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Original paper

Prevalence of gastrointestinal parasites among greater one-horned rhino in Chitwan National Park, Nepal

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ABSTRACT. Recently, many individuals of greater one-horned rhino (GOHR) were died with unknown reason in Chitwan National Park (CNP), Nepal. This has arisen chaos and confusion in the rhino conservation program of the country. This study was designed to determine the prevalence of gastrointestinal parasites among GOHR in the CNP. A total of 100 dung samples were collected opportunistically by the random sampling method. Dung samples were preserved in 2.5% potassium dichromate solution and analysed in the laboratory by the direct smear and concentration method (floatation and sedimentation). Eggs and larvae of gastrointestinal parasite were found in 91% examined samples. Altogether 13 different genera of parasites were identified with one protozoan i.e. *Eimeria* sp. (9%), nine nematodes i.e. *Strongyloides* sp. (65%), *Ascaris* sp. (16%), *Haemonchus* sp. (15%), *Dromeostrongylus* sp. (9%), *Oxyuris* sp. (8%), *Bunostomum* sp. (8%), *Chabertia* sp. (5%), *Trichostrongylus* sp. (4%) and *Nematodirus* sp. (2%), one cestode i.e. *Anoplocephala* sp. (16%) and two trematodes i.e. *Paramphistomum* sp. (31%) and *Fasciola* sp. (14%). Nematode parasites were found to be most prevalent (87% of samples) followed by trematodes (45%), cestodes (16%) and protozoans (9%). The study indicates a high prevalence of gastrointestinal parasites in the GOHR of CNP and identifies that there is need of strategic control measures to protect this endangered species from parasitic infection.

Keywords: wildlife parasites, conservation, nematodes, infection, prevalence

Introduction

Greater one-horned rhino (*Rhinoceros unicornis* Linnaeus, 1758, henceforth GOHR) used to be found across the entire northern part of the Indian sub-continent along the Indus, Ganges and Brahmaputra River basins from Pakistan to the Indian-Burmese border including Bangladesh and the southern lowlands of Nepal and Bhutan [1]. In Nepal, they are found in the riverine grasslands of the Terai and prefer the flood plain grasslands and adjacent swamps, waterholes and riverine forests [2]. GOHR is highly threatened large mammal species and are listed in the appendix I of the convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and is categorized as Vulnerable under the International Union for Conservation of Nature (IUCN) Red List [3]. Further, it is listed as a protected species by the National Park and Wildlife Conservation Act, 1973 of Nepal. Habitat loss and vicious poaching were the twin prominent drivers causing rhino population decline in Nepal [4]. However, infectious diseases are also an incipient threat to these threatened species [5]. Its negative scope can further be exaggerated with recent high unknown deaths of GOHR in Chitwan National Park (CNP) and its buffer zone. For instance, in a 12-month period from June 2018–June 2019, a total of 43 rhinos were recorded dead in CNP of which the reasons behind death of 20 rhinos were unknown [6]. Generally, the known causes of the rhino death include poaching, old age, fight with each other, natural calamities, tiger predation, trampling and disease [7]. Our research assumes that the parasites could harm the rhino more than anticipated.

Intestinal parasites are the parasites that can infect the gastrointestinal tract of humans and other animals [8]. They can thrive throughout the body but prefer the intestinal wall [9,10]. The most

favourable sites for gastrointestinal parasites are the duodenum, ileum, caecum and large intestine [11]. The gastrointestinal parasites are comprised of protozoan and helminth parasites. The intestinal parasitic worms associated to GOHRs are cestodes, trematodes and nematodes [12]. Protozoans can be directly infectious when they are passed through the faeces into the environment, but helminths require a period of maturation in the soil to become infectious, with some requiring the involvement of intermediate hosts [13]. Further, GOHRs are found to be infected with trematodes: *Fasciola* sp., *Paramphistomum* sp., cestodes: *Anoplocephala* sp. and various species of nematodes as *Kiluluma* sp., *Chabertia* sp., *Necator* sp., *Bunostomum* sp. and *Strongylus* sp. and coccidians [14,15]. Similarly, hookworms (Nematoda: Strongylida) are blood-feeding nematodes that parasitize the mammalian alimentary system, which causes chronic blood loss and creates a perfect environment for secondary bacterial infections, with significant inflammation in the mucosae, impairing digestion and absorption [16]. Furthermore, Trematode parasites cause watery diarrhoea, weakness, weight loss, other secondary infections and even mortality [17].

