Nepalese Journal of Zoology
Special Issue: International Conference on Zoology 2021
ISSN: 2705-4403 (Print) & 2705-4411 (Online)
www.cdztu.edu.np/njz
Volume 6 | Issue S1 | December 2022
https://doi.org/10.3126/njz.v6iS1.50527

Research Article

Prevalence of gastrointestinal parasites in bovines along the elevation gradients of Annapurna Landscape, Nepal

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Suggested citation: Dhakal, D. N., Adhikari, J. N., Regmi, R. S., Adhikari, S. and Bhattarai, B. P. 2022. Prevalence of gastrointestinal parasites in bovines along the elevation gradients of Annapurna Landscape, Nepal. Nepalese Journal of Zoology **6**(S1):25–34. https://doi.org/10.3126/njz.v6iS1.50527

Article History:
Received: 25 June 2022
Revised: 13 November 2022
Accepted: 25 November 2022

Abstract

Gastrointestinal (GI) parasitic infection is a great concern for livestock management in Nepal, which may vary with the age and sex of the animals, feeding practices, habitat types, and interaction with other domestic and wild animals. As a consequence of growing environmental changes, it is anticipated that temperature and precipitation variation in altitudinal gradients will influence parasite communities and their hosts with unpredictable impacts. In light of this, the current study aimed to explore the pattern of prevalence and factors affecting GI parasites in bovines along the altitudinal and environmental gradients of Chitwan Annapurna Landscape. A total of 600 faecal specimens of bovines were collected from six different blocks and examined microscopically in the Zoology laboratory of Birendra Multiple Campus, Bharatpur, Nepal in between 2019 to 2020. The presence of cysts, oocysts and eggs of parasites in faecal samples were detected morphometrically using standard qualitative and quantitative methods. Out of 600 faecal samples of cattle and buffaloes examined, 64.8% were found positive for one or more species of GI parasites. The prevalence of parasitic infection was significantly higher in cattle (68.8%) as compared to the buffaloes (62.7%, p=0.007). A total of 16 species (5 protozoans, 11 helminths) were reported. The prevalence of GI parasites was significantly lower with increasing annual rainfall, elevation, distance from the forest and water resources (p=0.0001) but significantly higher with increasing temperature (p=0.001). The calf (p=0.028) and sub-adults (p=0.028) were significantly infected than the adults. Despite the fact that a large number of the herders used veterinary drugs for deworming, GI parasites were still rampant in the cows and buffaloes. We conclude that GI parasites are serious problems in the cattle and buffaloes of Chitwan Annapurna Landscape. Hence, regular health checkups, deworming, cleanliness of the sheds, and water resources are suggested regularly.

Keywords: Elevation gradient; GI parasites; Livestock; Oocysts; Protozoan parasites

1 | Introduction

Since the beginning of time, raising livestock has been a crucial component of Nepal's agricultural systems. Bovines, particularly cows and buffaloes, are the main livestock in Nepal (MOALD 2020). The majority of the population (83%) lives in rural areas, where farming and livestock husbandry are their main sources of income. Livestock farming plays a vital role in the Nepalese economy, providing about 11.5% of the country's GDP (MOALD 2020). The main challenge of livestock farming is gastrointestinal (GI) parasites, particularly in low-elevation tropical regions with a humid, tropical climate where the temperature always favors larva hatching and growth (Derek Scasta 2015). The prevalence issues of livestock parasitism in high-elevation rangelands are still a dilemma to be fully identified and addressed by the livestock industry, academic research, or the general public. The producers with livestock grazing at high elevations assume that the harsh climate will limit parasite persistence and infestations (Derek Scasta 2015; Kaufmann et al. 2012). The most crucial risk factors for parasitic infections include grazing patterns, climate, nutritional deficiencies, the presence of intermediate hosts and vectors, and the quantity of infectious larvae and eggs in the environment. Meteorological parameters like temperature and precipitation can also have an impact on the abundance of parasite species as well as the severity of infection in the host species (Adedipe et al. 2014; Mas-Coma et al. 2008). Higher rate of transmission and mortality have been recorded from the areas of dense settlement and heavy flow of people. About 80% of people with COVID-19 have mild or asymptomatic disease, therefore, symptom-based control is unlikely to be sufficient (Anderson et al. 2020, WHO 2020a). The basic reproduction number (R0) of COVID-19 had a value of about 2.5 in the earlier days of outbreak (Liu et al. 2020) that has substantially been lowered with the impose of social distancing measures (Rothan & Byrareddy 2020). The greater the reduction in transmission demands early self-isolation and social distancing. Despite rigorous global efforts, COVID-19 is continuously spreading across the world causing significant illness and death (WHO 2020b). No vaccine or effective antiviral drug is likely to be available soon as the process will take time (Anderson et al. 2020).

