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Copromicroscopic study of gastrointestinal parasites in captive mammals at Central Zoo, Lalitpur, Nepal

Pitambar Dhakal[1] | **Hari Prasad Sharma**[1] | **Rachana Shah**[2] | **Parbat Jung Thapa**[2] | **Chiranjibi Prasad Pokheral**[2]

[1]Central Department of Zoology, Institute of Science and Technology, Tribhuvan University, Kirtipur, Nepal

[2]National Trust for Nature Conservation-Central Zoo, Lalitpur, Nepal

Correspondence
Hari Prasad Sharma, Central Department of Zoology, Institute of Science and Technology, Tribhuvan University, Kirtipur, Kathmandu, Nepal.
Email: hari.sharma@cdz.tu.edu.np

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Abstract

Background: Zoological gardens (Zoo) provide abode to various threatened animals or trafficked animals seized by the authorities, and injured and orphaned animals. Captive animals are more susceptible to infection as they are under significant stress due to diet and space which further dwindle their resistance to parasitic infections.

Objectives: This study was conducted to determine the prevalence and burden of gastrointestinal parasites in captive mammals housed at Central Zoo.

Methods: Fresh faecal samples from three orders of mammals including carnivora (n = 24), rodentia (n = 28) and artiodactyla (n = 35) were examined by direct smear, faecal floatation and sedimentation techniques, and the McMaster technique was applied to quantify parasite eggs per gram (EPG)/oocysts per gram (OPG) of a faecal sample.

Results: One or more parasite taxa were detected in 19.54% of the examined samples and five types of GIPs including one protozoon (*Eimeria* spp.) and four helminths (*Strongyloides* spp., *Haemonchus* spp. and *Trichostrongylus* spp. and hookworm) were recorded. The protozoan prevalence (6.89 %) was lower than helminths (12.64%). The *Eimeria* spp. was the most prevalent parasite (6.89%) with the highest OPG (427.77 ± 25.45SD) in spotted deer (*Axis axis*), and the highest prevalence was noticed among artiodactyla (34.28%) followed by carnivora (12.5%) and rodentia (7.14%). Artiodactyla had both single infection (25.71%) and double (8.57%) infection. The percentage of single infection (16.09%) was found to be higher than double infection (3.44%) among the captive mammals. The wild boar (*Sus scrofa*) had the highest EPG of 383.33 ± 76.37SD (*Strongyloides* spp.), while the spotted deer had the lowest EPG of 216.66 ± 76.37SD (hookworm).

Conclusions: Despite careful management practices, the parasitic infection may be attributed to the narrow enclosure, group housing and environmental contamination. The present finding provides baseline information on the parasitic infection in captive mammals, and can be used by zoo managers for the better life of captive animals.

KEYWORDS
captive mammals , Central Zoo, copromicroscopy, gastrointestinal parasites

