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Research Article

The Gastrointestinal Parasites in Habituated Group of Sulawesi Black-crested Macaque (*Macaca nigra*) in Tangkoko, North Sulawesi

Sitti Aisyah May Wulandari[1], Dyah Perwitasari-Farajallah[1,2]*, Erni Sulistiawati[3]

1) Animal Biosciences Graduate Program, Department of Biology, Faculty of Mathematics and Natural Sciences, IPB University, Jl. Raya Dramaga, Bogor, West Java, 16680, Indonesia.
2) Primate Research Center, IPB University, Jl. Lodaya II/5, Bogor, West Java, 16151, Indonesia.
3) Vocational School Study Program, IPB University, Jl. Kumbang No 14, Bogor, West Java, 16153, Indonesia.
* Corresponding author, email: witafar@apps.ipb.ac.id

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ABSTRACT

The Sulawesi black-crested macaque (*Macaca nigra*) is an endemic primate of North Sulawesi that is categorized as critically endangered (IUCN 2015). Endoparasite contributes to the decline of *M.nigra*. Therefore, this study aims to determine the prevalence of endoparasites in the Sulawesi black-crested macaque (*M. nigra*). We collected 80 fresh fecal samples representing all sex from the two habituated groups. We analyzed them using the direct examination technique (0.9% NaCl, iodine, methylene blue) and flotation technique with the modified McMaster test. A total of 15 endoparasite taxa were recorded and 78 of 80 samples were infected with at least one or several endoparasite taxa. Around 93.75% (75/80) samples were positive for protozoa (*Balantidium* sp., *Entamoeba* sp., *Giardia* sp., and *Isospora* sp.) and 88.75% (71/80) samples were positive for helminths (*Ancylostoma* sp., *Strongyloides* sp., *Haemonchus* sp., *Trichuris* sp., *Trichostrongylus* sp., *Ascarid* sp., *Diphyllobothrium* sp., *Echinococcus* sp., *Hymenolepis* sp., *Schistosoma japonicum* and *Schistosoma mekongi*). The abundance of protozoa was higher than helminth, although the number of helminth taxon (11) was higher. The average temperature and monthly rainfall did not affect the number of endoparasites (EPG). The prevalence was higher in females than males due to different social styles; female crested macaques are more tolerant than males. The group with a larger number of individuals had a higher prevalence of endoparasites. These results confirm the presence and high diversity of gastrointestinal endoparasites in *M. nigra*, which can help to understand transmission dynamics and zoonotic potential, as well as to consider conservation policies.

INTRODUCTION

The Sulawesi black-crested macaque (*Macaca nigra*) is an endemic primate of North Sulawesi that is categorized as critically endangered (IUCN 2015). The highest population is in Tangkoko with a density of 15 individuals/km^2 (Maneasa et al. 2021). Due to anthropogenic pressures such as hunting and habitat fragmentation, their population has decreased due to the loss of their natural habitat (O'Brien & Kinnaird 1997; Melfi 2010). Humans also can reduce the primate population density by threatening the primate's health through direct or indirect pathogen transmission due to their close phylogenetic proximity (Wolfe et al. 2007).

Humans contact *M. nigra* when they keep them as a pet or when humans enter their habitat to engage in illegal activities (Palacios et al. 2012). Protozoa and nematodes have been found in eight *Macaca* species in Sulawesi that are kept as pets by humans (Jones-Engel et al. 2004). Parasite transmission is also influenced by several factors such as climate, habitat type, and sex of the host (Benavides et al. 2012; Chapman et al. 2006). Female and male primates have behavioral differences (Duboscq et al. 2013) that may influence the probability of parasite encounter as the previous study showed that climate and home range also affect the presence of endoparasites (Wenz-Mücke et al. 2013; Boundenga et al. 2018).

As an endangered species, they become vulnerable to many threats including zoonotic disease. However, there have been no studies that investigate the presence of endoparasites in habituated *M. nigra* by biological and environmental factors. The information obtained can help to understand the parasite-host dynamic to avoid the pathogen transmission that can lead to the *M. nigra* population decline. Therefore, this study aims to analyze the prevalence of gastrointestinal parasites from different genders and groups through different seasons to fill the knowledge gap.