Limited number of researches have been conducted on the gastrointestinal parasites of rhinoceros species. In Nepal, single study to date, documented the trematode parasites in GOHR [18]. So, the present study is relevant to give continuity to gastrointestinal parasites research, as the Chitwan GOHR population holds second largest and unique sub-population after Kaziranga National Park of Assam, India [19]. Most importantly, our study aims to facilitate an understanding of the prevalence and intensity of gastrointestinal parasites, and provide baseline data for interpreting GOHR morbidity and mortality and contributing to the formation of policies and potential control strategies.

Materials and Methods

Study area

Chitwan National Park (CNP), a World Heritage Site, is located in four districts (Nawalpur, Parsa, Chitwan and Makwanpur) of Nepal. The park is situated in south-central Nepal in the tropical lowlands of the inner Terai (27°16.56'– 27°42.14'N and 83°50.23'– 84°46.25'E) and comprises a core area of 952 km^2 and a buffer zone of 729 km^2 (Fig. 1). The area was gazetted as the country's first national park to conserve keystone species including rhinos and other threatened species and their habitat [20].

Faecal analysis

GOHR has a unique habit of sharing common latrine thereby making a large pile of dung [21–23].

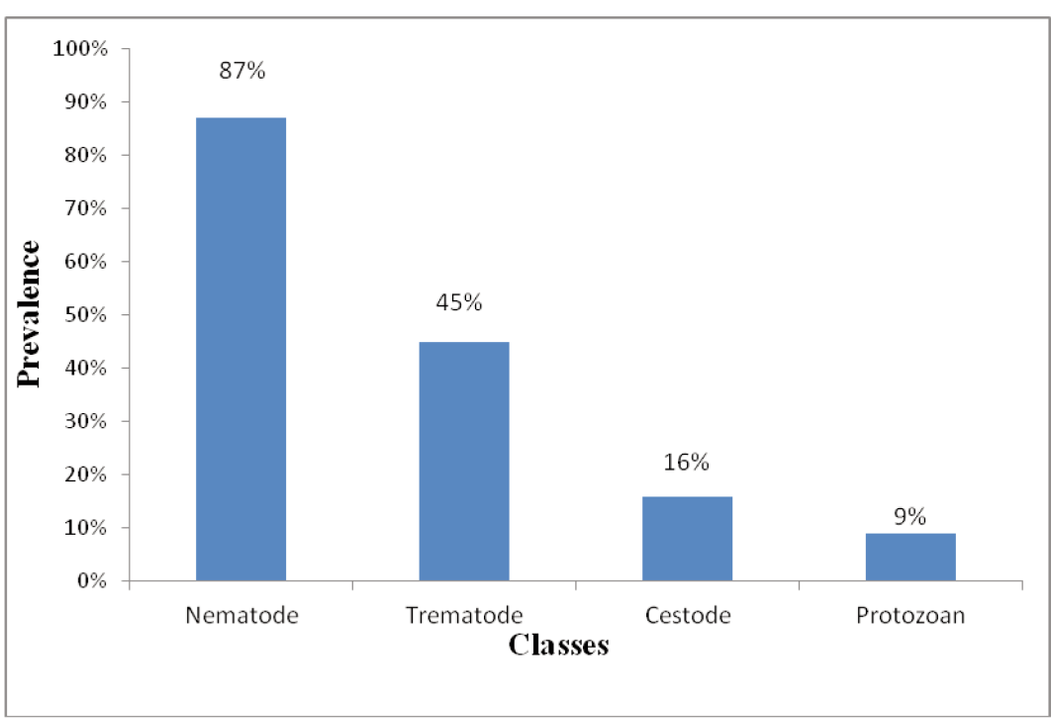

Figure 1. Class wise prevalence of gastrointestinal parasites in GOHR

Table 1. Genera wise prevalence of gastrointestinal parasites in 100 faecal samples of GOHR

Identified gastrointestinal parasites	Number of infected samples	Prevalence
Eimeria sp. with micropyle	6	6%
Eimeria sp. without micropyle	3	3%
Strongyloides sp.	65	65%
Haemonchus sp.	15	15%
Dromeostrongylus sp.	9	9%
Bunostomum sp.	8	8%
Oxyirus sp.	8	8%
Ascaris sp.	16	16%
Trichostrongylus sp.	4	4%
Chabertia sp.	5	5%
Nematodirus sp.	2	2%
Paramphistomum sp.	31	31%
Fasciola sp.	14	14%
Anoplocephala sp.	16	16%