1 | INTRODUCTION

Zoological gardens (Zoo) are the ex-situ conservation centres, mostly owned by the government, where various threatened animals are collected from different natural habitats in the course of rescuing the orphan, injured and problematic wild animals. They are kept in quarantine for a couple of weeks in close observation and treated. Based on animals' health conditions and behaviour, they are released either in the wild or kept in the zoo for exhibition, conservation education, aesthetic, recreational and research purposes (McElroy, 2015; Miller et al., 2004). The major objectives of captive management of animals are to promote the animal diversity and protect threatened species (Gracenea et al., 2002; Kelly & English, 1997; Parsani et al., 2001). Zoos have a major role in wildlife conservation, for example, 173 mammal species are in the verge of extinction on six continents (Ceballos & Ehrlich, 2002), and most of these species are used for captive breeding in zoos to preserve their gene (Alroy, 2015). These captive-bred animals are widely used for re-introduction programs in the wild for the long-term survival of the species. Central Zoo has an experience of reintroduction of some threatened species, for example, Blackbuck (*Antilope cervicapra*) in Shuklaphanta National Park and Wild water buffalo (*Bubalus arnee*) in Chitwan National Park. Animals mainly neonatal babies face survival pressure in captivity due to parasites transmitted probably from their parents or other sources including zoo keepers (Patterson-Kane & Piper, 2009). Several factors such as age, sex, environmental factors, husbandry practices, or management practices including keeping animals separately before introducing into a group, measures undertaken for prophylaxis of parasitic infections and their type, and efficacy of parasitic treatment are accelerating parasitic invasions in captive animals (Khan et al., 2009; Lamy et al., 2012; Muhammad et al., 2010; Parsani et al., 2001). In the wild, animals thrive in vast geographic areas where they are exposed to a variety of parasites, and naturally develop resistance against them (Achhami et al., 2016; Barbosa et al., 2020; Sharma & Achhami, 2022). In addition, the zoos are most often established near city areas with very limited space, and they have many animal species. Contrary to the wild state, stress conditions created by the captive environment can dwindle the resistance to parasitic diseases (Cordon et al., 2008; Gracenea et al., 2002). While they are released in the wild, captive-bred animals are more susceptible to the pathogens than wild animals (Kolodziej-Sobocinska et al., 2018). The captive-released animals can spread parasites rapidly in the wild. Therefore, it is essential to investigate the mode of parasite transmission in captive animals.

Parasites might be introduced into a zoological garden by several means through contaminated animal food (fruits, vegetables, infected meat or fish, etc.), intermediate and paratenic hosts (snails, ants, cockroaches and other insects, rodents, etc.), newly acquired parasitized animals, and infected zoo staff and visitors (Pencheva, 2013). Many species of helminths and protozoans are known to infect mammals. Helminths such as *Strongyloides*, *Trichuris*, *Nematodirus*, and other strongyles, *Toxacara*, *Moniezia*, and protozoan parasites such as *Giardia*, *Balantidium*, *Entamoeba*, and coccidian parasites are commonly reported gastrointestinal parasites (GIPs) in captive mammals worldwide (Barbosa et al., 2020; Goossens et al., 2005; Karim et al., 2021; Levecke et al., 2007; Li et al., 2015; Naz et al., 2021; Thawait et al., 2014). The presence of these parasites in the host may induce morbidity and mortality (Nath et al., 2012). The captive mammals can also serve as the reservoir of parasites for the domestic mammals, and some of these infections can be zoonotic which can be communicable to humans (Bogale et al., 2014) and raise public health concerns (Levecke et al., 2007).

Central Zoo has been home to 798 animals of 105 species (February–April, 2019). Among them, 286 animals are mammals (30 species), 21 reptiles (nine species), 232 fish (14 species), and 259 birds (51 species). Captive animals do not manifest alarming signs of parasitism provided that regular de-worming practices are carried out in zoological gardens (Parsani et al., 2001). Zoo animals living in captivity are susceptible to almost all types of diseases particularly helminth infestations being the major problem (Khatun et al., 2014; Mir et al., 2016). Due to congested animal husbandry practices having many individuals in a small area (six hectare), provision of various food supplies to the captive animals, and existing infrastructure having mud and soil floor might increase the potentiality of gastrointestinal parasite transmission from one individual to another at Central Zoo. Therefore, we aimed to investigate the gastrointestinal parasitic prevalence and intensity of infection in captive mammals.

2 | MATERIALS AND METHODS

2.1 | Study area

Central Zoo (27.6733°N; 85.3107°E) is the only Zoo of Nepal located in Jawalakhel, Lalitpur, Bagmati Province, with an area of six hectares. Originally established as a private zoo in 1932 by the Late Prime Minister Judda Shamsher Rana, Central Zoo was administered under His Majesty's Government of Nepal from 1950 to 1995, and later the responsibility for management was handed over to the National Trust for Nature Conservation. The Trust aims to develop Central Zoo as a centre for ex-situ wildlife research and conservation education. It also serves as a popular recreational centre for tourists and local people. Animals are not allowed to outdoor movements from the cage. The floor of captive animals' cages is made of soil which might be the source

TABLE 1 Total number of mammalian species sampled and infection pattern

Mammalian order	No. of samples examined	No. of host species infected	No. of individuals infected (prevalence %)
Carnivora	24	3	3 (12.5)
Rodentia	28	1	2 (7.14)
Artiodactyla	35	5	12 (34.28)
Total	87	9	17

of parasites. The animal management unit has been providing health services for the zoo animals by establishing an animal hospital within its premises.