MATERIALS AND METHODS

Materials

Fecal samples were collected from April 2018 to March 2019 in the Tangkoko, Bitung, North Sulawesi, Indonesia (1N 32'39", 125E 12'42"). A total of 80 fecal samples were collected every month during wet and dry seasons from two habituated groups (R1, n= 120; PB1B, n = 79). The samples were collected immediately after defecation and sprayed with 10% formalin (Gillespie 2006). Both groups are habituated and individually identifiable. The collection of environmental data in the form of temperature and rainfall was carried out every day at the same time using a thermometer and ombrometer.

Methods

Endoparasite Examination and analysis

The examination and identification of parasites were carried out from February to November 2021 at the Laboratory of Biosystematics and Animal Ecology, Department of Biology, Bogor Agricultural University. Fecal samples were examined using two methods (direct examination and flotation with the modified McMaster test) to maximize the detection of all possible endoparasites.

Direct examination: A small amount or about two milligrams of fecal for each sample was placed in three different object glasses. Each object glass was given 1-2 drops of a different solution (0.9% NaCl, iodine, and methylene blue) then mixed evenly before being covered with a coverslip. The slide was observed under a microscope with 10x and 40x magnification (Gillespie 2006).

Flotation method: Two grams of fecal sample were mixed with 28 ml of saturated salt solution. The precipitate was then pipetted into the *McMaster* glass until the two chambers are filled. The sample was then allowed to stand for five minutes before examining the number of eggs using a microscope with 10x and 40x magnifications. A saturated salt solution was obtained by adding 400 g of NaCl into 1.000 mL to get a saturated salt solution with a concentration of 40% and SG 1.18, which was measured using a hydrometer (WHO 2019).

The eggs/endoparasite cysts found were then identified through photos taken with 10x and 40x magnifications using a camera-embedded microscope Olympus CX31LEDRFS1. The identification was based on morphological characteristics of eggs/cysts such as size, shape, color, stage of development, and other unique characteristics of each species according to Chitwood et al. (1950), Cuomo et al. (2009), WHO (2019), and Zajac and Conboy (2012).

Calculation of Endoparasites Prevalence and Egg per gram (EPG)

Parasite prevalence was calculated by dividing the number of each sample with one or more endoparasites by the total number of samples examined and written in percentage (McKenna & Dohoo 2006), using the following formula:

$$\text{Prevalence} = \frac{\text{The number of each sample with one or several parasites}}{\text{The number of all samples examined}} \chi\ 100$$

The quantitative examination of gastrointestinal parasites was performed by flotation with a modified *McMaster* test. Following Zajac and Conboy (2012), the eggs/cysts found in both chambers within the grid of the *McMaster* glass were counted and multiplied by 50 to obtain the number of each parasite per gram of feces (EPG) and estimate the infection rate in each host (Thienpont et al. 2003).

Data Analysis

The significance of all parasitic taxa prevalence was determined using Kruskal-Wallis and the prevalence of endoparasites between sexes and different groups were compared using the Chi-square test (χ^2). We computed the parasite abundance of each month through a year with monthly rainfall and temperature using the Pearson and Spearman correlation. The whole analysis was carried out using the R program (R Core team 2018) with a confidence level of 0.05.

RESULTS AND DISCUSSION

Diversity and Prevalence of Endoparasites

A total of 15 endoparasite taxa were recovered from the stool samples. Almost all of the samples (78/80) were infected at least with one endoparasite taxon, which are nematodes (A1-F1), cestodes (A2-C2), trematodes (A3-B3), and protozoa (A4-D4) (Figure 1). Compared to data from the previous studies (Jonas & Melfi, in Hilser et al. 2013; Jones-Engel et al. 2004), to our knowledge, this is the first report of *Diphyllobothrium* sp., *Echinococcus* sp., *Schistosoma mekongi*, *Giardia* sp., and *Isospora* sp. found in *Macaca nigra*.