A total of 100 dung samples from free ranging GOHR were collected opportunistically from different sectors of CNP during the summer season i.e. May 2017. Since the samples were collected from wild habitat with random sampling method, we assumed these samples were from different individuals because we collected fresh samples within a short interval of time from different locations. Each fresh dung sample represented individual rhino which particularly helped to avoid resampling of same faeces. However, our research has limitation as parasites prevalence varies with seasons. About 50 gm of freshly voided dung sample were collected separately in labelled 50 ml vials and these samples were properly sealed, labelled with date, time and place. The vials contained 2.5% potassium dichromate that helps in maintaining the morphology of protozoan parasites and fixing helminthic eggs and larvae. The preserved samples were processed for microscopic examination. The eggs/cysts and larvae of different parasites were identified according to their morphology and quantitative estimation by using direct smear method, concentration method (floatation and sedimentation technique) [17] and Stoll's counting technique to determine mixed infection and intensity of parasites [24]. The floatation technique for this study was used based on the principle that lighter eggs of helminths and protozoans float on the medium having greater density [17]. Two grams of faecal samples were homogenized in a drop of Lugol's iodine solution. Any debris were removed from the solution, and the suspended faecal were examined for protozoan oocysts and helminths eggs. The smear was examined under microscope at 10× and 40×. Oocysts, eggs and larvae were identified on the basis of morphological characters (shape and size) [17]. A stage micrometer was used to measure the length and breadth of eggs, oocysts and larvae**.** The intensity of parasite infection was calculated depending upon the number of eggs/oocysts and larvae found per field [24].

Light infection = <2 eggs/cysts/larvae per field
Mild infection = 3–4 eggs/cysts/larvae per field
Moderate infection = 5–6 eggs/cysts/larvae per field
Heavy infection = >6 eggs/cysts/larvae per field

Statistical analysis

Chi-square tests were used to identify the differences on the prevalence of parasites on the Greater one-horned rhino, for that a 95% confidence interval at $P<0.05$ was considered for statistically significant. All analyses were performed in R program 3.5.2. [25] and SPSS 23 [26].

Results

Of total samples (n=100), 91% (n=91) showed prevalence of gastrointestinal parasites in GOHR of