2.2 | Sample collection and preservation

Between February–April, 2019, 87 faecal samples were collected from three orders (Carnivora, Rodentia, and Artiodactyla) of the captive mammals kept in Central Zoo. Of them, 24 samples were collected from nine species of carnivora, 28 from five species of rodentia and 35 from 10 species of artiodactyla (Table 1). Individual animals' faecal sample was collected; however, the samples from animals kept in the group were collected by observing them until defecation. The faeces were examined visually whether the faeces have blood, mucus, tapeworm proglottids, and adult; however, they were not found as such. The early-morning fresh faecal samples were picked up in the sterile well-labelled zipper plastic bags between 8:00 AM and 10:00 AM before cleaning the animal cages and surrounding with the aid of zoo keepers. The average daytime temperature at Central Zoo was 13.5°C, and there was no rainfall during the month of sample collection. No obvious clinical signs were observed except for one case of diarrhoea in a sloth bear. In all samples, preference was given to fresh samples to avoid the chances of environmental contamination. While sampling, the husbandry pattern of sampled animals was classified as single or group, and all the faecal samples were classified according to their consistency. The samples were fetched immediately to the Parasitology Laboratory of Central Department of Zoology, Tribhuvan University where the samples were transferred to the sterile vial with 2.5% potassium dichromate as a preservative.

2.3 | Laboratory process

2.3.1 | Direct and wet mount

All the samples were carefully examined by direct mount (Pourrut et al., 2011), saline, and iodine wet mount technique (Zajac & Conboy, 2012). Using a sterilized wooden applicator, a small portion of the faecal sample (≈1gm) was emulsified with normal saline (0.9% physiological saline) and 1% Lugol's iodine solution on a separate clean microscope glass slide, covered with a cover slip, and examined under 400× magnification. The iodine-stained smears were used to identify protozoan cysts and trophozoites.

2.3.2 | Faecal concentration method

A formalin-ether concentration method was used to demonstrate the presence of GIPs using a standard protocol (Becker et al., 2011). Approximately a half gram of faeces was suspended in 5 ml of 5% formalin and strained through a wire sieve to remove debris. The fatty content in the resulting filtrate was removed by emulsifying the sample with 5 ml of diethyl ether followed by centrifugation at 3000 rpm for 2o min. The supernatant (ether, faecal debris, and acetic acid formalin), after another centrifugation, was discarded, and the sediment was transferred to two glass slides, one with normal saline and another with 1% Lugol's iodine, and examined under high power (400× magnification).

2.3.3 | Identification and measurement of parasite oocysts and eggs

Qualitative procedures such as normal saline and iodine wet mount, flotation and sedimentation techniques were used with a slight modification. At least, two smears were prepared from each sample for each technique to identify eggs and oocysts based on their morphological characters (Foreyt, 2001; Soulsby, 1982; Taylor et al., 2007; Zajac & Conboy, 2012). The size of all identified parasite oocysts and eggs was measured using calibrated ocular micrometre under 400× magnification. The number of divisions on the ocular micrometre subtended by the parasite oocysts or egg was multiplied by the calibration factor (2.24 after calibration of ocular micrometre against stage micrometre) to obtain the exact size, and it was referenced with available data (Foreyt, 2001; Zajac & Conboy, 2012) (Table S1).

