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Gastrointestinal parasites of hard-ground barasingha (*Rucervus duvaucelii branderi*) of Kanha and Satpura Tiger Reserves, Madhya Pradesh, India

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Abstract

A total of 150 faecal samples were examined, comprising 77 samples from Satpura Tiger Reserve and 73 samples from Kanha Tiger Reserve. Overall, 102 samples (68.0%) were positive for one or more gastrointestinal parasites. The prevalence was significantly higher in Satpura Tiger Reserve (76.6%) compared to Kanha Tiger Reserve (58.9%) ($p < 0.05$). Species-wise analysis of the positive samples revealed strongyle infection in 57.62% samples from Satpura Tiger Reserve and 51.16% samples from Kanha Tiger Reserve. Amphistomes were detected with prevalence rates of 22.03% in Satpura and 18.60% in Kanha. *Eimeria* spp. was detected with prevalence of 15.25% in Satpura and 16.27% in Kanha. Cestodes infections (*Moniezia* spp.) were recorded at lower frequencies, representing 5.08% of infections in Satpura and 13.95% in Kanha. The overall prevalence of *Eimeria* spp. among positive samples was 15.68%. No significant differences were observed in the distribution of parasite groups between the two reserves ($P > 0.05$). Mixed infections were common and detected in 66.10% of positive samples from Satpura Tiger Reserve and 51.16% in Kanha Tiger Reserve, indicating continuous exposure of Barasingha to multiple infective stages in their habitat. Quantitative estimation of *Eimeria* spp. revealed 2006.31 ± 39.75 - 2350.76 ± 170.02 in Satpura and Kanha Tiger Reserve respectively. Whereas the mean EPG values for nematodes, cestodes and trematodes were 1617.82 ± 101.68 - 1954.90 ± 140.11 , 1984.51 ± 99.70 - 1650.57 ± 133.61 and 1357.87 ± 325.41 - 1821.45 ± 271.22 in Satpura and Kanha Tiger Reserves respectively.

Keywords: Hard-Ground Barasingha; *Rucervus duvaucelii branderi*; Gastrointestinal Parasites; Satpura Kanha, Tiger Reserve; Madhya Pradesh; Faecal Examination; Wildlife Health

Introduction

Barasingha of deer family known for social hierarchy wear 12 tines of antlers on the stag of male and gorgeous look of fawns with pretty females. Three sub-species of Barasingha called swamp deer are distributed across Indian sub-continent viz. the wetland Barasingha (*Rucervus duvaucelii duvaucelii*) resident of South Nepal and Dudhwa National Park of Uttar Pradesh are explicitly characterized by broad, splayed hooves with large skull. The hard-ground Barasingha (*Rucervus duvaucelii branderi*) endemic to Kanha Tiger Reserve is adapted to drier uplands with compact hooves whereas the eastern Barasingha (*Rucervus duvaucelii ranjitsinhii*) is native to the plains of Assam, with significant populations residing in Kaziranga National Park and Manas National Park. This subspecies is now considered extinct in Bangladesh (Prater, 2005) [13]. Wildlife health management plays a pivotal role in biodiversity conservation pertaining to sustenance of critically endangered species like hard-ground Barasingha (*Rucervus duvaucelii branderi*) (Singh & Gajadhar, 2014) [14]. However, sporadic efforts were made for health monitoring and disease diagnosis of Barasingha due to their elusive nature and high susceptibility for capture myopathy imposes limitations in direct handling and contributes to a lack of baseline hematological and biochemical data (Banerji et al., 2026) [3].

Materials and Methods

Freshly voided fecal pellets were collected from the individual Barasingha either of sex including young and adults from Kanha Tiger Reserve and Satpura Tiger Reserve covering different ranges. These faecal pellets were divided into two parts; one part kept in the buffered 10 % formalin whereas second part kept unpreserved maintained in the cool chain and brought to the laboratory for further investigations. Collected faecal pellets were processed to detect gastrointestinal parasites using flotation, sedimentation techniques and those found higher parasitic load were screened under McMaster eggs/oocysts counting method for intensity estimation of encountered infection with the help of standard procedures described by Palmer *et al.* (2013) ^[11].

