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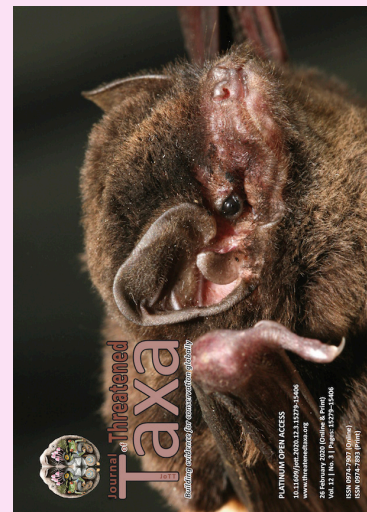
SHORT COMMUNICATION

PREVALENCE AND SEASONAL VARIATION OF GASTROINTESTINAL PARASITES AMONG CAPTIVE NORTHERN PIG-TAILED MACAQUE *MACACA LEONINA* (MAMMALIA: PRIMATES: CERCOPITHECIDAE)

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Prevalence and seasonal variation of gastrointestinal parasites among captive Northern Pig-tailed Macaque *Macaca leonina* (Mammalia: Primates: Cercopithecidae)

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Abstract: A study on the prevalence and seasonal variations of gastrointestinal parasites of 12 captive Pig-tailed Macaques *Macaca leonina* housed at Aizawl Zoological Park, Mizoram, India, was conducted. Fresh stool samples were collected on a monthly basis from the study animals was grouped for two seasons—summer (April–June 2017) and monsoon (July–September 2017). Samples were stored in 10% formalin until further processing. Three methods—direct smear, faecal floatation, and faecal sedimentation were used. Two categories of parasites—protozoa and nematodes were recorded. *Balantidium coli*, a protozoa, *Strongyle*, *Ascaris lumbricoides*, *Trichiuris trichiura*, and nematode parasites were recorded in different stages. Out of 71 samples analysed, 63 samples (88.73%) were positive with ova of gastrointestinal parasites. The prevalence of *Balantidium coli* was highest with 38.23% and 56.75%, followed by *Strongyle* 35.29% and 37.83% in summer and monsoon season, respectively. A variation on the prevalence of gastrointestinal parasites was assessed using chi squared tests between monsoon season and summer season. Variation was found to be significant ($\chi^2=20.569$, $P<0.05$ and $\chi^2=10.857$, $P<0.05$). The overall prevalence of gastrointestinal parasites was higher during monsoon season (91.89%) than summer season (85.29%).

Keywords: Aizawl Zoological Park, *Ascaris lumbricoides*, *Balantidium coli*, India, Mizoram, *Strongyle*, *Trichiuris trichiura*.

Non-human primates are susceptible to a variety of diseases caused by infection with gastrointestinal parasites, both in the wild and in captivity (Kuntz 1982). Captive animals are supposed to have low prevalence of parasites as anti-helminthic measures are practiced, however, infestation may be more due to unhygienic conditions of cages. Crowding of animals in cage, type of food and feeding practices are key factors in the development of endoparasites in zoo animals (Malan et al. 1997; Mul et al. 2007; Sanchez et al. 2009). The majority of primate pathogens culminate in chronic, sub-lethal infections (Goldberg et al. 2008) and parasite infections with low immune system can trigger deterioration of health (Glaser & Kiecolt-Glaser 2005; Coe 2011). Gastrointestinal parasite infected animals exhibit symptoms like watery diarrhea, hemorrhage, and dysentery; the animals may also develop renal infections that eventually lead to death (Leveck et al. 2007). Parasite load may affect the fitness of the host, influencing the survival and reproduction of the infected individuals (Boyce 1990; Hudson 1992; van Vuren 1996; Hilser et al. 2014). Knowledge about the

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profile of gastrointestinal parasites and their intensity in primates may help the zoo managers in developing better management plans to maintain the health of this threatened species, and to ensure local survival. This study is an attempt on captive Pig-tailed Macaque *Macaca leonina* to understand the parasitic profile of this threatened primate species so that the zoo authorities may undertake appropriate measures for prevention of parasite infection for this species and also for primates in general.

