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SHORT COMMUNICATION

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Comparative Evaluation of Three Fecal Examination Methods for Detecting Gastrointestinal Parasites in Captive *Macaca fascicularis*

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Abstract
Effective health monitoring of captive *Macaca fascicularis* requires reliable, sensitive diagnostic tools to detect gastrointestinal parasites. This study compared the diagnostic performance of three fecal examination methods, concentration-sedimentation, concentration-flotation, and the Kato-Katz technique, applied in parallel to fecal samples from 14 captive *M. fascicularis* at the Research Animal Facility Lodaya, Primate Research Center (PRC), IPB University, collected from October to December 2024. Concentration-sedimentation detected parasites in 78.6% of individuals (up to two parasite types per individual). The Kato-Katz method detected a single parasite type in 42.9% of individuals, while concentration-flotation yielded no positive results. Only protozoan parasites were identified: *Entamoeba* sp. and *Balantidium coli*. The detection of *B. coli* by the Kato-Katz method, theoretically unsuited for protozoan cysts, is attributed to its unusually large, thick-walled cysts. No helminth infections were found. These results confirm concentration-sedimentation as the most sensitive and appropriate routine diagnostic method for gastrointestinal parasite screening in captive macaques at this facility.

Keywords: endoparasites, Kato-Katz, long-tailed macaques, protozoa, sedimentation

1. Introduction

Macaca fascicularis is one of the most widely used non-human primates in biomedical and preclinical research, owing to its close phylogenetic proximity to humans and broad distribution across tropical mainland and insular Southeast Asia (Fooden 1995; Iwasaki *et al*. 2018). The validity of research conducted on this species is closely tied to the health status of captive individuals, making accurate and efficient routine health monitoring a central biosecurity and welfare concern (Musk 2020; Balansard *et al*. 2019).

Gastrointestinal parasitic infections are among the most prevalent health problems in captive primates, with potential to impair host fitness, alter behavior, and compromise physiological status (Agostini *et al*. 2023). Fecal examination is the most practical non-invasive diagnostic approach (Garedaghi 2020). Three methods are widely applied in veterinary parasitology: (1) concentration-sedimentation, in which centrifugation pellets denser parasite forms from fecal debris; (2) concentration-flotation, which uses a high-density salt solution to float lighter helminth eggs to the meniscus surface; and (3) the Kato-Katz method, a standardized thick-smear technique for quantifying helminth egg burden (Katz *et al*. 1972; WHO 2019; Soares *et al*. 2020). Each method is theoretically suited to different parasite morphologies, yet their comparative diagnostic sensitivity has rarely been formally evaluated in captive *M. fascicularis*.

At the Research Animal Facility Lodaya (RAFL), Primate Research Center IPB University (PRC-IPB), concentration-sedimentation is the only routine diagnostic method, and its performance relative to alternative techniques has not been assessed at this facility. This study aimed to: (1) directly compare the diagnostic sensitivity of three fecal examination methods applied in parallel to the same samples; (2) identify the gastrointestinal parasite species detected; and (3) provide baseline prevalence data to support ongoing health surveillance of captive *M. fascicularis* at RAFL, PRC-IPB.

2. Materials and Methods

2.1 Study Site and Animals

Fourteen captive *M. fascicularis* (seven individuals of each male and female; age range 2–15 years) housed at RAFL, PRC-IPB were included in this study. Six individuals were kept in sex-separated group cages and eight in individual cages (Table 1). Animals were categorized by age as juveniles (≤3 years), adolescents (4–9 years), or adults (≥10 years). All procedures were conducted under the supervision of the facility veterinary team and in accordance with the institutional animal welfare guidelines of PRC-IPB.

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Table 1. Demographic profile of captive *Macaca fascicularis* at RAFL, PRC-IPB.

No	Animal ID	Sex	Age (Years)	Housing Type	Age Category
1	Mf01	male	15	group	adult
2	Mf02	male	15	group	adult
3	Mf03	female	7	group	adolescent
4	Mf04	female	5	group	adolescent
5	Mf05	female	5	group	adolescent
6	Mf06	female	5	group	adolescent
7	Mf07	male	10	individual	adult
8	Mf08	male	9	individual	adolescent
9	Mf09	male	9	individual	adolescent
10	Mf10	male	2	individual	juvenile
11	Mf11	male	2	individual	juvenile
12	Mf12	female	11	individual	adult
13	Mf13	female	7	individual	adolescent
14	Mf14	female	5	individual	adolescent

Age categories: juvenile ≤3 years; adolescent 4–9 years; adult ≥10 years.

