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Prevalence Of Gastrointestinal Parasites In The Greater One-Horned Rhinoceros (Rhinoceros Unicornis) At Chitwan National Park (Cnp), Nepal

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Abstract:
***Background**: The study was conducted to assess the prevalence of gastro-intestinal parasites in Greater One-horned Rhinoceros (Rhinoceros unicornis) at Chitwan National Park, Chitwan, Nepal from February to December 2021. The Greater One-horned Rhinoceros has been listed in the vulnerable category, with the serious concern of conservation due to habitat destruction and various diseases including parasites*
***Materials and Methods**: The necessary data was collected from NTNC officials, forest rangers, security officers, and native people living in the buffer zone. Collecting samples from all of the Rhino roaming areas was challenging due to the arduous pathway and the chance of being attacked by a wild animal, so we chose to use the systematic random sampling method (Kakoti et al., 2019). The faecal samples were collected from 3 different locations within Chitwan National Park i.e., Sauraha and Golaghat in Chitwan district and Divyapuri in Nawalpur district, Nepal. Samples were collected along with GPS coordinates, from both the core and buffer zone areas where the livestock interface can be observed. A total of 109 recently voided fecal samples were collected in a zip lock bag without preservatives, labelled distinctly, and stored in a refrigerated box with ice before further investigations. Identifications of GI parasites were performed qualitatively with faecal floatation and sedimentation techniques at Department of Microbiology and Parasitology, Agriculture and Forestry University, Chitwan. Data were analyzed using SPSS (version 25.0) software for statistical analysis.*
***Results**: Among the samples tested 103 were found positive for 16 different genera of gastro-intestinal parasites from 4 different classes i.e., nematode, trematode, cestode, and protozoa. The prevalence of the nematodes (87.2%) was found highest followed by trematodes (65.1%), cestodes (38.5%), and protozoa (2.8%). Among the nematodes, the highest prevalence was revealed in Strongyloides sp. (54.10%). The co-infection study revealed that double infection (45%) showed the highest prevalence followed by a triple (26.7%) and single infection (23%). Prevalence based on location was higher at Divyapuri (100%) followed by Golaghat (97.1%) and Sauraha (92.6%). The prevalence of trematodes was higher at Divyapuri (83.3%) followed by Golaghat (82.9%) and lowest in Sauraha (54.4%).*
***Conclusion:** The study concluded that rhinoceros have a high burden of GI parasites, which could be the cause of mortality. Strategically planned wildlife treatment protocols are fundamental for wildlife conservation. Prioritizing research into the anthelmintic properties of plants and herbs should be required. Frequent surveillance of gastro-intestinal parasites in Greater One-Horned Rhinoceros should be carried out to reduce the risks associated with these parasites.*
***Key Word**: Greater One-Horned Rhinoceros, In- situ conservation, Qualitative Examination, Anthelminthic, Greater, Department of National Park and Wildlife Conservation (DNPWC), National Trust for Nature Conservation (NTNC)*

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I. Introduction

Nepal is renowned for its vast floral and faunal species, as well as its diverse natural ecosystem, which ranges from the southern lowland Terai region to the Northern High Himalayas (IUCN, 2008). Diversified metrological and landform conditions have played an important role in the variation in the flora and fauna in Nepal. There are 12 national parks, 6 conservation areas, 1 wildlife reserve, 1 hunting reserve, and 13 buffer zones respectively, and occupies about 23.39% of the total country's area which is dedicated to the in-situ conservation and protection of the biodiversity within various ecosystems all over the country.

Among the National parks, Chitwan National Park is the first national park that was established in 1973 occupying an area of 952.63 km (367.81 sq. mi). This National Park is wealthy with the various species of wildlife like the Royal Bengal tiger (Panthera tigres), Asiatic elephant (Elephas maximus), and the most iconic and symbolic endangered species of mammals, the greater one-horned Asiatic rhinoceros (Rhinoceros unicornis) (Bhuju et al., 2007). The greater one-horned rhinoceros is inhabiting around the Chitwan National Park, Bardia National Park, and Shuklaphanta National Park in Nepal. Recently few numbers of rhinos even started to inhabit the Parsa National Park due to suitable habitat. The national red list of mammals has labelled one-horned rhinoceros (IUCN, 2008) as a vulnerable species and the status of this species is secured under National Park and Wildlife Conservation Act, 1973. Poaching and habitat destruction have been a serious threat to the existence of the one-horned rhinoceros whereas the disease and the larger population in an isolated habitat with some limited restricted areas for them have also been a great threat (Kakoti et al., 2019).