MATERIALS AND METHODS

Study site

The study was conducted at Aizawl Zoological Park that covers an area of 65ha and is situated 14km away from Aizawl, the state capital. The zoo maintains seven species of primates, which includes four species of Vulnerable (VU) primates, such as, Stump-tailed Macaque *Macaca arctoides*, Northern Pig-tailed Macaque *Macaca leonina*, Bengal Slow Loris *Nycticebus bengalensis*, and Capped Langur *Trachypithecus pileatus*, one Near Threatened (NT) species, Assamese Macaque *Macaca assamensis*, one Endangered (EN) primate, Western Hoolock Gibbon *Hoolock hoolock*, and one Least Concern (LC) primate, Rhesus Macaque *Macaca mulatta*.

Subjects

The Northern Pig-tailed Macaques study group included a total of 12 individuals, seven males and five females. They live in an enclosure of 850m² area with two adjacent indoor rooms which can be opened or closed by sliding doors. They are fed with fruits and vegetables every day. Water is available ad libitum. For the control of parasitic infection, piperazine hydrazine liquid 61% is used by the zoo authorities. The piperazine hydrazine liquid is mixed with water which is given to the monkeys for drinking. This treatment is done once in three months as recommended by the veterinarian.

Faecal sample collection

This study was conducted during April 2017–September 2017. Fresh stool samples were collected each month from the study group and grouped into two seasons summer (April–June 2017) and monsoon (July–September 2017) for meaningful inference. Animals were in captive conditions, hence monthly variations were not cognizable. Samples were examined macroscopically for the presence of larval or adult of various parasites at different stages. Samples were collected in the morning hours and were stored in 10%

formalin at the sampling site for further processing as per the procedures mentioned by Gillespie (2006). The collection tubes labeled with date and time of collection were shaken vigorously to homogenize sample and storage solution.

This study was undertaken with the permission of the Chief Wildlife Warden, Department of Environment, Forest and Climate Change under the permission number A.33011/4/2011-CWLW/Vol.II/388-89.

Sample processing

Three methods as recommended by Gillespie (2006) were used for the identification of parasitic infection, i.e., direct smear, faecal floatation, and faecal sedimentation.

Direct Smear: A thin smear of faecal material with normal saline was prepared on a slide and observed under the microscope.

Faecal floatation: Approximately 1g of faeces was placed into a 15ml centrifuge tube. The tube was filled 2/3rd with de-ionised water and homogenized with a wooden spatula, then centrifuged for 10min at 1,800rpm. The supernatant was decanted and the faeces was re-suspended in sodium nitrate (NaNO₃) solution. The tube was filled to the meniscus with NaNO₃ and a cover slip was placed on the mouth of the tube and left for 10min. The cover slip was removed and placed on a labelled slide. Single slide for each individual sample was observed under a microscope with 10X and 40X magnifications. Presence of parasitic helminths and protozoa were observed and photographed.

Faecal sedimentation: One gram of the preserved faecal sample was homogenized in a centrifuge tube, topped up and thoroughly mixed with 7–10 ml of 10% formal saline solution which also served as the fixative. The resulting suspension was strained into a clean centrifuge tube using a fine sieve to remove debris. Three milliliters of diethyl ether was then added. The mixture was stoppered, mixed, and centrifuged for 3min at 2,000rpm. Debris and fat which formed a floating plug were dislodged using an applicator stick and the supernatant was discarded. Using a Pasteur pipette, a drop each of the remaining sediment was transferred to a clean glass microscope slide to make a wet smear. Lugol's iodine solution (0.15%) was used to stain the slide. Sediments were further screened and analysed for identification of parasites and their different stages.

Chi-square test was used to assess the variation on the prevalence of each gastrointestinal parasite between winter and summer seasons. Chi-square test was carried out with SPSS version 18.0.