Primates are generally contaminated with gastrointestinal endoparasites through direct transmission from the soil, water, and zoonotic links in their natural habitat (Klaus et al. 2017), Invasion then occurs when the host ingests something that has been contaminated with endoparasite eggs (Cuomo et al. 2009). The Sulawesi black-crested macaque spends 60% of their daily activity on the ground looking for food than on trees, making them a semiterrestrial primate (O'Brien & Kinnaird 1997). The prevalence of nematodes in terrestrial primates was higher than in arboreal primates (Huffman et al. 2013).

Statistical analysis showed a significant difference between the total prevalence of all endoparasite species found (X^2 = 422.14, df = 14, p-value <2.2e-16). The protozoa group had the highest prevalence of 93.75% (75/80), followed by nematodes 86.25% (69/80), cestodes 26.25% (21/80), and trematodes 26.25% (21/80). This result shows that protozoan parasites had higher prevalence and abundance, in which *Balantidium* sp. and *Entamoeba* sp. were responsible for most of the infection (Table 1). In contrast, *Trichuris* sp. and *Diphyllobothrium* sp. from the worm group was not found in the McMaster test. We can assume that the abundance of these parasites was low.

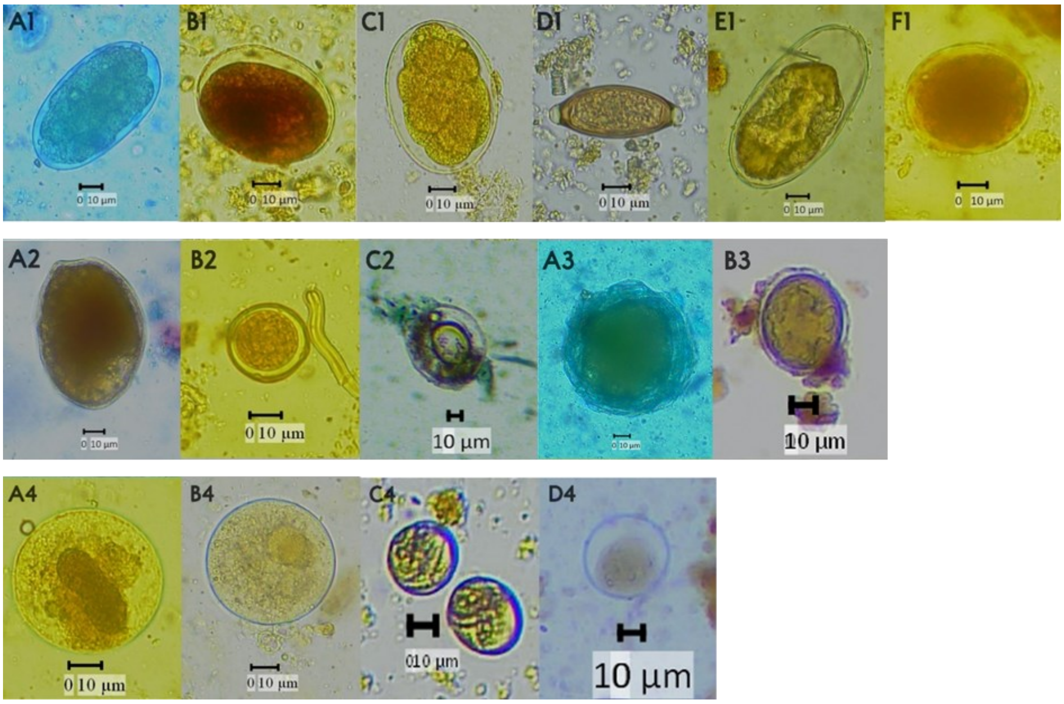

Figure 1. Endoparasites found in the fecal of *M. nigra* using saline, iodine, methylene blue and flotation methods. A1. *Ancylostoma* sp., B1. *Strongyloides* sp., C1. *Haemonchus* sp., D1. *Trichuris* sp., E1. *Trichostrongylus* sp., F1. *Ascarid* sp., A2. *Diphyllobothrium* sp., B2. *Echinococcus* sp., C2. *Hymenolepis* sp., A3. *Schistosoma japonicum*, B3. *Schistosoma mekongi*, A4. *Balantidium* sp., B4. *Entamoeba* sp., C4. *Giardia* sp. and D4. *Isospora* sp.