Parasitic infestation is one of the most common problems in Rhinoceros and gastrointestinal nematodes are the most common and widely distributed helminths parasite in these species. *Strongyloides sp.* is the most common cause of gastrointestinal nematodiasis in Rhinoceros. There are massive infestations with certain species that can directly cause the death of rhinoceros affected, and on the other, there are parasites less dangerous by themselves but may act as a predisposing factor for the development of secondary deficiency and infectious diseases bringing about a negative impact on their health and reproduction (Borg et al., 2014)limited restricted areas for them have also been a great threat (Kakoti et al., 2019). Intestinal helminthic worms associated with the one-horned rhinoceros are nematoda, cestoda, and trematoda. The duodenum, ileum, caecum, and large intestine are the most suitable regions for gastro-intestinal parasites (Cuomo et al., 2009). Parasites can disrupt the host's reproduction and survival directly by causing pathological changes such as blood loss, tissue damage, spontaneous abortion, congenital deformity, and death, as well as impacting the host's immune system and risking its health (Thawait et al., 2014)

II. Material And Methods

Study Design: The necessary data was collected from NTNC officials, forest rangers, security officers, and native people living in the buffer zone. Collecting samples from all of the Rhino roaming areas is challenging due to the arduous pathway and the chance of being attacked by a wild animal, so we chose to use the systematic random sampling method (Kakoti et al., 2019).

Study Location: The current research was conducted at 3 separate locations in Chitwan National Park (CNP) i.e. Sauraha, Golalghat, and Divyapuri). The faecal samples were collected from 3 different locations within Chitwan National Park i.e., Sauraha and Golaghat in Chitwan district and Divyapuri in Nawalpur district, Nepal. Chitwan National Park is located in Nepal's, subtropical inner Terai lowlands,
bordering the districts of Chitwan, Nawalpur, Makwanpur, and Parasi. It ranges from roughly 100 meters (330 feet)
in the river valley to 815 meters (2674 feet) in the Churia highlands (DNPWC, 2021).

Study Duration: February 2021 to December 2021.

Sample size: A total of 109 recently voided fecal samples.

Identification of the dung in the field:

Freshly voided rhino dung samples were collected on foot or occasionally by using elephant rides mostly early in the morning. The Greater One-horned Rhinoceros mostly deficate repeatedly at a certain site, resulting in a heap-like structures. It is not much difficult to identify the fresh rhino dung on the basis of location, smell and textures; however, the following criteria were taken into consideration (Bhattacharya & Chakraborty, 2015).

- Size (diameter) of the dung balls: adult, sub- adult, and calf 13.8 cm (1.1 cm), 9.9 cm (0.6 cm), and 5.2 cm (0.5 cm), respectively.
- Color of the new defecations: greenish-black to deep greenish-brown.

- Texture of the dung ball: spongy and wet to varying degrees. The texture of new defecations deteriorated over time, becoming mushy and brittle, dry, and the colour progressively changed to a straw colour (these were discarded).
- Height of the dung heap: 0-3 meters taking around 55 days for its formation.

Sample collections

Three separate sites inside Chitwan National Park were sampled: Sauraha, Golaghat (Chitwan district) and Divyapuri area (Nawalpur district) (Appendix 1). Dung samples were collected with the assistance of veterinarians, forest rangers, government workers, and NTNC officials. The dung samples were labelled well with GPS coordinates, date and representative of rhinos living in core as well as buffer zone areas where the livestock interface is distinct. A total of 109 faeces bolus samples were collected from a heap of dung and placed in a zip lock bag without preservatives, marked with a distinct identification number along with the date, time, and sampling site. The sample was chosen based on the excrement visual and physical characteristics and only recently voided samples were collected. Freshly voided field samples were collected in a sealed plastic bag and stored in a refrigerated box with ice before being delivered to the Department of Microbiology and Parasitology, Agriculture and Forestry University, Chitwan, for further investigation.