RESULTS

The study animals were found to be infected with two major groups of parasites: protozoa and nematodes. Four species of parasites, namely, *Balantidium coli* (protozoa), and *Strongyle*, *Ascaris lumbricoides*, and *Trichiuris trichiura* (nematodes), were recorded. Photos of the ova of all species recorded are given on Image 1. Out of the total 71 samples analyzed during the study, 63 samples (88.73%) were found to be positive with ova of gastrointestinal parasites, however, seasonal variations in the rate of infestation and different parasite species recorded also varied. In summer, out of 34 faecal samples, 85.29% were infected with parasites. In this season, protozoan infestation was found to be more (38.23%) and among nematodes, infection with *Trichiuris trichiura* (35.29%) was highest, followed by *Strongyle* (26.47%), and *Ascaris lumbricoides* (23.53%). The overall prevalence of gastrointestinal parasites was higher during monsoon season (91.89%) than summer season (85.29%). In the monsoon season, the rate of infection with *Balantidium coli* was high (56.75%), followed by *Strongyle* and *Trichiuris trichiura* (37.83% and 37.83%, respectively) and *Ascaris lumbricoides* (27.02%). Seasonal comparison of prevalence (%) of all four types of parasites is given in Figure 1. The prevalence of *Balantidium coli* was also highest in both the seasons,

followed by the whipworm *Trichiuris trichiura*.

On comparing the prevalence of infection between the summer and monsoon seasons, it was found that infection with *Balantidium coli* and *Strongyle* was significantly higher during monsoon season than summer season ($\chi^2=20.569$, $P<0.05$ and $\chi^2=10.857$, $P<0.05$, respectively). There was, however, no significant variation on the prevalence of *Ascaris lumbricoides* and *Trichiuris trichiura* between the two seasons ($\chi^2=3.611$, $P=0.164$ and $\chi^2=3.782$, $P=0.151$, respectively).

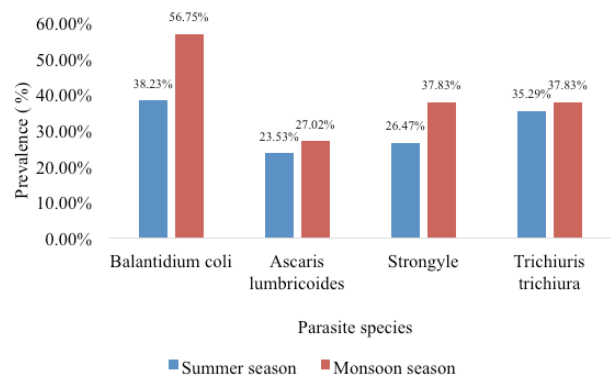


Figure 1. Comparison of the prevalence of gastrointestinal parasites during summer and monsoon season.