The helminths' free-living stages (cestodes, nematodes, and trematodes) are subject to the environmental circumstances in their specific microhabitats and depend on their own energy reserves until they come across a susceptible host that can provide a sufficient resource (Pietrock & Marcogliese 2003). Because of wildlife, animal husbandry, and climatic variations, high-elevation rangelands are distinct from lower elevations, and thus free-grazing livestock on high elevation may increase the opportunity for parasite transmission because they share the same foraging ground (Adhikari et al. 2018, Adhikari et al. 2021). (Derek Scasta 2015). As a result, even minor changes in climate or weather conditions can have an impact on the prevalence of infectious diseases (Cunsolo Willox et al. 2014). Many previous studies have shown that long-term climate warming promotes the rapid geographic spread of several infectious diseases such as malaria, helminthiasis, foot and mouth disease, and so on (Cunsolo Willox et al. 2014; Ostfeld & Brunner 2015). Extreme weather events may provide opportunities for more clustered disease outbreaks in unusual locations and times (Epstein, 2004). Many of the most prevalent infectious diseases, especially those spread by insects and mollusks, are extremely sensitive to changes in the environment (Morley 2010). These global changes have posed a significant challenge to human society, biodiversity, and livestock husbandry in the era of sustainable development (Weng et al. 2013). Climate change is a significant contributor to the increased prevalence of gastrointestinal parasites and other infectious diseases in humans and livestock (Cunsolo Willox et al. 2014; Epstein 2004).

High altitudinal variation of the Himalayan countries like Nepal supports various climatic variations and is greatly and swiftly affected by climate change (Dhakal et al. 2013; Manandhar et al. 2010; Pandey & Bardsley 2015). Therefore, little change in temperature supports the shifting of the disease from lower elevations to high elevations. Under these scenarios, it is quite relevant to explore the patterns of prevalence of GI parasites, especially in bovines, along the elevation gradients of Nepal as there is dearth of information pertaining to it. Therefore, the current study was designed to investigate the pattern of prevalence of gastrointestinal parasites in bovines along the elevation gradients of Chitwan Annapurna Landscape (CHAL), Nepal.

2 | Materials and methods

2.1 | Study area

This is a descriptive cross-sectional study comprising 600 livestock from December 2019 to May 2020. The study area was divided into six study blocks taking into account the altitudinal gradients and landscapes. The study blocks were A: Baseni-Padampur area, B: Jugedi-Kabilas area, C: Ghumaune-Keshabtar area, D: Vimad area, E: Panchase area, and F: Ghandruk area (Fig. 1). Open-ended semi-structured questionnaire was employed to assess demographic information of the livestock owners, their knowledge on livestock infections and treatments, and feeding mechanism used for their livestock.

2.2 | Sampling procedure and sample collection

Dry, clean, disinfectant-free, wide-necked and well-labelled plastic containers were distributed to the livestock owners and asked to bring about 10gm stool sample. Age and sex-stratified random sampling technique was used to collect samples. Three strata of age groups were created as 1-6 months of age- calves, 7 months- 2 years- young stock (sub-adult), and above 2 years- adult (Wade et al. 2000). Samples were collected from six blocks regarded as the altitudinal gradients (500m elevation gradients) as mentioned earlier. In each block, a total of 100 stool samples were collected in the early morning (6:00am – 11:00am) and evening (3:00pm – 6:00pm). About 10gm of stool was taken in a vial following the physical examinations of the stool and preserved in 25ml 2.5% potassium dichromate solution ($K_2Cr_2O_7$). Livestock owners were interviewed to assess their knowledge about the livestock diseases, intestinal parasites, treatment methods and other pertinent information.