2.3.4 | Estimation of parasite burden

Parasite positive faecal samples were subjected to the McMaster technique (McMaster counting slide, 2 cells, ALL GLASS, Vetlab Supplies Ltd.) with a slight modification to quantify the eggs/oocysts per gram (EPG/OPG) of the faecal sample. Saturated sodium chloride (specific gravity = 1.20) was used as a floatation solution for coccidian oocysts and nematode eggs (Cringoli et al., 2004). Two gram of faecal sample was homogenized in 28 ml of floatation solution. The faecal suspension was then strained three times through a double layer of gauze pad to remove debris. Both the chambers of a McMaster slide were loaded with the faecal suspension, allowed to stand for 5 min. A volume of

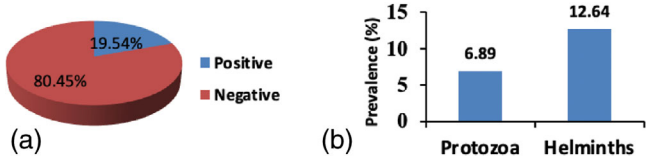

FIGURE 1 (a) Overall prevalence of gastrointestinal parasites (GIPs) in captive mammals. (b) Prevalence of protozoa and helminth parasites in captive mammals

0.15 ml of faecal suspension in each chamber was examined for protozoan oocysts and helminth eggs, and the EPG/OPG was calculated by multiplying the total number of oocysts/eggs (sum of both chambers) by 50 (Levecke et al., 2011)). The same procedure was repeated three times for each parasite positive faecal sample to increase the efficacy of the test, and the value of EPG/OPG was expressed as mean ± standard deviation (SD).

The formula used to calculate EPG/OPG was as follows:

$$\text{EPG/OPG} = \frac{n}{0.30} \times \frac{V}{m},$$

where n is the total number of eggs or oocysts in two counting chambers, 0.30 is the volume of the counting chamber, V is the volume of homogenized faecal sample ($V = 30$ ml), and m is the weight of faeces ($m = 2$ g).

2.4 | Data analysis

The parasitic prevalence was analyzed based on the number of parasite positive samples, and the total number of faecal samples examined from captive mammals belonged to three orders.

3 | RESULTS

3.1 | Prevalence GIPs

Out of 87 faecal samples examined, 19.54% were found to be shedding ova and/or oocyst of at least one species of GIPs (Figure 1a). In total, five GIPs were encountered—one protozoan (*Eimeria* spp.) and four helminths (*Strongyloides* spp., *Haemonchus* spp., *Trichostrongylus* spp., and hookworm) (Figure 2, Table 2); however, none of the trematodes and cestodes were recorded. The prevalence of protozoan and helminth parasites was recorded to be approximately 7% and 12.64%, respectively (Figure 1b). Three species of carnivora (jungle cat [*Felis chaus*], Himalayan black bear [*Ursus thibetanus*], and sloth bear [*Melursus ursinus*]), one species of rodentia (guinea pig [*Cavia porcellus*]), and five species of artiodactyla (blackbuck, spotted deer [*Axis axis*], barking deer [*Muntiacus vaganialis*], sambar deer [*Rusa unicolor*], and wild boar [*Sus scrofa*]) were infected with at least of one kind of GIP (Table 3). None of the carnivora and rodentia had a double infection, and among artiodactyla 25.71% had a single infection, while 8.57% had a double infection (Figure 3). Two individuals of spotted deer had a double infection, one with *Eimeria* and *Haemonchus*, and the other with *Eimeria* and hookworm, and only one individual of barking deer was infected with *Eimeria* and *Trichostrongylus* (Table 4).

3.2 | Parasitic burden

The parasitic load in captive mammals was estimated based on the number of eggs/oocysts per gram of faecal examination according to the McMaster technique. The highest OPG of *Eimeria* spp., the only protozoan detected, was recorded to be 427.77 ± 25.45 in spotted deer, while among the helminth parasites, the highest EPG with regard to *Strongyloides* spp. (383.33 ± 76.37) was recorded in wild boar, followed by *Haemonchus* spp. (283.33 ± 57.73) in spotted deer, *Trichostrongylus* spp. (233.66 ±144.33) in barking deer, and hookworm (216.66 ± 76.37) in spotted deer (Table 4).