2.2 Sample Collection and Preparation

Fecal samples were collected each morning individually in October–December 2024, immediately after defecation, placed in labeled fecal collection tubes, and transported on ice to the Pathology Laboratory, PRC-IPB for same-day examination. Each sample was manually homogenized using a wooden applicator stick and then divided into three approximately equal portions by weight for simultaneous processing by each of the three diagnostic methods.

2.3 Diagnostic Methods

2.3.1 Concentration-sedimentation.

One g of feces was mixed with 10 mL of 10% formalin, filtered through double-layered gauze into a conical tube to a final volume of 7 mL, supplemented with 3 mL of ethyl acetate, vortexed, and centrifuged at 2500 rpm for 5 minutes. The resulting sediment was examined microscopically at 100× and 400× magnification. Infection intensity was scored semi-quantitatively per field of view as: (+) 1–3, (++) 4–6, (+++) 7–10, or (++++) >10 parasite forms (Rosmanah pers. Comm. 2024).

2.3.2 Concentration-flotation

Approximately 3 g of feces was homogenized in a saturated NaCl solution (specific gravity ~1.20), surface debris was removed, and the solution was filled to form a convex meniscus at the tube rim. A glass slide was placed on the meniscus and left for 20 minutes, then examined at 100× and 400× magnification (Ballweber *et al*. 2014).

2.3.3 Kato-Katz method

Feces were filtered through a nylon mesh, and the material collected on the upper side was loaded into a standardized mold (6 mm diameter, 1.5 mm depth) on a glass slide. The sample was covered with cellophane pre-soaked in glycerol–malachite green solution overnight and examined at 100–400× magnification (WHO 2019).

2.4 Parasite Identification

Parasites were identified based on morphological characteristics using a Nikon Eclipse compound microscope, including cyst and egg size, shape, wall thickness, number of nuclei, presence of vacuoles, pseudopodia, cilia, or other diagnostic features, following standard keys (Zajac and Conboy 2012; WHO 2019; Said *et al*. 2022).

2.5 Statistical Analysis

Infection prevalence was calculated as the proportion of individuals who tested positive within each group. Fisher's Exact Test was performed in RStudio (v. 2024.12.1) to test for associations between infection status and sex, age category, or housing type, with a significance threshold of $\alpha = 0.05$.

3. Results

Among the three methods, concentration-sedimentation detected gastrointestinal parasites in 11 of 14 individuals (78.6%), identifying one or two parasite types per positive individual. The Kato-Katz method yielded positive results in 6 of 14 individuals (42.9%), detecting a single parasite type in each. Concentration-flotation yielded negative results for all 14 samples (Table 2).

Only protozoan parasites were detected. *Entamoeba* sp. was observed in both cyst form (11–20 µm, 1–2 nuclei with bubble-like appearance) and as trophozoites with irregular outline and pseudopodia. *Balantidium coli* was found as round-to-oval cysts (40–60 µm, thick wall with indistinct internal structures) and ciliated trophozoites (Figure 1). All *B. coli*-positive individuals were also positive for *Entamoeba* sp.; no individual harbored *B. coli* alone. No helminth eggs or larvae were detected by any method.

Infection intensities were predominantly low to moderate for both parasites (Table 3). One individual (Mf14) exhibited a very high *Entamoeba sp*. intensity score (++++), while all *B. coli* positive individuals

Table 2. Fecal examination results using three diagnostic methods in 14 captive *M. fascicularis* at RAFL, PRC-IPB.

No	Animal ID	Sex	Age (Years)	Housing	Consentration-Sedimentation	Consentration-Floatation	Kato-Katz
1	Mf01	male	15	group	*Entamoeba sp.*	Negative	Negative
2	Mf02	male	15	group	*Entamoeba sp.*	Negative	Negative
3	Mf03	female	7	group	*Entamoeba sp., B. coli*	Negative	*B. coli*
4	Mf04	female	5	group	*Entamoeba sp., B. coli*	Negative	*B. coli*
5	Mf05	female	5	group	*Entamoeba sp., B. coli*	Negative	*B. coli*
6	Mf06	female	5	group	*Entamoeba sp.*	Negative	Negative
7	Mf07	male	10	individual	Negative	Negative	Negative
8	Mf08	male	9	individual	*Entamoeba sp., B. coli*	Negative	*B. coli*
9	Mf09	male	9	individual	Negative	Negative	Negative
10	Mf10	male	2	individual	*Entamoeba sp., B. coli*	Negative	*B. coli*
11	Mf11	male	2	individual	*Entamoeba sp.*	Negative	Negative
12	Mf12	female	11	individual	*Entamoeba sp., B. coli*	Negative	*B. coli*
13	Mf13	female	7	individual	Negative	Negative	Negative
14	Mf14	female	5	individual	*Entamoeba sp.*	Negative	Negative