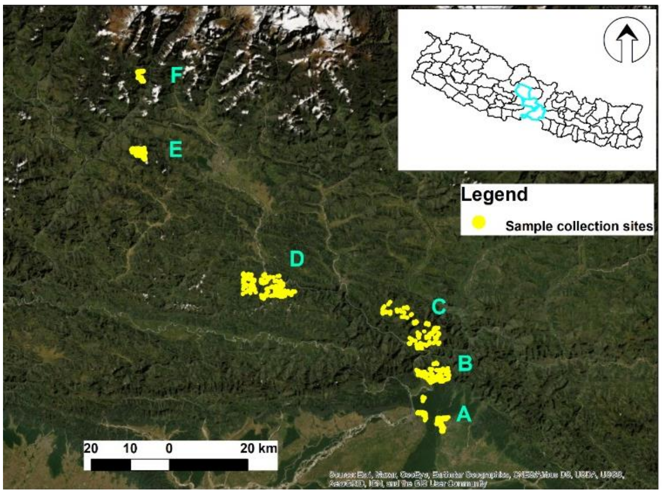

Figure 1. Study area showing the study blocks and sampling points

2.3 | Microscopic examination of faecal sample

All the collected samples were brought to the laboratory of the Department of Zoology, Birendra Multiple Campus, Bharatpur, Nepal. The faecal samples were microscopically examined for the presence of trophozoites, cysts, oocysts, eggs, and larvae of gastrointestinal parasites by concentration method viz. floatation and sedimentation techniques (Khadka et al., 2021; Zajac & Conboy 2012).

2.3.1 | Floatation technique

About 3 gm of faecal sample was taken and ground with about 20 ml of water. The faecal solution was filtered with a filter and poured into a centrifuge tube up to 12 ml mark and centrifuged at 1000 rpm for 5 minutes. The centrifuge tube was taken out and the upper part of the water was removed with the help of a pipette. Centrifuge tube was again filled with ZnSO4 solution up to 12 ml mark and centrifuged at 1000 rpm for 5 minutes. ZnSO4 was added up to the tip of the tube, a drop of methylene blue was added in it and covered with covers slip and left for 5 minutes. Then, the coverslip was taken out gently, placed on a microscopic slide, and examined.

2.3.2 | Sedimentation technique

The sediments of centrifuged contents of flotation technique were taken for eggs detection. The sediment content was poured into the watch glass and stirred gently. Small drop of sediment mixture was taken with the help of a pipette, placed on the slide, and one drop of iodine solution was added to stain eggs if present and examined under the microscope. The suspected samples were further stained with modified Zeihl-Neelsen stain to confirm the oocyst.

2.3.3 | Modified acid-fast staining

Owing to difficulty in detection of oocysts of the coccidian species such as *Cyclosporine*, *Cryptosporidium* and *Cystoisospora* with the routine stain like trichrome, modified acid-fast staining was applied for the identification of those parasites (Center of Disease Control and Prevention, 2021). The protocol for this technique was followed according to the CDC (Centers for Disease Control and Prevention) recommendation with slight modifications (Center of Disease Control and Prevention 2021). A portion of the sediment obtained after sedimentation was kept in the slide and allowed to dry at room temperature. Later on, fixation was done using absolute methanol for 30 s, followed by staining with carbol-fuchsin for 1 min, and washed with distilled water. It was further de-stained with acid alcohol for 2 min followed by washing with distilled water and counter-staining with malachite green (2 min). Finally, the slide was washed with distilled water and dried at 60 °C for about 5 min. The dried slide was observed at 1000× magnification using immersion oil.