4 | DISCUSSION

In this study, we confirmed that three orders of captive mammals at Central Zoo were infected by intestinal parasites. We can speculate that the parasitic infection might be acquired through contaminated food and a poor immune system (Northrop-Clewes & Shaw, 2000; WHO, 2002). Foods supplied to captive mammals were not examined for parasites; however, the majority of slotter animals in Kathmandy valley are with parasitic infection (see Joshi et al., 2003). This study is not based on any immunological evidence in concerning parasite prevalence. Captive conservation of wild animals generally results in severe stress with concomitant increased output of corticosteroids which further compromises their innate resistance by immunosuppression (Mbaya & Nwosu, 2006). We did not perform any stool test related to the stress level of the captive animals, and no such study has been conducted so far at Central Zoo. The intestinal parasites in captive animals were also recorded frequently in other zoos such as Rangpur recreational garden, Bangladesh (Khatun et al., 2014). However, the overall low prevalence (≈20%) of parasites was recorded in Central Zoo in comparison to Rangpur, Bangladesh (60%). Contrary to our result, a higher prevalence of parasitic infection (61.5%) with 18% of protozoans and 54.5% of helminths has been reported from two Italian zoological gardens (Fagiolini et al., 2010). Similarly, Kolapo and Jegede (2017) documented a 62.9% prevalence of overall GIP infection rate for all the captive animals except avian species. The low parasitic prevalence at Central Zoo might be due to efficient preventive measures such as daily removal of dung, a regular deworming schedule of twice a year, and a drainage system have been maintained by the zoo administration to reduce the environmental contamination. The prevalence of helminths (only nematodes) in this study was approximately double (12.64%) of that of protozoa (6.89%) which can be related to the soil-borne infection cycle of helminths and chances of soil contamination with parasitic stages. Generally, Central Zoo does not perform any activities for deworming the possibly contaminated

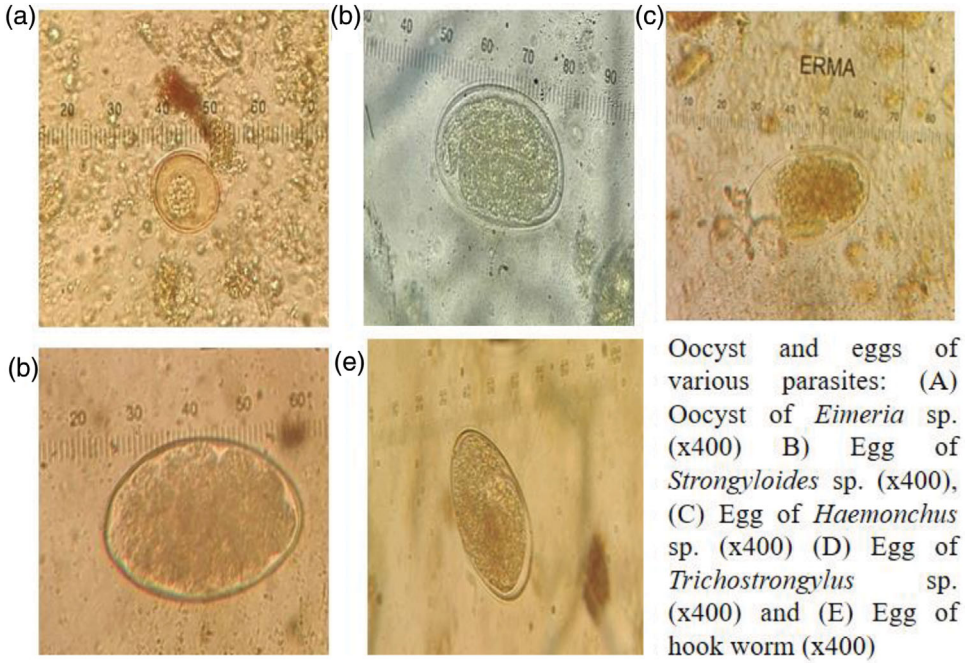

FIGURE 2 Gastrointestinal parasites in captive mammals at Central Zoo

TABLE 2 Prevalence of gastrointestinal parasites (GIPs) in three orders of mammals in Central Zoo