Microscopic analysis

The samples were microscopically analysed at the Department of Microbiology and Parasitology, Agriculture and Forestry University (AFU) Chitwan. The qualitative examination was conducted using direct microscopic examination and concentration methods (Sedimentation and Floatation techniques) according to Soulsby (1982) for the identification of eggs, cysts, oocysts, and larvae of various gastrointestinal parasites

Qualitative examination procedure:

- Floatation technique

This technique ensures that the eggs float in the flotation liquid, allowing the eggs to be identified, and was used to identify nematode and cestode eggs in feces. A beaker was filled with 3 gm of dung sample and 42 ml of water, then the sample was lightly mixed with a mortar and pestle. Using a tea strainer, the solution was then filtered. The filtrate solution was placed in a 15 ml centrifuge tube and centrifuged for 5 minutes at 2000 rpm (Paudel et al., 2022). The water in the tube was replaced with a saturated sodium chloride solution and centrifuged once more (Soulsby, 1982). More saturated sodium chloride solution was added after centrifugation to create a convex surface at the top of the tube, then one drop of methylene blue (to stain) and a coverslip were placed for a few minutes. The coverslip was then removed and placed on a slide, then was studied at 10X and 40X magnification. Photographs of cysts and eggs were obtained, and the color, shape, and size of the eggs were used to identify them.

- Sedimentation technique

The detection of trematode eggs is accomplished using this method. Because trematode eggs are slightly heavier than other eggs, sediments from centrifuged contents were used to detect eggs. After analyzing the floating section of the saturated salt solution, the sediment was gently removed from the test tube and placed into the watch glass, where it was gently mixed. To make a second slide, one drop of the mixture was taken. Iodine wet mount solutions were used to stain the sample (Soulsby, 1982); past works of literature, and online sources were used to identify oocysts, eggs, and larvae based on morphological features (form and size).

Statistical analysis

The data obtained from the microscopic analysis of the dung samples were subjected to ANOVA and analyzed statistically using SPSS. The Person Chi-square test was used to estimate the significance among categorical variables.

III. Result

Microscopic findings

The microscopic examination, larval culture and analysis of DNA by Gel electrophoresis of obtained larvae of nematodes revealed 16 different genera of gastrointestinal parasites in the Rhino dung samples.The identification was based on morphology including color, size and texture of the eggs, oocysts, cysts and larvae of gastro intestinal parasites (Table 1).

Table 1. Identification of different genera of the gastro-intestinal parasites in Greater One-horned Rhinoceros at Chitwan National Park

S.No.	Class of GI parasites	Genera identified	No. of positive samples
1	**Nematodes**		
		Strongyloides sp.	59
		Haemonchus sp.	16
		Strongylus sp.	10
		Toxocara sp.	32
		Chabertia sp.	14
		Trichuris sp.	3
		Ascaris sp.	41
		Dictyocaulus sp.	7
		Oesophagostomum sp.	2
		Trichostrongylus sp.	1
2	**Trematodes**		

		Schistosome sp.	33
		Fasciola sp.	34
		Paramphistomum sp.	40
3	**Cestodes**	*Anoplocephala sp.*	19
		Moniezia sp.	30
4	**Protozoa**	*Eimeria sp.*	3

Parasitic class wise prevalence of GI parasites

Out of 109 fecal samples tested, 103 samples were found positive for 16 different genera of gastro-intestinal parasites from four different classes i.e., nematode, trematode, cestode, and protozoa. The prevalence of the nematode parasites (87.2%) was highest followed by trematodes (65.1%), cestodes (38.5%), and protozoa (2.8%) (Fig.2).

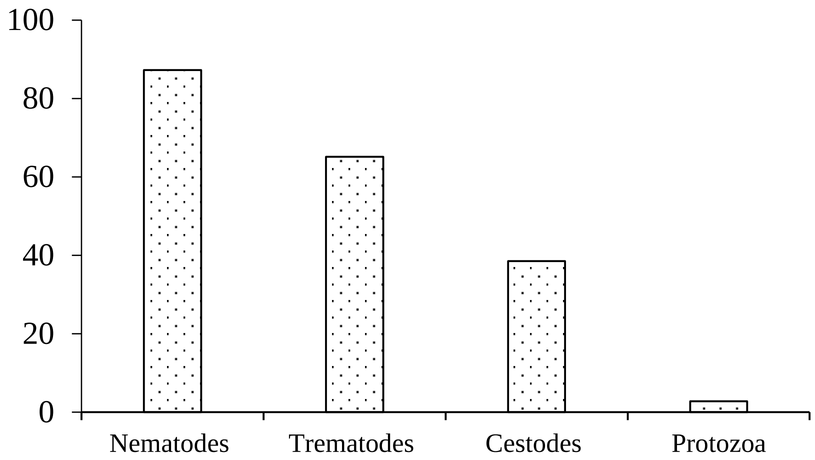

Figure 2. Class-wise prevalence of gastrointestinal parasites in Greater One-horned Rhinoceros