2.3.4 | Identification of the eggs, cysts, and larva

All the samples were observed using a compound light microscope (Olympus Model–HSA, India) at different magnifications of 40×, 100×, 400× and 1000×. Identification of the eggs, cysts, and larva was made by comparing their structure, color, and size in the published literature, journals and books (Al-Aredhi 2015; Soulsby 1984 & Taylor et al. 2007).

2.4 | Data analysis

The collected data were encrypted and entered into a Microsoft Excel spreadsheet. Data were statistically analyzed using PAST and R version 4.0.2. The linear regression model was used to show the direct relation of a number of parasites present in the samples with environmental variables such as rainfall, temperature, altitude, and distance from forest and water. Generalized linear model (GLM) was used to find the relation between presence of parasites with the age of the hosts, feeding method, and other parameters.

3 | Results

3.1 | Prevalence of parasites and their modes of infection

The current study reported that 64.8% of the samples were positive for GI parasites, among them 62.7% positive were in buffalos and 68.8% positive were in cows clearly indicating that cows were significantly more infected than the buffalos (z=3.379, p=0.0007). The laboratory investigation of faecal samples showed that triple infection of the parasites was the highest (38.6%) followed by double (27.2%), multiple (21.1%), and single (13.1%) infection.

3.2 | Association of different variables with GI parasitic infections of bovines

Among 600 total samples collected, majority (n=474) were from female cattle and among them 65.4% were found to have at least one parasite. Similarly, among samples collected from male cattle (n=126), 61.9% were reported with GI parasites. However, there was no significant association between gender of cattle and presence of GI parasites (p>0.05). Large number of samples was collected from the adult livestock, followed by calf and sub-adult. Rate of infection was observed higher in calves 68.1% (107 out of 157) than in any other groups i.e., adult (62.8%) and sub-adult (67.1%) although statistically they were not associated (p>0.05). Stall feeding was adopted by a larger portion of respondents (57.7%), and higher rate of parasitic infection was observed in those livestock (66.8%) in comparison to the livestock which were mostly free ranging (62.2%). However, no significant association was found between the mode of feeding and parasitic infection (p>0.05). Parasitic infection rate was noted higher in the livestock of both chunks of respondents who lacked knowledge about livestock infection and those who lacked knowledge about GI infection. It was found that 62.3% of the respondents had knowledge about the livestock infection and 38.3% respondents had knowledge about GI infection in particular. Owners' prior knowledge on livestock infection and GI infection both were found to be significantly associated with the occurrence of parasitic infections in their cattle (p<0.05). Larger numbers (78.8%) of GI parasites were encountered in those livestock which were not treated with medicines at any point of time than those which were treated with medicines (61.4%) and a strong association was noted between medicine treatment of the livestock and parasitic infection (p<0.05). From each of the six study areas, 100 samples were taken. Cattle of Padampur area were found most infected (89%), whereas the cattle of Panchase and Lumle-Ghandruk area were least infected (42% and 43% respectively) by GI parasites. This finding was also statistically significant (p<0.05) (Table 1).

Table 1. Association of different variables with parasite infections

Attributes	**Sample size**	**Infection rate (%)**	**p-value**
Sex			
Male	126	78(61.9%)	0.465
Female	474	311(65.4%)	
Age			
Adult	352	221(62.8%)	
Sub-adult	91	61(67.1%)	0.449
Calf	157	107(68.1%)	
Method of feeding			
Stall feeding	346	231(66.8%)	0.247
Free ranging	254	158(62.2%)	
Knowledge about livestock infection			
Yes	374	219(58.6%)	0.00
No	226	170(75.2%)	
Knowledge about GI infection			
Yes	230	135(58.7%)	
No	370	254(68.7%)	0.013
Treatment of livestock			
Medicines	482	296(61.4%)	
None	118	93(78.8%)	0.00
Location			
Padampur	100	89(89%)	
Jugedi-Kabilash	100	74(74%)	0.00
Ghumaune-keshavtar	100	71(71%)	
Bhimad	100	70(70%)	
Panchase	100	42(42%)	
Lumle- Gnadruk	100	43(43%)	

3.3 | LM and GLM for few variables affecting parasitic infection

The linear model (LM) showed that temperature played a significant role in the infections by GI parasites. The infection rate was observed higher with the increase in temperature but the infection rate decreased with increased elevation gradients, annual rainfall, as well as increase in the distance of water resources and nearest forest (Table 2, Fig. 2).