	Orders of Captive mammals			
GIPs	Carnivora ($n = 24$)	Rodentia ($n = 28$)	Artiodactyla ($n = 35$)	Total samples ($n = 87$)/prevalence (%)
Eimeria	–	–	6 (17.14)	6 (6.89)
Strongyloides	2 (8.3)	–	1 (2.85)	3 (3.44)
Haemonchus	–	–	3 (8.57)	3 (3.44)
Trichostrongylus	–	–	4 (11.42)	4 (4.59)
Hookworm	1 (4.1)	2 (7.14)	1 (2.85)	4 (4.59)
Total infected number	3 (12.5)	2 (7.14)	12 (34.28)	17 (19.54)

Note: En-dash indicates parasite not detected, and number in parenthesis indicates prevalence (%) of the parasites. n = number of samples.

TABLE 3 Double infection pattern among Artiodactyla

Artiodactyla with double infection	No. of cases with double infection	Combination of GIPs
Spotted deer	1	*Eimeria* + *Haemonchus*
Spotted deer	1	*Eimeria* + hookworm
Barking deer	1	*Eimeria* + *Trichostrongylus*
Total	3	

Abbreviation: GIPs, gastrointestinal parasites.

soil. The veterinarian and technical staff of Central Zoo follow the treatment of all captive animals by mixing the recommended dose of the prescribed drugs (albendazole and thiabendazole as anthelmintics and amprolium and metronidazole are used as anti-protozoan) with their preferred food. However, the provided animal feed mixed with medicine may be avoided by the animal because of the smell of the drug or sometimes may be taken. This might cause the continuous burden of parasitic infection and in most cases failure of treatment. The current deworming processes at every 6-month interval need to be revised.

This study reported one protozoan parasite (*Eimeria* sp.) and four nematodes (*Strongyloides* sp., *Haemonchus* sp., *Trichostrongylus* sp., and hookworm). However, trematodes and cestodes were not observed. This might be due to the absence of essential intermediate hosts as they have very complex life cycle patterns requiring at least one

TABLE 4 Captive mammal detected positive for gastrointestinal parasites (GIPs) and respective parasite load

Mammalian sampled	Housing condition	Total animals	Total samples	No. of cases	Parasite detected	Parasite load (mean EPG/OPG ± SD)
Carnivora						
Jungle cat	Group	4	2	1	*Strongyloides* sp.	266.66 ± 28.86
Himalayan black bear	Group	4	4	1	*Strongyloides* sp.	283.33 ± 76.37
Sloth bear	Group	4	4	1	Hookworm	150 ± 50
Rodentia						
Guinea pig	Group	50	12	2	Hookworm	175 ± 25
Artiodactyla						
Black buck	Group	2	2	1	*Eimeria* sp.	350 ± 50
Spotted deer	Group	29	15	3	*Eimeria* sp.	427.77 ± 25.45
				2	*Trichostryongylus* sp.	200 ± 50
				1	*Haemonchus* sp.	283.33 ± 57.73
				1	Hookworm	216.66 ± 76.37
Barking deer	Group	18	10	2	*Eimeria* sp.	366.66 ± 76.37
				1	*Haemonchus* sp.	166.66 ± 28.86
				1	*Trichostrongylus* sp.	233.66 ± 144.33
Sambar deer	Single	1	1	1	*Haemohcnu* sp.	200 ± 50
Wild boar	Group	3	2	1	*Strongyloides* sp.	383.33 ± 76.37

Note: Mean EPG/OPG ± SD is calculated from three times observations for each host.