Qualitative Assessment of enteric parasites:

Floatation method: Approximately 2-3 g of faecal material was collected from each sample and crushed using a pestle and mortar with a small amount of water to form soft slurry. More water was added to obtain a uniform suspension, which was filtered through a single mesh nylon tea strainer into a beaker. The filtrate was then transferred to a 15 ml. centrifuge tube and centrifuged at around 3000 rpm for 5 minutes. The supernatant was discarded and the sediment was broken, again mixed with water and centrifuged at 3000 rpm for 5 minutes. The process of washing the faecal sample with water was repeated at least 3 times until the supernatant appeared clear. The sediment was then thoroughly mixed with Sheather's sugar solution (prepared by dissolving 450 g sugar in 355 ml water and 10 ml of 10% formalin as preservative) and gently topped to form a convex meniscus. A clean circular 18mm ('0' no.) cover slip was carefully placed over the meniscus and the tube was again centrifuged for 3 minutes at 500 rpm gradually increasing the speed to 1000 rpm for 7 minutes. After allowing the tube to stand undisturbed for about 12 minutes, the cover slip was removed, placed on a clean glass slide and examined under a compound microscope using 10x to 40 x magnifications for the presence of parasite eggs and oocysts.

Formalin-Ether sedimentation method: For screening of heavier eggs, about 2-3 g of faecal material was mixed with 10 ml of water in a 50 ml glass beaker to obtain pasty consistency. Addition of 10 ml 10% buffered formalin was done to obtain a homogenous mixture. The mixture was triturated to form a uniform suspension and filtered through single 50 mesh nylon sieve. The strained material was left to settle down for 15 minutes, after which the supernatant was discarded, retaining the sediment. About 2 ml of sediment was transferred to a centrifuge tube, to which 5 ml of normal saline (0.9% NaCl) and equal volume of diethyl ether or petroleum ether was added, vigorously shaken (approximately 15-20 times) and allowed to stand for 10 minutes till there was formation of ring at the ether and normal saline junction. The tube was then centrifuged at 3000 rpm for 3 minutes. The upper ether layer and intermediate layer with formalin and debris were decanted carefully, leaving the heavier sediment at the bottom. The sediment was rinsed twice using 3 ml of normal saline (mixing with Normal saline and centrifuging at 3000 rpm for 2 min.) and about 1-2 drops of sediment were spread over clean grease-free glass slide, covered with a cover slip and microscopically examined under 100 x and 400x.

Quantitative estimation: Coccidian oocysts were quantified using the modified McMaster method while Stoll sedimentation techniques were applied for OPG/EPG

counting according to Soulsby (1982) ^[16]. The initial five steps were similar to the Sheather's sugar flotation method but only 2 g of faecal material was measured carefully for the estimation. The faecal sediment obtained was mixed thoroughly with about 30 ml of tap water to obtain a homogenous suspension. This mixture was then combined with 30 ml of a saturated sugar solution to form a uniform suspension. After proper mixing, the suspension was aspirated using a 10 ml pipette and loaded into McMaster eggs/oocyst counting chamber (about 0.15 ml per chamber = 0.30 ml total) and allowed to stand for about 3-5 minutes to settle. The oocysts visible within the grid area of the chamber were then counted under a compound microscope under dry magnification. The final oocyst concentration per gram of faeces (OPG) was calculated by applying the formula: OPG (Average number of oocysts in 1 cm² grid) x 200 (dilution factor).

Prevalence of gastrointestinal parasites was calculated as percentages based on the number of positive samples relative to the total number of samples examined. Quantitative parasitological data, including eggs per gram (EPG) and oocysts per gram (OPG) of faeces, were summarized using mean $\pm$ standard error (Mean $\pm$ SE). Sporulation percentages of *Eimeria* spp. Oocysts and micrometric measurements (length and width) were also expressed as Mean $\pm$ SE to describe central tendency and variability. The Chi-square test of independence was applied to assess differences in overall prevalence of gastrointestinal parasitism between Satpura Tiger Reserve and Kanha Tiger Reserve. The test was also used to evaluate differences in the distribution of major parasite groups between the two reserves. Statistical analysis using the chi-square test revealed a significant difference in overall gastrointestinal parasite prevalence between the two reserves ($P < 0.05$). Where expected frequencies in contingency tables were low, Fisher's Exact Test was applied to ensure validity of the statistical inference.

Results and Discussion

Gastrointestinal parasitism has pivotal importance specifically in wildlife health management, influencing reproductive performance, behavioral patterns, survival rates and potentials of habitat vicinity. Surveillance of parasitic infections in wild hosts can contribute to minimizing extinction risks and improving conservation outcomes (Maharjan *et al.*, 2025) ^[8]. Overall, 102 samples (68%) were found positive for gastrointestinal parasitic infections. A higher prevalence 59 (76.6%) was recorded in Satpura Tiger Reserve as compared to Kanha Tiger Reserve 43 (58.9%). (Table 01). The findings of the present study revealed more or less similar trend of prevalence as Tiwari (2007) ^[18]. Encountered 57.03 % gastrointestinal parasitism in Barasingha of Kanha Tiger Reserve whereas in Satpura Tiger Reserve it appears to be first report of surveillance of GIP after their translocation from Kanha Tiger Reserve. This significant variation indicates differences in environmental conditions, host density, grazing behaviour or parasite transmission dynamics between Satpura and Kanha Tiger Reserves. The species-wise distribution of gastrointestinal parasites in Barasingha of Satpura Tiger Reserve revealed nematode infections were predominant (57.62%), followed by Amphistomes (22.03%), *Eimeria* spp. (15.25%) and *Moniezia* spp. (5.08%). A similar trend was observed in Kanha Tiger Reserve, where nematodes constituted the highest prevalence (51.16%), followed by Amphistomes (18.60%), *Eimeria* spp. (16.27%) and *Moniezia* spp. (13.95%).