Table 2. Linear model (LM) showing factors affecting distribution of GI parasites

Model	Intercept	Std. error	r^2	t	p-value
Temperature (°C)	-0.98	0.33	0.11	8.31	0.0001
Annual rainfall (mm)	3.38	0.29	0.05	-5.69	0.0001
Elevation (m)	2.38	0.09	0.09	-8.14	0.0001
Distance from forest (m)	2.97	0.11	0.21	-12.86	0.0001
Distance to water resources (m)	2.79	0.13	0.12	-8.83	0.0001

The Generalized linear model (GLM) showed that the age of the host played a crucial role in the infection of bovines by GI parasites. The calf and sub-adult were significantly infected than the adult. Livestock that relied on grass only were significantly less infected by the GI parasites than those which relied on both feeding. Cows were found more infected than the buffalos. Similarly, it was noted that the free grazing livestock were more infected than the stall feeders. Above all, female livestock were seen more vulnerable to parasitic infection than the males (Table 3).

3.3 | Distribution of GI parasites

Altogether 16 different GI parasites were identified from the bovines which comprised of 5 species of protozoan parasites and 11 species of helminthic parasites. The most frequently isolated helminthic parasite was *Bunostomum* sp., found in 29.3% faecal sample, whereas the least encountered was *Cooperia* sp. (3.1%). *Buxtonella* sp. and *Cryptosporidium* (each 17.7%) were the most frequent among the protozoan parasites (Figs. 3-5).

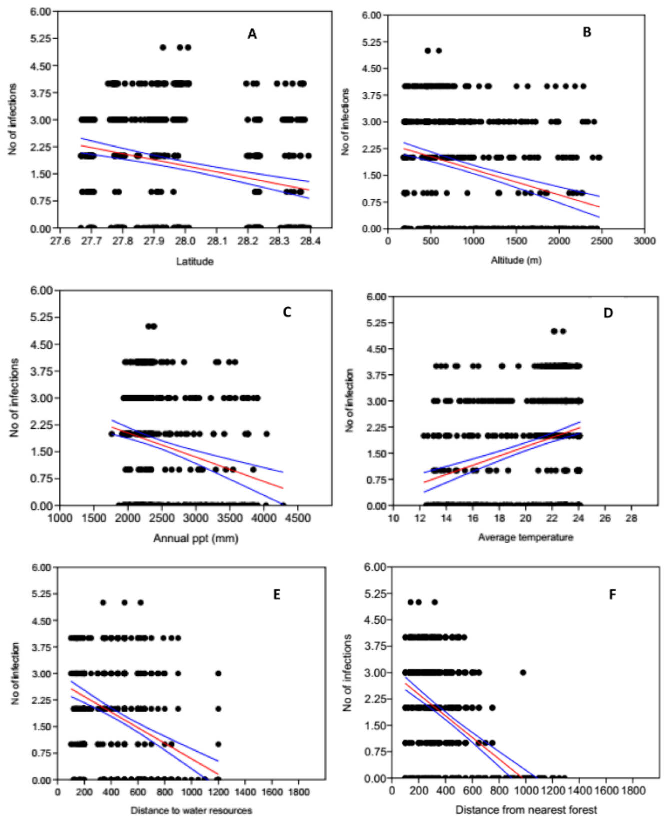

Figure 2. Linear model showing the relation of number of parasites infected (showing in dots) with different environmental variables. A- Latitude, B-altitude, C- annual rainfall, D- annual temperature, E-distance to water sources, F-distance from nearest forest