Table 1. The endoparasites prevalence and infection state of Sulawesi black-crested macaque.

No.	Endoparasite taxon	Number of positive samples	Prevalence (%)	*Mean EPG±SE	Infection
	Nematodes				
1	*Ancylostoma* sp.	44	55	62.50±20.71	light
2	*Strongyloides* sp.	38	47.5	71.43±12.05	light
3	*Haemonchus* sp.	31	38.8	62.50±8.33	light
4	*Trichostrongylus* sp.	20	25	50.00±6.52	light
5	*Ascarid* sp.	43	53.8	40.00±23.02	light
6	*Trichuris* sp.	3	3.75	0.00±0.00	-
	Cestodes				
1	*Diphyllobothrium* sp.	7	8.75	0.00±0.00	-
2	*Hymenolepis* sp.	6	7.5	50.00±5.61	light
3	*Echinococcus* sp.	8	10	50.00±5.61	light
	Trematodes				
1	*Schistosoma japonicum*	11	13.8	50.00±6.52	light
2	*Schistosoma mekongi*	6	7.5	50.00±4.16	light
	Protozoa				
1	*Entamoeba* sp.	64	80	497.0±596.7	light
2	*Balantidium* sp.	66	82.5	1101±1411.9	moderate
3	*Giardia* sp.	8	10	75.00±17.54	light
4	*Isospora* sp.	4	5	50.00±11.23	light

*mean EPG is calculated using data from modified *McMaster* test only. Standard errors were shown in the table.

A previous study of eight macaque species in Sulawesi also showed that protozoan has a higher prevalence than nematodes (Jones-Engel. et al 2004). The high prevalence is probably because these amoebas have a small size, large feeding strategy, and are able to make cysts (Schuster & Ramirez-avila 2008). The high prevalence of the protozoan group may indicate that the water *Macaca nigra* drunk is contaminated since protozoa are water-based parasites (Finlay et al. 2013).

Endoparasite Prevalence Between Different Gender

The prevalence between male and female *M. nigra* was not significant (p = 0.33). For both sexes, *Balantidium* sp. and *Entamoeba* sp. have a high prevalence (Table 2). For other taxa, females had a slightly higher prevalence than males, especially for helminths, including *Strongyloides* sp. (50 vs 42,5), *Trichostrongylus* sp. (32.5 vs 20), *Ascarid* sp. (62.5 vs 42,5), *Trichuris* sp. (5 vs 2.5), *Diphyllobothrium* sp. (12.5 vs 7.5), *Hymenolepis* sp. (10 vs 5), *Schistosoma japonicum* (15 vs 12.5), and *Giardia* sp. (12.5 vs 7.5).

This result may be due to the different behavior between female and male *Macaca nigra* including social style and diet. The females are engaged in physical contact such as grooming and playing with others more often than males (Sueur et al. 2011; Duboscq et al. 2013). Furthermore, the matrilineal system that *M. nigra* adopt makes females accompanied by an infant for most of their daily activity. The degree of second-hand contact then increases via their offspring (Mul et al. 2007). Meanwhile, males *M. nigra* rarely make physical contact because they prefer to rest more (O'Brien & Kinnaird 1997).

Regarding dietary behavior, *M. nigra* females significantly eat more often than the males measured by percentage of time feeding (3.9 vs 2.9) (O'Brien & Kinnaird 1997). Females primates also forage more than the males due to their duty as mothers that have to feed their offspring (Bicca-Marques 2003). Therefore, females *M. nigra* have a higher susceptibility to parasite infection. This aligns with previous research reporting that females are more likely to be infected with a higher parasite prevalence, more severe symptoms, and slower immune development than males (Escobedo et al. 2010; Mul et al. 2007).

Table 2. Endoparasite prevalence (helminth and protozoa) between gender.