Utmost negligible differences were evident in the prevalence of individual parasite groups between the two reserves; chi-square test indicates no statistically significant difference in parasite infection ($P > 0.05$) (Table 01). However, mixed

infection of gastrointestinal parasites in barasingha was found in 39 (66.1%) samples in Satpura Tiger Reserve whereas in Kanha Tiger Reserve, it was 22 (51.16%) with more than one parasitic species of strongyles and coccidian protozoans.

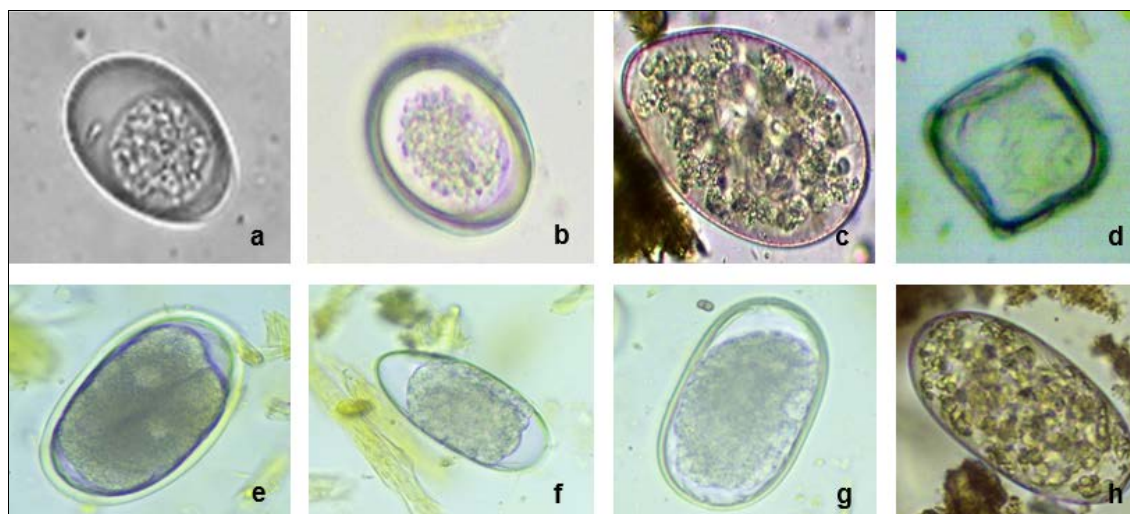


Fig 1: Showing gastrointestinal parasitic eggs and cysts recovered from free ranging hard-ground Barasingha: a & b. Immature oocysts of *Eimeria*, c. *Amphistome* d. *Moniezia* e, f & g. Strongyles h. *Amphistome*.

Subsequently, location-wise variations observed in gastrointestinal parasitism amongst wild ungulates (Tiwari, 2007) [18] in Kanha Tiger Reserve and by Gupta *et al.* (2011) [6] in protected areas of Madhya Pradesh. Banerji *et al.* (2026) [3] also documented variation in parasitic prevalence among Barasingha populations of Kanha and Satpura Tiger Reserves, attributing these differences to environmental and management factors. The overall prevalence observed in the

present study (Table 01) falls within the range similar to Khatun *et al.* (2014) [7] and Pangen (2021) [12], documented high prevalence of gastrointestinal parasites in free-ranging cervids, indicating that parasitism appears common ecological process in sylvatic as well as domestic cycle which spread the infection in wild and domestic animals those uses common grazing field and water resources.

