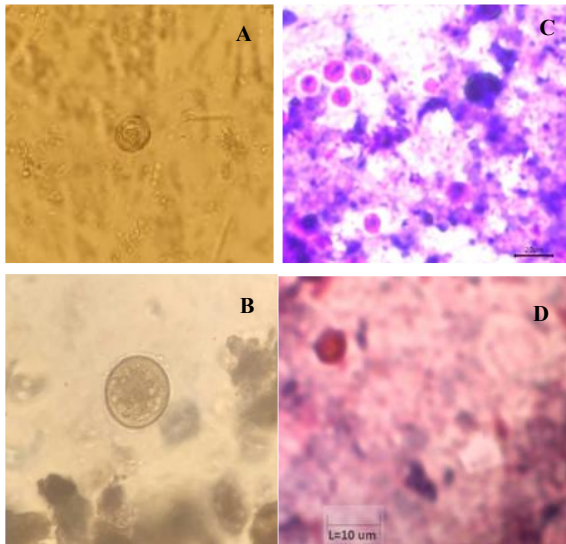


Figure 1. Examination of long-tailed monkey faeces (A). Oocyst *Eimeria* sp. (40x) (B). *Balantidium* sp. Cysts (40x) (C). *Cyclospora cayetanensis* oocyst (100x) (D). *Cryptosporidium parvum* oocyst (100x)

Azmy *et al.* [17] conducted a study of 100 soil samples examined around the landfill in Denpasar and found that 5% of the soil studied contained oocysts from the Coccidia family. He described that the tropical environment was the optimum environment for protozoan development. Long-tailed macaque live in groups where one group consists of males and females [18]. MacIntosh *et al.* [19] stated that animals living in the same population have a high likelihood of disease transmission among these animals.

Symptoms caused by infection of *Eimeria* sp. oocysts were diarrhea, bloody faeces and the presence of mucus. The stool could be red to black and died epithelial cell could be seen microscopically [20]. Although the faeces of long-tailed monkeys did not show any symptoms such as blood, the results showed oocysts of *Eimeria* sp. sp. was highly presented in stool samples.

Protozoal infections of other genera such as *Balantidium* sp. (Figure B) could be identifies by the presence of cysts in long-tailed macaquefaeces samples in two sub-districts in Sabang Municipality. In the research of Selian *et al.* [15], 40% of protozoa such as *Balantidium coli* were found in the faeces of the Sumatran orangutan (*Pongo abelii*) in the Jantho Pine Nature Reserve, Aceh Besar District.

The transmission of protozoal infestations in this study occurred through contaminated food withoocysts in the soil. Research conducted by Lestari and Mulyowati [21] found two trophozoid *Balantidium coli* with oval and gray-green characteristics on the body of the *Chrysomya megachepala* fly which was collected at the Surakarta Legi Market.

Cyclospora cayetanensis oocysts (Figure C) were also found in long-tailed macaque faeces in Sabang Municipality.

Cyclospora cayetanensis oocysts are 8-10 μ m in size and round in shape, have very thin walls (<1 μ m) and are colorless [22]. The diameter of the *Cyclospora* oocysts that can be obtained through the stool examination reaches an average of 8-10 μ m which indicates that these oocysts are oocysts of *Cyclospora cayetanensis*. The transmission of *Cyclospora cayetanensis* could be through contamination of food, water, and soil with oocysts from *Cyclospora cayetanensis*. Wahdini *et al.* [23] examined student stool samples where two people were positive for *Cyclospora* sp. This could be attributed to poor hygiene of the students in washing the fruit. *Cyclospora cayetanensis* in the digestive tract destroyed epithelial cells in the duodenum and jejunum [22]

The results of this study also indicated that long-tailed macaque have been infected by *Cryptosporidium parvum* oocysts (Figure D) in each sub-district in Sabang Municipality. Protozoa of the genus *Cryptosporidium* are often found in wild primates and can potentially transmitted to humans or livestock [24]. Long-tailed macaque stool samples showed a consistency of liquid (predominantly containing water) and mushy (solid-like but not hard) which indicates that the monkey had diarrhea. According to Wijayanti [25], Cryptosporidiasis are a zoonotic disease caused by *Cryptosporidium* where the parasite infects the small intestine causing acute diarrhea. Transmission occurs through intermediate hosts such as rats or environments that have been contaminated with oocysts such as soil and water.

The oocysts of the genus *Cryptosporidium* are 3-8 μ m in diameter, round or oval in shape. *Cryptosporidium parvum* oocysts have an oval shape, are round and range in diameter (4.6 - 5.4 x 3.8 -4.7 μ m) [26]. The average size of the oocysts obtained in this study was (4-8 μ m) in diameter. Oocysts from *Cryptosporidium* have been widely distributed in Indonesian waters, such as in the sea, ponds or rivers. Oocysts can survive in humid environmental conditions, cold environments, and can even survive in chlorinated water [25, 27]. Given that the geographical conditions of Sabang City are surrounded by oceans, there is a possibility for *Cryptosporidium* oocyst contaminates the waters in Sabang City and could be a potential transmission to the community, both local people and tourists. Maryanti *et al.* [28] described that *Cryptosporidium* infection in humans usually occurs in humans under five years of age. Wijayanti [25] also stated that the prevalence of diarrhea caused by *Cryptosporidium* in infants and children has reached 5 - 15%, but it does not rule out that adults could also be infected.

4. CONCLUSION

Based on the results of the research conducted, four types of protozoa was infected long-tailed macaque, namely *Eimeria* sp., *Balantidium* sp., *Cyclospora cayetanensis* and *Cryptosporidium parvum* in two sub-districts in Sabang Municipality. In Sukakarya subdistrict, the highest infestation of protozoa was caused by *Cyclospora cayetanensis* with 73% prevalance rate, while in Sukajaya

subdistrict the highest infestation was *Balantidium* sp. with 53% prevalence rate.

AUTHORS' CONTRIBUTIONS

All authors equally contributed to the preparation and editing of the manuscript.

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