Parasites taxa	Sex				Comparison test	
	Male		Female		X	P-Value
	N/40	P(%)	N/40	P(%)		
Helminth						
Ancylostoma sp.	22	55	22	55	0.81	0.36
Strongyloides sp.	17	42.5	21	50	0.20	0.65
Haemonchus sp.	18	45	13	35	0.46	0.49
Trichostrongylus sp.	8	20	12	32.5	1.03	0.30
Ascarid sp.	17	42.5	26	65	2.45	0.11
Trichuris sp.	1	2.5	2	5	0.00	1.00
Diphyllobothrium sp.	3	7.5	4	10	0.00	1.00
Hymenolepis sp.	2	5	4	10	0.18	0.67
Echinococcus sp.	5	12.5	3	7.5	0.01	0.70
S.japonicum	5	12.5	6	15	0.00	1.00
S.mekongi	4	10	2	5	0.18	0.67
Protozoa						
Giardia sp.	3	7.5	5	12.5	0.13	0.70
Entamoeba sp.	32	80	32	80	0.00	1.00
Balantidium sp.	34	85	32	80	0.08	0.76
Isospora sp.	3	7.5	1	2.5	0.26	0.60

*Notes: N = Number of samples; P = Prevalence.

Although females are more susceptible to infection, there are several types of parasites that are higher in males *M. nigra*. This is probably because males still have possible transmissions through food contamination and physical contact although they rarely occur, such as fighting and grooming (Engelhardt et al. 2017). Thus, transmission from others possibly happens during these contact. However, male and female primates generally still show the same response when they were infected with parasites, which is reducing activity and increasing duration of rest (Ghai et al. 2015).

Endoparasite Prevalence Between Group

The results show that *Balantidium* sp. has a higher prevalence among other taxa for both groups (87.5% and 77.5%). Meanwhile, for the other ten taxa, the R1 group has a higher prevalence (Table 3). However, the significant differences in prevalence occur only for *Strongyloides* sp. (X2 = 12.80, df=1 $p<0.0003$), which is higher in the R1 group than in PB1B. The incidence in the R1 population may be related to the high contact rate with humans as intermediate hosts for some endoparasites as the home range of R1 is in the recreation areas of Tangkoko. It was reported by Saroyo (2010) that 53.8% of visitors come to the recreation area to see *Macaca nigra*.

The presence of humans in the primate's habitat can cause behavioral changes; they spend more time on the ground and eat more food from humans, this change is correlated with parasite infection (Wenz-Mücke et al. 2013). Human and nonhuman primates are known to share a wide range of gastrointestinal parasites because their phylogenetic proximity will increase the chance of pathogen's exchange (Wolfe et al. 2007). According to Jones-Engel et al. (2004) and Hasegawa et al. (1992), pet macaques have been infected with parasites commonly found in human populations in Sulawesi and the endoparasites burden were higher in pets than wild *M. nigra*. Thus, the endoparasites may be transmitted by other species or humans which live sympatrically with *Macaca nigra*.

Table 3. Endoparasite prevalence between different groups.

Endoparasites	Group				Comparison test	
	R1		PB1B		X2	P-value
	N/40	P(%)	N/40	P(%)		
Helminth						
Ancylostoma sp.	22	52.5	22	55	0.00	1.00
Strongyloides sp.	28	67.5	10	25	12.80	0.0003
Haemonchus sp.	12	32.5	19	47.5	1.30	0.25
Trichostrongylus sp	13	35	7	17.5	2.32	0.12
Ascarid sp.	24	60	19	47.5	0.45	0.50
Trichuris sp..	2	5	1	2.5	0.00	1.00
Diphyllobothrium sp.	5	12.5	2	5	0.62	0.42
Hymenolepis sp.	5	12.5	1	2.5	1.62	0.20
Echinococcus sp.	6	15	2	5	1.25	0.26
S.japonicum	6	15	5	12.5	0.00	1.00
S.mekongi	3	7.5	3	7.5	0.00	1.00
Protozoa						
Giardia sp.	2	5	6	15	1.25	0.26
Entamoeba sp.	34	85	30	75	0.70	0.40
Balantidium sp.	35	87.5	31	77.5	0.77	0.37
Isospora sp.	2	5	2	5	0.00	1.00

*Notes: N = Number of samples; P = Prevalence.