Prevalence of nematodes

Among 109 samples examined, 95 samples were found positive for nematodes belonging to 10 different genera. *Strongyloides sp*. was the most common nematode with the highest prevalence rate i.e., 54.1% followed by *Ascaris sp*. (37.6%), *Toxocara* sp. (29.4%), *Haemonchus sp.* (14.7%), *Chabertia sp*. (12.8%), *Strongylus sp*. (9.2%), *Dictyocaulus sp*. (6.4%), *Trichuris sp*. (2.8%), *Oesphhagostomum sp*. (1.8%) and *Trichostrongylus sp*. (0.9%), respectively (Fig. 3).

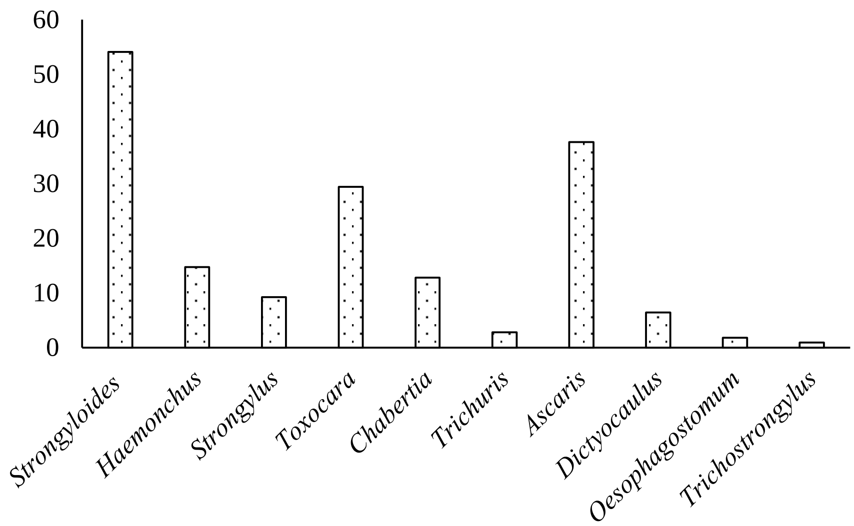

Figure 3. Prevalence of nematodes in Greater One-horned Rhinoceros

Prevalence of trematodes

Out of 109 samples, 71 samples were found positive for trematodes. Three different genera of trematode parasites were identified i.e. *Paramphistomum* sp., *Schistosoma* sp., and *Fasciola* sp. Among the identified parasites highest prevalence was shown by *Paramphistomum* sp. (36.7%) followed by *Fasciola* sp. (31.2%) and *Schistosoma* sp. (30.3%) (Figure 4)