Table 3: Generalize Linear Model (GLM) value of the infection rate of Gastrointestinal parasites in cows and buffalo

Model	Estimate (β)	Std. Error	z value	Pr(>|z|)	Significance
(Intercept)	2.3322	0.706	3.304	0.0009	***
Age[T.Calf]	0.3741	0.1708	2.19	0.028	*
Age[T.Sub-adult]	0.5324	0.2121	2.51	0.012	*
Food[T.Grass]	-0.4565	0.1622	-2.815	0.004	**
Host[T.Cow]	0.5218	0.1544	3.379	0.0007	***
Rear.type [T.Free]	0.7438	0.714	1.042	0.297	
Rear.type[T.Stal]	0.4788	0.708	0.676	0.499	
Sex[T.Male]	-0.2532	0.173	-1.464	0.143	

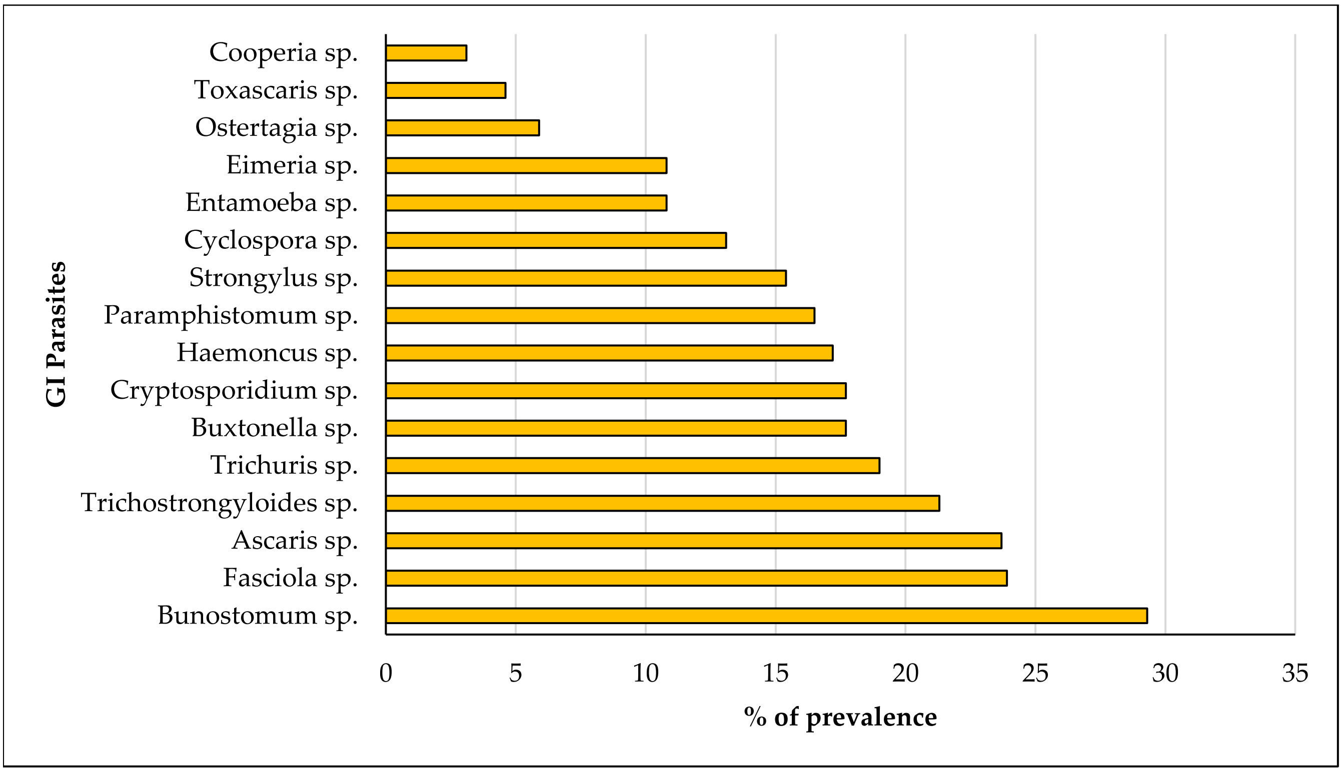

Figure 3: Frequency of different GI parasite

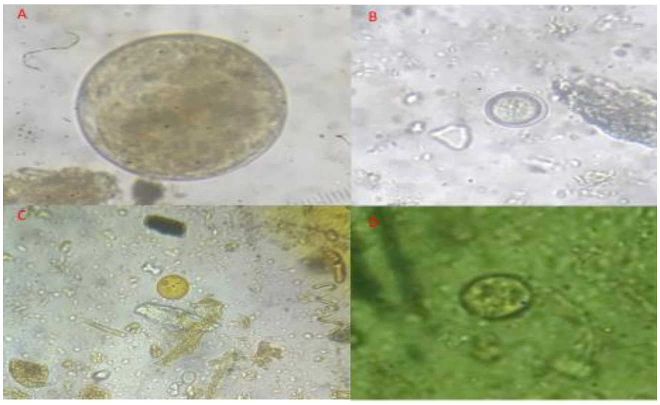

Figure 4. GI parasites (Protozoan) found in livestock A. Cyst of *Buxtonella* sp. (400X). B. Oocyst of *Eimeria* sp. (400X), C. Cyst of *Entamoeba* sp. (400X), D. Cyst of *Cyclospora* sp. (400X)