Moreover, group size also plays an important role in parasite exchange due to the mobility of the host and the transmission route of the parasite (Patterson & Ruckstuhl 2013). The R1 group has a higher population density than the other groups, making them have a wider home range (Rismayanti

2020). The largest group will travel further but it includes more fragmented areas with human density and spends longer time to forage than the smaller group does (O'brien & Kinnaird 1997). Thus, the chance of the R1 group encountering more parasites from the inter-intraspecies transmission is greater than the PB1B group which lives in the primary forest. Previous studies have reported that the diversity and prevalence of endoparasites appear to be higher in fragmented locations with a higher density of human appearance (Gillespie & Chapman 2008; Boundenga et al. 2018; Joesoef et al. 2018).

There was no significant difference in the endoparasites prevalence between both groups except *Strongyloides* sp. It is most likely because they still live in the same habitat and geographic area. In addition, groups R1 and PB1B were the groups that most often passed each other and shared the same sleeping tree more than other groups (Rismayanti 2020). Therefore, the diversity of endoparasites found in these two groups does not differ much. As Jones-Engel et al. (2004) reported, there are no significant differences in the endoparasite prevalence of Macaca sub-groups in the Sulawesi area. The biased parasitism is most likely found in primates exposed to different environmental conditions or different host-specific (Boundenga et al. 2018).

Climates Impact on Endoparasites Abundance

Pearson's correlation showed that rainfall was positively correlated with parasite abundance that invaded each host ($r = 0.18$), but it was not significant ($t = 0.59868$, $df = 10$, p-value = 0.562). Conversely, the monthly temperature negatively correlated ($r = 0.46$) with the abundance of endoparasites, but it was also not significant ($S = 417.69$, p-value = 0.132). The correlation value of 0.18 is very weak, while 0.46 is quite strong (Dancey et al. 2004). Although this is not significant, we can provide evidence on the correlation of average climate with endoparasite abundance; endoparasite abundance tends to increase when rainfall also increases (Figure 2) but tends to be low when temperatures are high (Figure 3).

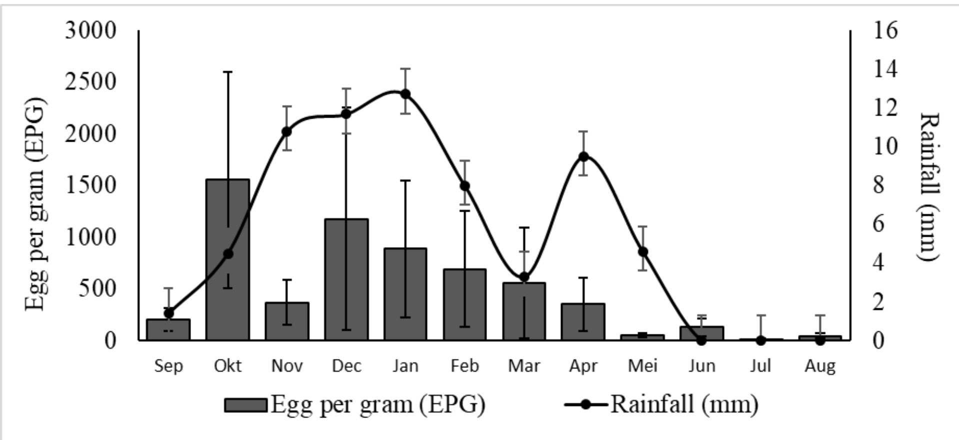

Figure 2. Average rainfall and EPG on each month. Error bars indicate standard errors.