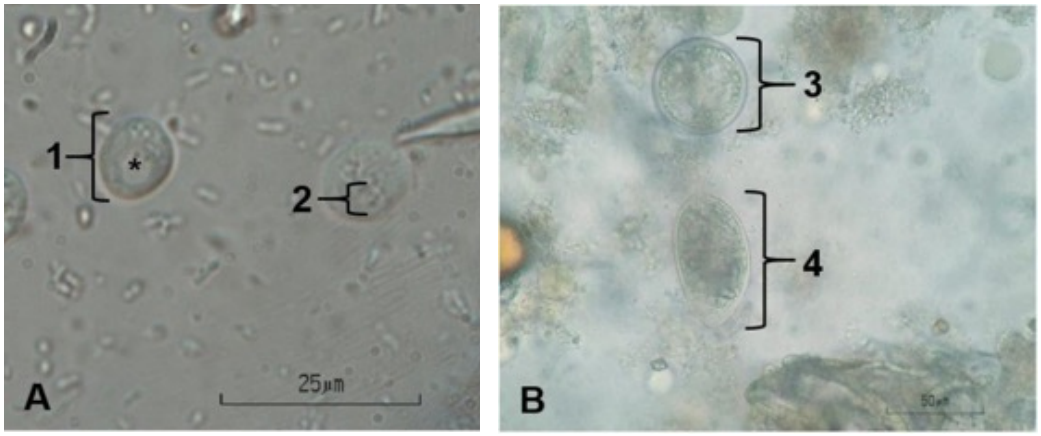

Figure 1. Morphological forms of gastrointestinal parasites. (A) *Entamoeba* sp. (B) *Balantidium coli, Entamoeba* sp. was found in cyst form (1), with visible organs: nucleus (2) and vacuole (*). Meanwhile, *B. coli* was found as a cyst (3) and a trophozoite (4) and examined using the concentration-sedimentation method.

Table 3. Semi-quantitative infection intensity of *Entamoeba* sp. and *Balantidium coli* per individual in 14 captive *M. fascicularis* at RAFL, PRC-IPB.

No	Animal ID	Sex	Age (Years)	*Entamoeba sp.*	*Balantidium coli*
1	Mf01	male	15	+	-
2	Mf02	male	15	+	-
3	Mf03	female	7	+	+
4	Mf04	female	5	++	+
5	Mf05	female	5	+++	+
6	Mf06	female	5	+	-
7	Mf07	male	10	-	-
8	Mf08	male	9	+	+
9	Mf09	male	9	-	-
10	Mf10	male	2	++	+
11	Mf11	male	2	+	-
12	Mf12	female	11	++	+
13	Mf13	female	7	-	-
14	Mf14	female	5	++	-

Scoring based on parasite forms per field of view: (+) 1–3; (++) 4–6; (+++) 7–10; (++++) >10; (–) negative.

showed low intensity (+). No clinical signs were observed in any animal during the study period, and fecal consistency remained normal throughout.

Overall infection prevalence was 78.6%. Females showed a higher prevalence (85.71%) than males (71.43%), and group-housed individuals were all infected (100%) compared to 62.5% of individually housed animals. Juveniles showed the highest prevalence (100%), followed by adolescents (83.33%) and adults (66.67%). None of these differences were

statistically significant (Fisher's Exact Test: sex, p = 1.00; age, p = 1.00; housing type, p = 0.21).