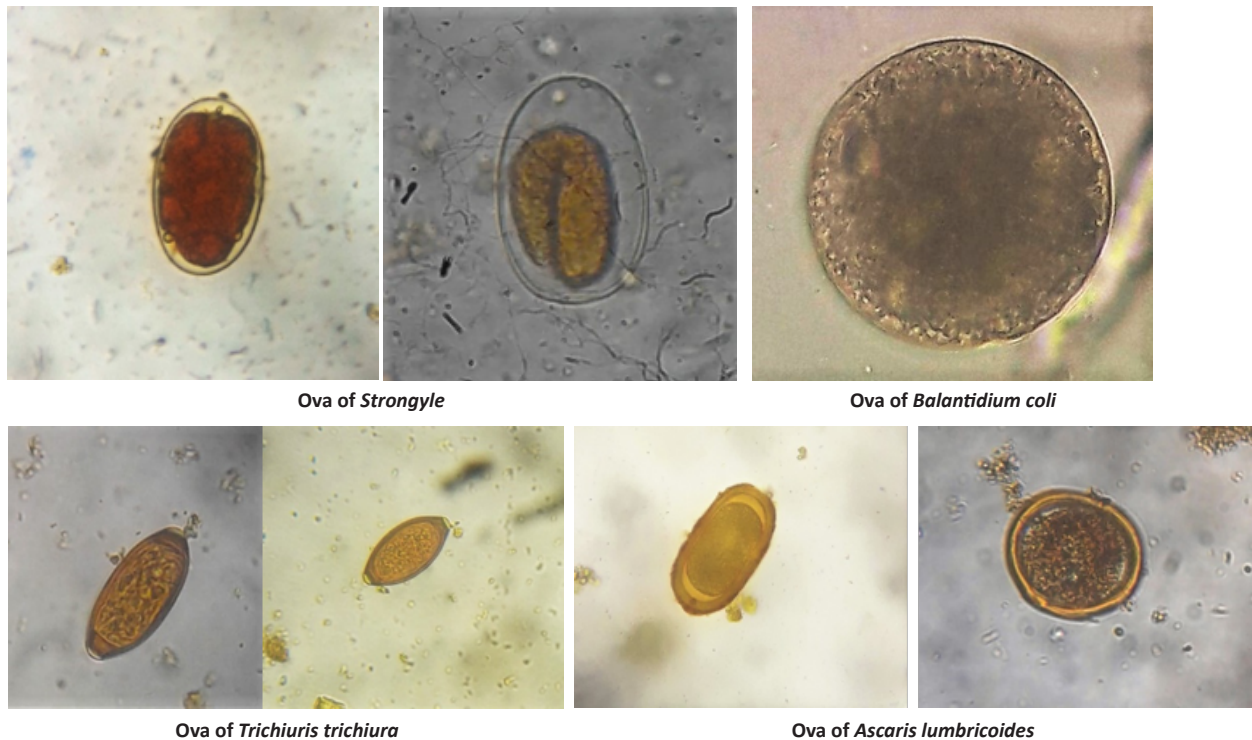


Image 1. Different gastrointestinal parasites reported in Pig-tailed Macaque.

DISCUSSIONS

Several parasitic infections have been reported in non-human primates, both in captivity (Levecke et al. 2007; Cordon et al. 2008; Nath et al. 2012; Barbosa et al. 2015; Margono et al. 2015) and in the wild (Legesse & Erko 2004; Parr 2013; Kouassie et al. 2015). The prevalence observed in the present study (88.73%) was higher than that reported by Opara et al. (2010) in captive animals, with prevalence rates of 62.5% and 61.5%, respectively. Parasitic diseases are reported to be common to zoo animals in tropical countries due to the climatic factors that favor the development of parasites such as light, temperature, and humidity (Opara et al. 2010). The two groups of parasites were also reported in Belgium Zoo in prosimians, old world monkey, new world monkeys, and some apes (Levecke et al. 2007). The protozoa and nematodes are highly prevalent even in wild non-human primates (Kouassi et al. 2015). The present study also indicates high prevalence of protozoa (*Balantidium coli*) in both the seasons as compared to nematodes (Figure 1), which is similar to the study conducted by Levecke et al. (2007) in Belgium on captive primates. Trematodes and cestodes were not detected in this study. This could be because these parasites require an intermediate host for their transmission and that are less likely in the captive environment (Atanaskova et al. 2011).

Attendants of enclosures of these animals could act as vehicles for cross transmission. Also, the animals serve as potential reservoirs that could transmit gastrointestinal parasites to zoo keepers and possibly to visitors. This study further shows the need for an anti-helminthic program such as early season treatments to prevent infection in animals under captivity, regular passive surveillance for parasitic infections, and effective treatment programs. Moreover, it has been observed that confinement of wild animals in zoo makes them more prone to different parasitic infections despite proper attention for feeding, water, and maintenance of hygiene in captivity (Kashid et al. 2002). The nematodes and some coccidian parasites have a direct life cycle, without any intermediate host and are transmitted by feco-oral route through contaminated feed, water, and soil and have the potential to accumulate in a captive environment (Thawait et al. 2014). The environmental contamination could be through contaminated water or fodder, and zoo workers have also been reported to play a role in transmission by acting as vectors and transmitting parasites through their shoes, clothes, hands, food, or with working tools (Adetunji 2014; Otegbade & Morenikeji 2014). Based on this study, it is recommended that upgraded and more effective

regular preventive as well as prophylactic measures are needed to be included in the management schedule of these animals at regular interval. Physical and chemical based hygiene are also needed as a part of management programs for captive animals.

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