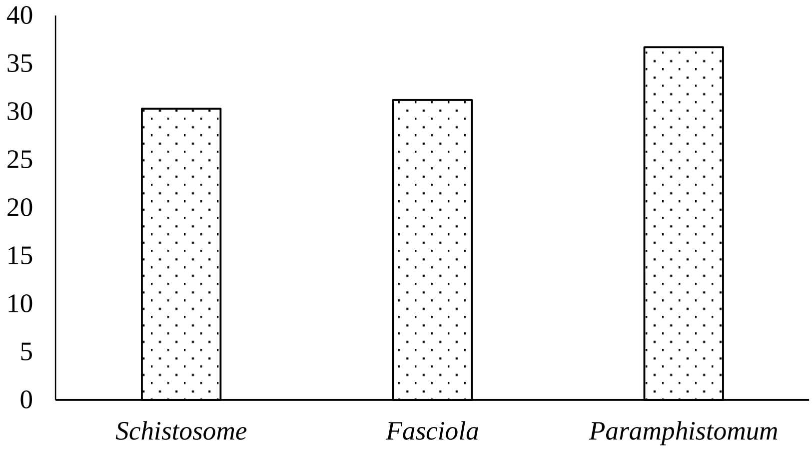

Figure 4. Prevalence of trematodes in Greater One-horned Rhinoceros

Prevalence of cestodes

Among 109 samples, 42 samples were found to be positive for cestodes. Two genera of Cestodes were identified i.e. *Anoplocephala* sp. and *Moneizia* sp. where the higher prevalence was shown by *Moniezia* sp. (27.50%) followed by *Anoplocephala* sp. (17.40%) (Figure 5).

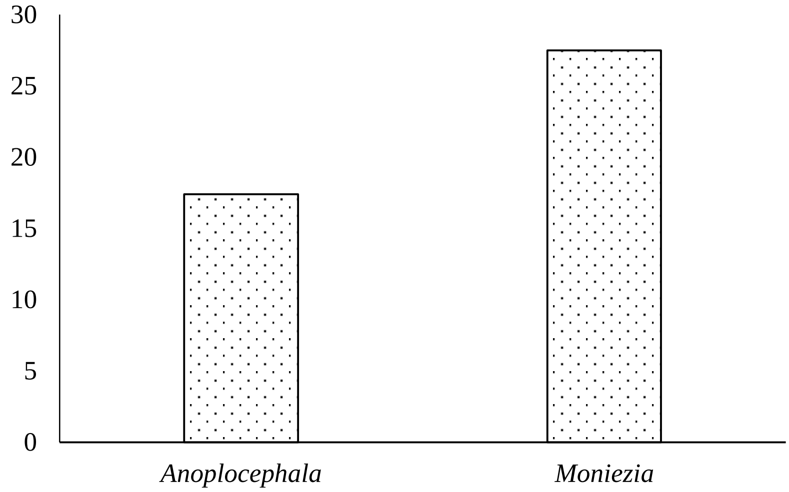

Figure 5. Prevalence of cestodes in Greater One-horned Rhinoceros

Prevalence of GI protozoan parasites

Among 109 samples, 3 samples were found to be positive for protozoan parasites. The identified genera of protozoa was *Eimeria* sp. having a prevalence rate of 2.8% (Figure 6).

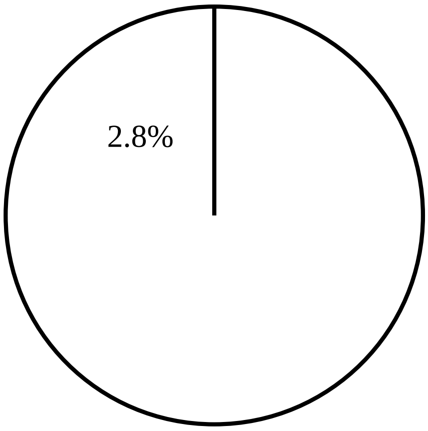

Figure 6. Prevalence of protozoan in Greater One-horned Rhinoceros

Prevalence of different types of parasitic infections

Table 2 shows the prevalence of different types of parasitic infections by different GI parasites were recorded and categorized as infections by single, double and triple parasitic genera. The prevalence rate was recorded as 25, 49, and 29 % for single, double, and triple species (Table 3). Among these, double infection (45%) was recorded as the highest prevalence followed by a triple (26.7%) and single infection (23%).

Table 2. Prevalence of different types of infection in Greater one-horned Rhinoceros in Chitwan National Park

Parasitic infection	Frequency	Percentage %
Single infection	*25*	*23*
Nematode	21	19.3
Trematode	4	3.7
Double infection	*49*	*45*
Nematode and Trematode	33	30.3
Nematode and Cestode	12	11.0
Trematode and Cestode	4	3.7
Triple infection	*29*	*26.7*
Nematode, Trematode and Cestode	26	23.9
Nematode, Trematode and Cestode	3	2.8
Non infected	*6*	*5.5*
Total	109	100

Overall prevalence status of parasites based on locations

Table 3 presents the prevalence status of parasites based on locations. Prevalence of parasites based on location was higher at Divyapuri (100%) followed by Golaghat (97.1%) and Sauraha (92.6%).