Table 1: Prevalence percentage of Gastro intestinal parasites recovered in free-ranging Barasingha

Tiger Reserve	Positive for GIP (%)	<i>Eimeria</i> spp. (%)	<i>Moniezia</i> spp. (%)	<i>Amphistomes</i> (%)	Strongyles (%)	χ^2	p-value
Satpura (n=77)	59 (76.62)	9 (11.68)	3 (3.89)	13 (16.88)	34 (44.15)	2.57	0.46
Kanha (n=73)	43 (58.90)	7 (9.58)	6 (8.21)	8 (10.95)	22 (30.13)		

No significant difference ($P > 0.05$)

Strongyles constituted the predominant group of gastrointestinal parasites in both tiger reserves in the present study, with prevalence of 57.62% in Satpura and 51.16% in Kanha, a pattern that is consistent with earlier observations in wild ruminants. Singh *et al.* (2009) [15] recorded strongyles infections in 26.15% of free-ranging cervids and the consistent predominance of nematodes across diverse ecosystems has been attributed to their direct life cycles, high egg output, and greater adaptability to environmental fluctuations, which enable efficient survival and transmission under favourable grazing conditions (Banerji *et al.*, 2026) [3]. The prevalence of *Eimeria* spp. observed in the present study (15.68% overall among positive samples) may be considered moderate when compared with earlier reports. Banerji (2024) [2] documented comparatively lower prevalence in Barasingha, reporting 5.55% in Kanha and 8.33% in Satpura Tiger Reserve, whereas Pangen (2021) [12] recorded substantially higher prevalence in swamp deer from Nepal, with oocysts possessing micropyle in 11.25% and without micropyle in 26.25% of samples. Higher levels of coccidia infection have also been reported by Mir *et al.* (2016) [9] in captive ungulates (38%) and by Woodbury *et al.* (2014) [19], who observed *Eimeria* spp. in up to 95% of bison herds. These comparisons

indicate that the prevalence recorded in the present study falls within the broad range reported for cervids and other wild ruminants, with variation likely influenced by host species, age structure, seasonality, and diagnostic methodology. Mixed parasitic infections were frequently observed in earlier studies, indicating that concurrent infections are a common feature of gastrointestinal parasitism in wild herbivores. Gupta *et al.* (2011) [6] reported mixed infections in the majority of wild herbivores examined, while Allwin *et al.* (2016) [1] documented concurrent infections in nearly 70% of gaurs and domestic cattle at wildlife-livestock interface zones. Bhaydiya (2018) [4] reported gastrointestinal parasitism in 47.03% of free-ranging gaurs in Satpura Tiger Reserve, and Banerji (2024) [2]. Observed mixed parasitic infections in 61% of Barasingha samples from Satpura and 50% from Kanha Tiger Reserve. The present findings are therefore well within the range reported for the region and support earlier observations that parasite prevalence and composition may vary between reserves within the same geographical zone, largely due to differences in grazing pressure, water availability, and microclimatic conditions rather than intrinsic host susceptibility.

Table 2: Overall mean OPG/EPG value of Gastro intestinal parasites of free-ranging Barasingha

Place of Work	Mean of Gastrointestinal parasite species $\pm$ SE			
	<i>Eimeria</i> spp.	Trematodes	Nematodes	Cestodes
Satpura Tiger Reserve (n=77)	2006.31 $\pm$ 39.75	1821.45 $\pm$ 271.22	1954.90 $\pm$ 140.11	1650.57 $\pm$ 133.61
Kanha Tiger Reserve (n=73)	2350.76 $\pm$ 170.02	1357.87 $\pm$ 325.41	1617.82 $\pm$ 101.68	1984.51 $\pm$ 99.70

The quantitative parasitic burden expressed as mean egg/oocysts per gram of faeces EPG/OPG of *Eimeria* spp. in Satpura Tiger Reserve was recorded as 2006.31 $\pm$ 39.75, while in Kanha Tiger Reserve it was 2350.76 $\pm$ 170.02 (Table 02). Mean EPG values for nematodes and Amphistomes also indicated moderate to high infection intensities in both reserves. Maximum coccidian OPG values of 79.31 were recorded (Tiwari, 2007) ^[18] in Barasingha of Kanha Tiger Reserve whereas Pal *et al.* (2015) ^[10] reported mean EPG values of 43.86 in yaks. The comparatively higher OPG values observed in the present study may reflect differences in host species; seasonal inputs sample collection and methodological sensitivity. Surveillance of 246 faecal samples from swamp deer (Talukdar, 2020) ^[17] also encountered similar trend of gastrointestinal parasite occurrences as reported in the present study. Although quantitative analysis of the present study also prevailing clinical or subclinical phase of coccidiosis as diarrhoea, occult blood in fecal pellets and dullness conditions may increase stress intensity (Banerji *et al.*, 2026) ^[3]. Encouraging outcomes of the present study suggest about needs of epidemiological studies including seasonal impact on *Eimeria*-induced coccidiosis in free-ranging Barasingha while surveillance of antelopes, cervids and bovid in and around these tiger reserves may clarify the cross-species transmission (Griffith *et al.*, 2021) ^[5] recorded in European bison.

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No conflict of interest as the fecal sample collected were processed using non-invasive techniques without disturbing the animals.

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