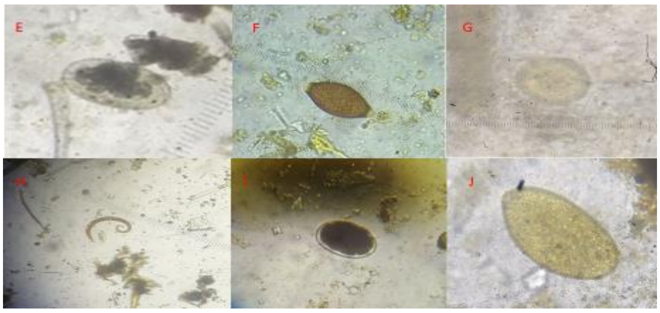

Figure 5. GI parasites (Helminthes) found in livestock E. Egg of *Haemonchus* sp. (400X), F. Egg of *Trichuris* sp.(400X) G. Egg of *Ascaris* sp. (400X) H. Larva of *Strongloides* sp.(400X), I. Egg of *Strongloides* sp. (400X), J. Egg of *Fasciola* sp. (400X)

4 | Discussion

Protozoa and helminths may infect the livestock's gastro-intestinal tract causing a plethora of infections. Control of GI parasitic infections in animals requires a robust knowledge of the disease epidemiology, history of infection and understanding of the pasture and farm management practices, and agroclimatic conditions such as temperature and rainfall. The level of infections in animals depend on the numbers of parasitic eggs and coccidial oocysts developed inside the host, level of host susceptibility, health and immunological status of the host animal.