Table 3. Overall prevalence status of parasites based on locations in Greater One-horned Rhinoceros in Chitwan National Park, Chitwan

Status	Frequency	Prevalence %	$\chi 2$ value	P-value
Locations				
Sauraha	63	92.6		
Golaghat	34	97.1	1.268^{NS}	0.531
Divyapuri	6	100.0		

IV. Discussion

A total of 109 dung samples were collected from various locations within Chitwan National Park (CNP) at varying times, in which 103 fecal samples (94%) were found positive for multiple gastrointestinal parasites. The prevalence of intestinal parasites appears to be relatively higher but it's lesser compared to O'Connor et al. (2018) in Kaziranga National Park, which revealed 100% parasitic prevalence. Similarly, a higher prevalence than our present result was reported by (Rahman and Day, 2014; Kethmini et al., 2016 and Fagoilini et al., 2010), which revealed a 100% prevalence of parasites. Whereas our present prevalence rate was comparatively higher than some of the studies carried out at global context, reported as 58.57%, 61.90%, 30%, and 56% (Kakoti et al., 2019; Chakraborty & Islam, 1993; Palmieri et al., 1980; Hariyadi et al., 2008). Likewise, in the national context research by Shahi & Gairhe (2019) revealed 82.5% parasitic prevalence. Variation in the result could be due to the differences in the number of samples studied, physiological state of the rhinoceros, habitats, topographical, seasonal variation, and the interaction between definitive host, intermediate host, and parasites.

Out of 109 fecal samples tested, 103 samples were found to be positive for 16 different genera of gastro-intestinal parasites from 4 different classes i.e. nematode, trematode, cestode, and protozoa. The prevalence of the nematode parasites (87.2%) was highest followed by trematodes (65.1%), cestodes (38.5%), and protozoa (2.8%). This is as per the data reported by Shahi & Gairhe (2019), i.e. nematode (48.80%), trematode (44.23%), and cestode (7.69%). Likewise, this is also consistent with the records revealed by Palmieri et al. (1980) who stated the nematodes (50%) as the most abundant parasite in Javan rhinoceros, followed by trematodes (45%) and cestodes (30%). Whereas it is different as reported by O'Connor et al. (2018) in Kaziranga National Park where the prevalence is chronicled as trematode (100%), nematode (94%), and cestode (56%). In addition, it contradicts with Chakraborty and Islam (1993) who concluded that trematode (46%) infection has been the most prevalent, followed by a nematode (21%), protozoan (4%), and cestode (3%). It might be due to the difference in the habitats between 2 different National parks where Kaziranga NP is comparatively swampier than the Chitwan National Park.

During this study 16 different genera of gastrointestinal parasites were reported among which 10 were different genera of nematodes, 3 were trematodes, 2 were cestodes, and 1 was protozoa i.e. *Eimeria*. Among 109 fecal samples examined, 95 samples were found to be positive for nematodes revealing 10 different genera of parasites. Recorded nematodes were *Strongyloides* sp. (54.10%), *Ascaris* sp. (37.60%), *Toxocara* sp. (29.40%), *Haemonchus* sp. (14.70%), *Chabertia* sp. (12.80%), *Strongylus* sp. (9.20%), *Dactyocaulus* sp. (6.40%), *Trichuris* sp. (2.80%), *Oesophagostomum* sp. (1.80%) and *Trichostrongylus* sp. (0.9%) respectively. These parasites have a direct lifecycle and animals become infected after ingesting the larva or contaminated soil as the larva can survive in the environment and can remain infective up to six months so the animal gets easily infected with the different species of parasites. *Strongyloides sp*. was the most common nematode with the highest prevalence rate of 54.1%.

Strongyloides sp. worms occur in both parasitic and free-living forms in a host. Present prevalence is higher than reported by Shahi & Gairhe (2019) i.e. 18.18%. But lesser than reported by (Paudel et al., 2022; Kethmini et al., 2016; Fagoilini et al., 2010) who reported 65%, 100%, and 100% respectively. Similarly, (O'Connor et al., 2018; Chakraborty & Islam, 1993) reported prevalence at 94% and 20.23% in Kaziranga National Park which is higher than our study. Free-living stages of the gastrointestinal nematode are heavily affected by climatic conditions where the extreme temperature determines their survival and development. Moisture is needed for the development and progression of larva from soil to pastures, whereas the rainfall and vegetation could be the limiting factors on the transmission. Which may influence the pattern of inter variability in infection pattern (Morgan & van Dijk, 2012), and could be the determent factors for the variability in the prevalence rate of infection.