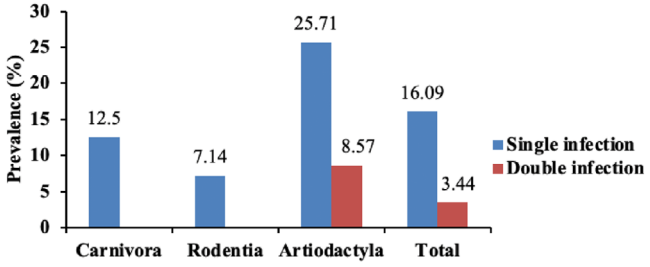

FIGURE 3 Pattern of single and double infection of gastrointestinal parasites (GIPs) in captive mammals

primary host and one or more intermediate hosts for the regular continuation of their races and subsequently transmitted to the susceptible host (Atanaskova et al., 2011). However, there was no previous record of the presence of any responsible intermediate hosts in and around Central Zoo, and we did not see any snail species as we visited Central Zoo in February–April. Among all the parasites recorded, *Eimeria* had the highest rate of prevalence (6.89%) and was only found in artiodactyla. The occurrence of this enteric protozoan parasite can be generalized by the simplicity of its lifecycle because it does not require any intermediate hosts and the oocysts are immediately infective when excreted (Tanyuksel & Petri, 2003; Thompson & Monis, 2004).

Among the artiodactyles, 12 individuals were infected, and the most common parasites were *Eimeria* and *Trichostrongylus*. The mixed infection in our study was found only in artiodactyla which might be due to the presence of animals of different age groups in the same cages. If the foods especially grass served to animals get contaminated before they were brought to the zoo, animals can be infected with the parasites following ingestion. Damp and improper cleaning of the animals' enclosures can be accountable for increased susceptibility to infections (Ortiz et al., 2007). In this study also, we found that both males and females have an equal risk of parasitic infection when they are brought to captive and provided with the same type of shed and food (Bacha & Haftu, 2014); however, gender-wise analysis of parasitic infection was not performed in this study. Fagiolini et al. (2010) reported the faecal quantification of EPG for strongyle nematodes ranging from 100 to 1800 (highest in black buck—*Antilope cervicapra*), and OPG of *Eimeria* to be 500 in American bison (*Bison bison*) and 750 in fallow deer (*Dama dama*) which was relatively higher than in our results. Generally, the deer and some birds are particularly susceptible to stress after encountering artificial breeding, high population density in the cage, visitor disturbances, and regular changing food types (Hu et al., 2018; Sharma et al., 2020). The exhaustive husbandry of wild animals in a zoo and zoological parks might be some of the reasons why higher animal density in the enclosure and their proximity to other animals provides the opportunity for accelerated transmission of parasites (Moudgil & Singla, 2013). However, we did not compare the parasite burden in relation to the density of the host animals in the enclosure owing to the smaller sample size.

5 | CONCLUSIONS

In conclusion, the captive mammal species at Central Zoo are susceptible to various parasitic infections despite careful management

practices. Besides deworming schedule, an emphasis needs to be laid on faecal analysis before administration of deworming and applying a more focused approach to minimize the chances of infection. We recommend deworming the captive animals at every 4-month interval to minimize the potential parasitic infection. Diagnosis at the molecular level of the parasite is essential to determine the chances of cross transmission of parasites among the various orders of mammals and their zoonotic potential. At the same time, the immunological status of the suspected animals needs to be carried out for the assessment of their immunity in relation to parasitic burden.

AUTHOR CONTRIBUTIONS

Conceptualization, sample collection, laboratory examination, writing original manuscript, and reviewing and editing: Pitambar Dhakal. *Conceptualization, supervision, and reviewing and editing manuscript*: Hari Prasad Sharma. *Reviewing and editing manuscript*: Rachana Shah, Parbat Jung Thapa, and Chiranjibi Prasad Pokheral.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

ETHICS STATEMENT

This research was performed under the non-invasive sampling methods. The research was permitted by Central Zoo, Lalitpur, Nepal.

ORCID

Hari Prasad Sharma https://orcid.org/0000-0002-0708-1769

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