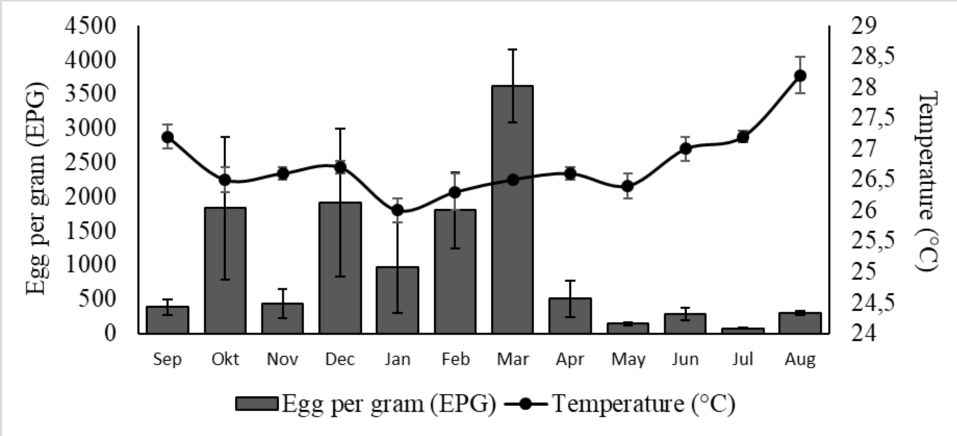

Figure 3. Average temperature and EPG on each month. Error bars indicate standard errors.

The highest parasite abundance occurred in October-March with the highest rainfall (12.2 mm) and the lowest temperature (26 ^{o}C). On the contrary, the lowest parasite abundance was in May-August with a lower rainfall range (4.6 - 0 mm) and a higher temperature (26.4 - 28.6 ^{o}C). Our finding is similar to the findings of previous studies which also showed a positive correlation between rainfall and parasite richness in primate populations (Benavides et al. 2012; Joesoef et al. 2018). Climate factors such as temperature and rainfall can temporally influence the presence of endoparasites. Some species of endoparasites have favorable environmental conditions to survive and to complete their life cycle (Cuomo et al 2009). A wet environmental condition during the rainy season supports the development of worm eggs and makes the rate of parasitic infection during this season increases (Joesoef et al. 2018).

As for the primates, the climate change in every season influences the availability and distribution of foods that will change the primate's daily ranges and activities (Hurtado et al. 2017). These changes include food composition, feeding intensity, and range of foraging movement. They eat fruit 4.6 times in the rainy season while in the dry season this number decreases to 3.7 times (O'Brien & Kinnaird 1997). Changes related to seasons will indirectly affect the possibility of *Macaca nigra* being infected by endoparasites. Even though the number of parasite's eggs in the rainy season is lower than in other seasons (Thienpont et al. 2003) because the feeding activity of Sulawesi black-crested monkeys increases, the possibility of being exposed to parasites through eating is still high.

Meanwhile, because of the high temperatures in the dry season, they will rest more and reduce their social activities (O'Brien & Kinnaird 1997). Even so, food scarcity in dry seasons will lead to host stress that causes immunosuppression and makes them vulnerable during this period; thus, the parasite load will increase (Chapman et al. 2006). Therefore, *M. nigra* still face the risk of being infected by endoparasites at every season because there is no clear pattern of the climate-gastrointestinal link mechanism.

CONCLUSION

This study reveals that there is an increase in the number of taxa and the prevalence of endoparasites found in *Macaca nigra* from Tangkoko, Bitung, North Sulawesi compared to previous studies. All of the taxa in this study have been found in other primate species including humans. This indicates that there is a potential zoonotic transfer between the two sympatric living species. There is no gender bias but the females have a slightly higher prevalence than the males. This, may be due to behavioral differences between the females and the males such as social style and diet, where the females eat and engage in social activities more often than males. The R1 group with more individuals had a higher prevalence of parasites than PBIB. This is probably because R1 travel more and have a wider home range, including fragmentation areas with human disturbance. The environmental factors show that rainfall is positively correlated, while temperature is negatively correlated to EPG.

AUTHORS CONTRIBUTION

S.A.M.W. contributed to designing the research concept, data examination, analyzed the data, and authored the manuscript. D.P.F and E.S contributed equally to this manuscript, developed the research concept, authored, reviewed, and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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