71 samples were found to be positive for trematodes i.e. 65.1%. Three different genera of parasites were identified i.e. *Paramphistomum* sp., *Schistosoma* sp., and *Fasciola* sp. The highest prevalence was shown by *Paramphistomum* sp. (36.7%) followed by *Fasciola* sp. (31.2%) and S*chistosoma* sp. (30.3%). This is contrary to the finding from Shahi & Gairhe (2019) who reported a higher prevalence of *Fasciola* sp. (33.33%) followed by *Paramphistomum* sp. (30.30%) and *Schistosoma* sp. (21. 21%). This variation in the prevalence could be related to geographic and climatic differences, as well as the research methods used. Similarly, the presence of *Schistosome* sp. is favoured by the research carried out by Devkota et al. (2014) who stated the sharing of the same species of *Schistosome Bivitellobilharzia nairi* in Greater One-Horned Rhinoceros and Elephant at Chitwan national park. Two different genera of cestodes have been identified i.e. *Anoplocephala* sp. 17.40% and *Monenzia* sp. (27.50%). Shahi & Gairhe (2019) reported a lower prevalence of Moniezia sp. 12.12%. A lower prevalence of 3% was recorded for *Anoplocephala* sp. from one-horned rhinoceros (Chakraborty & Islam, 1993). On the other hand, the higher prevalence was recorded from Javan rhinoceros and black rhinoceros i.e. 30% and 87% respectively by (Plamieri & Purnomo, 1980; Stringer, 1997). In the present study, the prevalence of *Eimeria* sp. was found to be 2.8% which is lower than 3.5% and 9% reported by (Chakraborty and Islam, 1993; Paudel et al., 2018). The prevalence of parasites is influenced by animal species, distribution patterns, meteorological circumstances, and habitat preferences (Boomker and Van, 1994).

Among these 3 types of infection, double infection (45%) showed the highest prevalence followed by a triple (26.7%) and single infection (23%). The heavy infection of *Strongyloides* sp., *Haemonchus* sp., *Ascaris* sp., *Toxocara sp.*, *Fasciola* sp., *Moniezia* sp. was encountered in this study.

V. Conclusion

The overall prevalence of endoparasites was found to be 94.49% comprising of 16 different genera from 4 different classes i.e. nematodes, trematodes, cestodes, and protozoa showing the higher prevalence for nematodes followed by trematodes, cestodes, and protozoa. The identified parasites were 10 nematodes, 3 trematodes, 2 cestodes, and 1 protozoan.

Strongyloides sp. (54.10%) was found to be higher followed by Ascaris sp. (37.60%), Toxocara sp. (29.40%), Haemonchus sp. (14.70%), Chabertia sp. (12.80%), Strongylus sp. (9.20%), Dactyocaulus sp. (6.40%), Trichuris sp. (2.80%), Oesophagostomum sp. (1.80%) and Trichostrongylus sp. (0.9%) respectively. Among the identified trematodes highest prevalence was shown by Paramphistomum sp. (36.7%) followed by Fasciola sp. (31.2%) and Schistosoma sp. (30.3%). Whereas among the cestode the prevalence shown by Moniezia sp. was 27.50% followed by Anoplocephala sp. (17.40%) and the protozoa identified was Eimeria sp. having a prevalence rate of 2.8%.

This study revealed that the greater one-horned rhinoceros of Chitwan National Park (CNP) has a significant prevalence of gastrointestinal parasite infection. The high incidence of gastro-intestinal parasites indicates a subclinical illness that could become pathogenic under stressful conditions. This research realized that it is essential to take extra precautions to prevent this parasitic infection to maintain the health of the Greater one-horned rhinoceros in Chitwan National Park. This study concludes that rhinoceros have a high burden of gastrointestinal parasites, which could be the cause of mortality in the study areas. There is a risk of pathogens being transferred from livestock to wildlife due to increased livestock encroachment on protected areas. This calls for a need to research diseases and infections harming the Greater One-Horned Rhinoceros. Strategic Planning of wildlife treatment protocols is fundamental for wildlife conservation like rhinoceros.

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