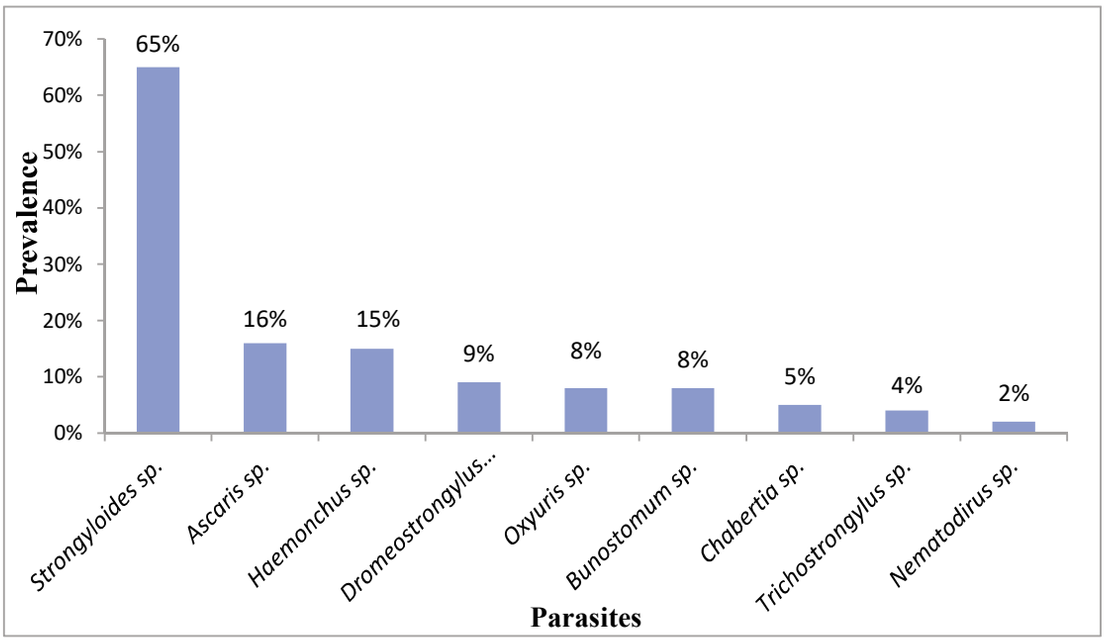

Figure 2. Prevalence of nematode parasites in GOHR

CNP. The microscopic examination of 100 dung samples of GOHR revealed 13 different genera of gastrointestinal parasites (Tab. 1).

Prevalence of protozoans, nematodes, trematodes and cestodes

Regarding class wise distribution of gastrointestinal parasites, nematodes showed the highest prevalence followed by trematodes, cestodes and protozoans (Fig. 1). Only nine samples out of 100 samples were found to be positive for protozoan parasites. The prevalence of *Eimeria* sp.

Table 2. Intensity of infection with parasites

Class	Parasites	Light (+)	Mild (++)	Moderate (+++)	Heavy (++++)
Nematodes	*Strongyloides* sp.	20 (30.7%)	31 (47.6%)	9 (13.8%)	5 (7.6%)
	Ascaris sp.	4 (25.0%)	5 (31.3%)	7 (4.8%)	–
	Haemonchus sp.	2 (13.3%)	4 (26.7%)	7 (46.7%)	2 (13.3%)
	Dromeostrongylus sp.	1 (11.1%)	6 (66.7%)	2 (22.2%)	–
	Oxyuris sp.	–	–	3 (37.5%)	5 (62.5%)
	Bunostomum sp.	5 (62.5%)	3 (37.5%)	–	–
	Chabertia sp.	3 (60.0%)	2 (40.0%)	–	–
	Trichostrongylus sp.	4 (100.0%)	–	–	–
	Nematodirus sp.	2 (100.0%)	–	–	–
Trematodes	*Paramphistomum* sp.	2 (6.4%)	9 (29.0%)	14 (45.2%)	6 (19.4%)
	Fasciola sp.	2 (14.3%)	9 (64.3%)	3 (21.5%)	–
Cestodes	*Anoplocephala* sp.	3 (18.8%)	5 (31.3%)	3 (18.8%)	5 (31.3%)
Protozoa	*Eimeria* sp. with micropyle	–	–	–	6 (100.0%)
	Eimeria sp. without micropyle	–	–	–	3 (100.0%)

Explanations: + = less than 2 ova per field i.e. light infection, ++ = 2–4 ova per field i.e. mild infection, +++ = 4–6 ova per field i.e. moderate infection, ++++ = 6 or more ova per field i.e. heavy infection

with micropyle was 6% and without micropyle was 3%. There was no significant difference in the prevalence of *Eimeria* sp. with and without micropyle (χ^2=1 and *P*=0.317). Among 100 samples examined, 87 samples were found to be positive for nematode parasites with nine different genera (Fig. 2).

Out of 100 samples, 45 dung samples were found to be positive for trematodes (31% *Paramphistomum* sp. and 14% *Fasciola* sp.). Sixteen samples were found to be positive for cestodes.

Intensity of infection with parasites

The intensity of parasitic infection has been assessed based upon the number of eggs or oocysts and larvae found per field (Tab. 2).

Our study revealed the differences in the faecal load between different parasites. Six samples were found with heavy infection with *Eimeria* sp. without micropyle and *Anoplocephala* sp. while five samples were with high intensity of *Strongyloides* sp., *Oxyuris* sp. and *Anoplocephala* sp., three samples with *Eimeria* sp. with micropyle and two samples with *Haemonchus* sp. Similarly, 14 samples were infected with *Paramphistomum* sp., nine samples with *Strongyloides* sp. seven each samples with *Ascaris* sp. and *Haemonchus* sp., three each samples with *Oxyuris* sp., *Fasciola* sp. and *Anoplocephala* sp. and two samples with *Dromeostrongylus* sp. showed the moderate infection. Maximum one sample showed the mild infection of *Strongyloides* sp. followed by nine each samples with *Paramphistomum* sp. and *Fasciola* sp.; six samples with *Dromeostrongylus* sp., five each samples with *Ascaris* sp. and *Anoplocephala* sp., four samples with *Haemonchus* sp., three samples with *Bunostomum* sp. and 2 samples with *Chabertia* sp. 20 samples with *Strongyloides* sp. showed light infection followed by 5 samples with *Bunostomum* sp., four each samples with *Ascaris* sp. and *Trichostrongylus* sp., three each samples with *Chabertia* sp. and *Anoplocephala* sp., two each samples with *Haemonchus* sp., *Nematodirus* sp. and *Fasciola* sp. and one sample with *Dromeostrongylus* sp. were found to show the light infection. Overall number of heavy infection was found to be less compared to light, mild and moderate infection.