4. Discussion

The superior performance of concentration-sedimentation over the other two methods is consistent with its underlying working principle. Centrifugation at 2500 rpm effectively pellets denser protozoan cysts and helminth eggs from fecal debris, enabling reliable detection across a broad range of parasite morphologies regardless of density (WHO 1991; Soares *et al*. 2020; Manser *et al*. 2016). In contrast, the saturated NaCl flotation solution (specific gravity ~1.20) does not provide sufficient buoyancy to float the relatively dense cysts of *Entamoeba* sp. and *B. coli*, which accounts for the complete absence of detection by the flotation method (Ballweber *et al*. 2014; Pouillevet *et al*. 2017). This finding is consistent with previous comparative studies in non-human primates demonstrating the superiority of sedimentation-based approaches for protozoan detection (Pouillevet *et al*. 2017).

The detection of *B. coli* by the Kato-Katz method was unexpected, as this technique was originally designed for helminth egg quantification and is generally considered unsuitable for protozoan cysts, owing to the absence of a debris-separation step and potential glycerol-mediated damage to cyst integrity (Khanna *et al*. 2014; Ngwese *et al*. 2020). The successful detection observed here is attributable to the unusually large cyst diameter of *B. coli* (40–60 µm) and its characteristically thick, resistant cyst wall, which conferred resilience to glycerol exposure and maintained cyst visibility. Notably, *Entamoeba* sp. cysts (11–20 µm) were not detected by Kato-Katz, consistent with the prediction that smaller, more fragile cysts are less likely to be detected. This finding suggests a potential ancillary utility of the Kato-Katz method for detecting particularly large and morphologically robust protozoan cysts, which may be of practical value in facilities without centrifugation equipment.

Protozoa are the predominant gastrointestinal parasites reported from captive *M. fascicularis* (Phosuk *et al*. 2024), and the two species identified here *Entamoeba* sp. and *B. coli* are well-documented asymptomatic intestinal commensals in primates (Nakauchi *et al*. 1990; Levecke *et al*. 2010; Köster *et al*. 2022). The parasite assemblage at RAFL is less diverse than that recorded at the Dramaga facility of PRC-IPB, where additional protozoan species (*Giardia sp*., *Endolimax nana*, *Iodamoeba butschlii*) and gastrointestinal helminths (ascarid, strongylid, and trichurid nematodes) have been recorded (Deansyah 2021; Rosyid *et al*. 2024). The absence of helminths at RAFL most plausibly reflects effective routine antiparasitic management and strict hygiene protocols at this facility. Persistence of protozoan infections despite these measures is consistent with waterborne transmission via contaminated water or food, as the cysts of both *Entamoeba* spp. and *B. coli* are environmentally resilient and survive under high-humidity conditions (Joesoef *et al*. 2018; Walochnik 2018).

Although no clinical signs were observed in any animal, including the individual with a very high *Entamoeba sp*. intensity score, ongoing monitoring of infection intensity is warranted. Higher parasite loads may increase the risk of subclinical or overt disease, and the pathogenic species *Entamoeba histolytica* has previously been confirmed by molecular methods in *M. fascicularis* at PRC-IPB (Khairunnisa 2024). The absence of clinical disease in this study may reflect stable gut homeostasis and a robust host immune response that suppresses parasite proliferation (Uribe-Querol and Rosales 2020). The uniform prevalence across demographic groups likely reflects standardized husbandry conditions, such as consistent diet, cleaning schedules, and veterinary oversight, that minimize differential exposure, as observed in other captive primate populations (Boundenga *et al*. 2021; Mapagha-Boundoukou *et al*. 2024).

Several limitations of this study should be noted. The sample size of 14 individuals is small, limiting statistical power to detect demographic differences. Parasite identification was based solely on morphological criteria; molecular methods were not applied. This is particularly relevant for *Entamoeba* sp., as morphology cannot distinguish the pathogenic *E. histolytica* from the non-pathogenic *E. dispar* and *E. moshkovskii.* Finally, the single sampling period (October–December 2024) may not fully capture seasonal or longitudinal variation in parasite dynamics. Future studies should expand sample size, incorporate repeated sampling, and apply PCR-based identification to resolve *Entamoeba* species identity and evaluate zoonotic risk.

In conclusion, concentration-sedimentation demonstrated the highest diagnostic sensitivity and is confirmed as the most appropriate routine fecal examination method for gastrointestinal parasite surveillance in captive *M. fascicularis* at RAFL, PRC-IPB. The unexpected detection of *B. coli* by the Kato-Katz method further suggests this technique may serve a supplementary role where sedimentation equipment is unavailable. The exclusive detection of protozoa and the absence of helminths indicate that current deworming and sanitation protocols are effective, though continued vigilance and molecular parasite characterization are recommended.

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