Discussion

Our study prevalence of gastrointestinal parasites compares to other studies. The high prevalence rates of gastrointestinal parasites were also recorded from one-horned rhinoceros in captivity of Bangladesh [27], black rhino in the National Zoological Garden, Sri Lanka [28] and animals of order Perissodactyla in a zoological garden in Italy [29] which showed the total parasitic prevalence of 100%. Conversely, the lower infection rates of gastrointestinal parasites were reported in some of the previous studies in India and Nepal regarding the gastrointestinal parasites in GOHR [15,18] and in Javan rhinoceros from Indonesia [30,31] where the prevalence rates were 61.9%, 50% 45% and 56% respectively. The variation in the prevalence might be due to the difference in the number of samples examined, difference in the species of rhinoceros from which the samples were collected and difference in the location from where the samples were collected because the variation in topographical location can influence the rate of prevalence [32]. Interestingly, the present study revealed that GOHR of CNP were infected by both helminths and protozoan parasites. Among four classes of parasites, nematodes were highly prevalent followed by trematodes, cestodes and protozoan parasites. The findings were in accordance with the findings of Palmieri and Purnomo [30] who documented highest prevalence of nematodes followed by trematodes and cestodes (50%, 45% and 30% respectively) in Javan rhinoceros. However, it was contrary to the study of Chakraborty and Islam [15] who reported higher trematode infection followed by nematode, protozoan and cestode infection (46.4%, 20.2%, 3.5% and 2.3% respectively). From the economic and sanitary point of view, coccidian parasites are the most prevalent among protozoans. Coccidian parasites infect large numbers of wild animals including rhinoceroses. *Eimeria* sp. is the most common coccidian parasite among wildlife and livestock. There are two distinct types of *Eimeria* sp., with micropyle and *Eimeria* sp. without micropyle. There are cases of protozoan infection in rhinoceros but literatures regarding the *Eimeria* infection are scanty. *Eimeria* sp. infection in Javan rhinoceros of Ujung Kulon National Park, Indonesia has been reported by Bliss [33]. In the present study, the prevalence of *Eimeria* sp. was found to be 9% which is higher than 3.5% in GOHR of Kaziranga National Park, India [15]. Helminths (including, nematodes, cestodes and trematodes) infections are common in humans, livestock and wild animal

populations, having key ecological and evolutionary roles through the energetic costs they impose on their hosts [34].

Strongyloides sp. causes strongyloidosis. *Strongyloides* sp. was found to be the most prevalent parasites in this study which could be due to more conducive environment for the development of the pre-parasitic stages in the hot and humid environmental conditions of the CNP. In the present study, the prevalence of *Strongyloides* sp. is higher than in Javan rhinoceros (10%) in Indonesia [30]. Another nematode parasite recorded in this study is *Oxyuris* sp. This parasite lives in the caecum, colon and rectum. The presence of worms in the intestine rarely produces clinical signs, but the female worms can cause perineal pruritus [35]. Previously, *Oxyuris* sp. was recorded from black and white rhinoceroses of Africa [36,37]. There appear few reports of *Bunostomum* sp. infection in Java rhinoceros [30,38]. The prevalence of *Bunostomum* sp. reported in this study was lower than 25% in Javan rhinoceros in Indonesia [29]. Of the greatest interest was the prevalence *Ascaris* sp., *Haemonchus* sp., *Dromeostrongylus* sp., *Chabertia* sp. and *Nematodirus* sp. which have not been reported previously in any species of rhinoceros globally. *Trichostrongylus* sp. is a common parasitic round worm of cattle and the adults of various species are mainly found in the small intestine, caecum and stomach [39]. There is very little evidence of *Trichostrongylus* infection in rhinoceros. *Trichostrongylus* infection in rhinoceros has been reported previously in Javan rhinoceros [30,38] the present study revealed prevalence of *Trichostrongylus* sp. in GOHR of CNP which was similar to the finding of Palmieri and Purnomo [30] who reported 5% prevalence of *Trichostrongylus* sp. in Javan rhinoceros in Indonesia. Trematode parasites cause watery diarrhea, weakness, weight loss, decreased milk production, reduced product quality mortality and other secondary infections in animals [17]. Prevalence of trematode parasites in GOHR was found to be 45% that might be due to its semi-aquatic mode of life, access of parasites to intermediate hosts, and sharing similar habitat with other infected individuals. Not only GOHR, but there are also many threatened wildlife species including musk deer and red pandas are vulnerable to parasite/disease transmission from infected individuals including livestock due to living in the same habitat or using same resources for food [40,41]. Trematode parasites have been recorded from various species of rhinoceros both globally and in Nepal as well [18,27,28,30,31,38]. The recorded trematode parasites in the present study were *Paramphistomum* sp. and *Fasciola* sp. Present study revealed prevalence of *Paramphistomum* sp. in GOHR which was slightly higher than the prevalence recorded from Our study observed high prevalence of gastrointestinal parasites among GOHR in CNP when compared Javan rhinoceros in Indonesia [29] and GOHR of India [15]. However, higher infection of *Paramphistomum* sp. was recorded by Chakraborty and Islam [15] in GOHR where the prevalence rate was 50%. Similarly, the prevalence of *Fasciola* sp. in the present study was found to be lower than 44% reported from Javan rhinoceros in Indonesia [31]. Previously, *Fasciola* sp. was reported by [38] from Javan rhinoceros in Indonesia.

The only cestode parasite recovered in this study was *Anoplocephala* sp. which has been reported frequently in previous studies in the global context. Cycle of cestodes is complicated involving one or more intermediate hosts. Requirement of intermediate host to complete its life cycle is the main differential factor for differential prevalence of cestode parasites. This might be the reason for low prevalence of cestodes in comparison to other helminthes. Lower prevalence (2.3%) of *Anoplocephala* sp. was recorded from one-horned rhinoceros in India [15]. On the other hand, the higher prevalence was recorded from Javan rhinoceros in Indonesia and black rhinoceros in Europe with 30% and 87.3% respectively [30,42]. Animal species, geographic distribution, climatic conditions and habitat preference each contribute towards determining the species composition of parasites [43]. This might be the reason for the variation in the results of the previous and present studies. The consequences of fecal egg load of parasite depend on type of parasite as well as where it resides in the host body. Mild infection of parasites was recorded the most which is asymptomatic condition and does not cause the diseases in animals. The heavy infection indicates symptomatic condition and causes the serious diseases in animals. Heavy infection of *Strongyloides* sp. causes anaemia due to erosions and ulceration of small intestinal mucosa and consequential development of hemorrhagic enteritis. Similarly, heavy infection of *Paramphistomum* sp. causes decreased appetite, listlessness, weight loss, diarrhea, dehydration and even death [44]. Heavy infection of *Eimeria* sp. in

animals is associated with loss of appetite, dehydration, and watery sometimes bloody diarrhea [45]. Clinical signs associated to infection of *Haemonchus* sp. include pallor, anaemia, oedema, lethargy and depression [46] and *Oxyuris* sp. in case of heavy infection is supposed to cause perineal pruritis.

In conclusion, the study indicated high prevalence of gastrointestinal parasitic infection in GOHR of CNP. The high prevalence of gastrointestinal parasites indicates subclinical infection which may flare up under stress conditions and can cause pathogenicity. Further, in CNP and its buffer zone, there are large areas used for unmanaged livestock grazing especially in buffer zone community forests which could represent opportunities for transmission of parasites from livestock to rhinos and *vice versa*. Thus, the result generated from this study emphasizes the importance of making the special measures to control this parasitic infection in order to safeguard the health of greater one-horned rhinoceros in CNP. With all the findings we recommend to reduce the risk of intestinal parasitic threats for welfare of GOHR. For this we emphasize, to conduct molecular level study for accurate identification of parasites up to species level as our study is confined to genus level, seasonal study of parasitic prevalence in GOHR and frequent surveillance of gastrointestinal parasites should be carried out to reduce the risks associated to these parasites.

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