In the current study, out of 600 faecal samples examined, 64.8% samples were found positive for one or more GI parasites. There exists a great variation in the prevalence of GI parasites in the cattle in different regions of the world as reported by previous studies. A study conducted in dairy cattle in Taiwan has reported a prevalence of 86.9% (Hung et al. 2014), 81.0% in Bangladesh (Nath et al. 2016), 75.0% in India (Rabbani et al. 2010) and 85.42% in Mustang, Nepal (Acharya et al. 2016). Some other studies meanwhile have recorded a lower rate of infection as in the studies carried out by Singh et al. (2012), Adhikari et al. (2013) and Ayele et al. (2020) where the rates were 16.98%, 21% and 29.3% respectively. In this study, 62.7% parasites were from buffaloes and 68.8% were from cows. Similar study done in Gujrat, India have found a lower infection rate in both the cows and buffaloes with a prevalence rate of 42.1% and 36.0% respectively (Gunathilaka et al. 2018). This variation in infection among cows and buffaloes and also in the overall rate may be attributed to drastic differences in sample size, sampling procedure, geographical distribution, host factors and climatic conditions (Gunathilaka et al. 2018). There was no significant difference in the parasitic infection of male animals (61.9%) and female animals (65.4%) in our study ($p > 0.05$). Similar study done by Maharana and his friends investigated that GI infection rate of females (44.2%) was slightly higher than the males (32.1%) (Maharana et al. 2016). But some other studies have found infection in males higher than in females like the one done by Gunathilaka and his colleagues (32.1% in males and 6.7 % in females) (Gunathilaka et al. 2018). The similar rate of infection among males and females may be related to the similar rearing practices adopted for animals of both sexes as both sexes are equally important in those areas because of the use of male animals for breeding and farming and use of females for milk (Maharana et al. 2016). In our study, calves (68.1%) were found slightly more prone to GI parasitic infection than other age groups with no statistical association ($p>0.05$). Our observation is in array with the similar studies by Gunathilaka and Maharana who have also found the calves being more infected than any other age groups (18.2% and 77.8% respectively) (Gunathilaka et al. 2018; Maharana et al. 2016). A previous study performed in Zimbabwe has shown that the susceptibility and pathogenicity of GI infections are greater in young animals than the adult ones (Pfukenyi et al. 2007). Although the variations in prevalence of GI parasites in different age group is seen often which is difficult to explain, the reasons can be attributed to the immunological status, negligence in nourishment of calves and also grazing conditions (Höglund et al. 2013). In the current study, 57.7% of animals were found to be stall-fed and the prevalence of GI infection among them was 66.8%, whereas the prevalence of GI infection in free ranging animals was 62.2%. However, the method of feeding was not associated with GI infection ($p>0.05$). Fairly similar rate of GI infection in both the cases- free ranging and stall feeding-may be because the grasses the stallfeeding animals were fed usually came from the same sources the free-ranging animals were grazed. Moreover, similar rearing practices for both types of animals might have contributed to a similar prevalence rate.

To our astonishment, we found that 37.7% owners didn't have knowledge about any kind of livestock infections at all and 61.7% had no knowledge about the GI infections. Livestock owners of the Baseni-Padampur area (block A) were more familiar with the infection of the parasites whereas the herders of the Ghandruk area (block F) were less familiar with the infection of parasites to their livestock. Respondents of Bhimad (Block D) and Ghumaune-Keshabtar (Block C) had the greatest and least knowledge about the infection of GI parasites to their livestock respectively. It is imperative that the GI parasitic infections in the livestock reared by these owners were quite high (75.2% and 68.7% respectively) and the association was statistically significant ($p<0.05$). We observed that 80.3% livestock had history of treatment with some kinds of medicines in the case any abnormality was noticed in the behavior of livestock such as-when livestock stopped feeding, decreased milk production, foul-smelling of the dropping etc. The rate of parasitic infection was seen higher in the livestock which were not treated with any medicines (78.8%) as compared to those which were treated with medicines (61.4%) at some point of their life. A strong association was found between treating the livestock with medicines and the occurrence of parasitic infections ($p<0.05$).

In the current study, GI infection was found most prevalent in bovines located at lower altitude or high temperature region as evidenced by occurrence of 89% GI infection in the livestock of Padampur area which lies in Terai region of the country. The rate of GI infection was found low as the altitude increased or temperature decreased and thus the lowest GI infection was observed in the livestock of Panchase and Lumle-Ghandruk area with a prevalence of 42% and 43% respectively. Padampur area is highly humid area located at a lower elevation with temperature averaging around 23.2ºC annually. Waller believed that at low elevation, higher humidity and higher temperature always favors hatching and larval development (Waller 1997). This may be the main reason behind a higher rate of GI infection at Padampur and decreasing rate of GI infection along the elevation gradient.

The present study recorded *Buxtonella* sp. and Cryptosporidium sp. (both 17.7%) as the most frequently isolated protozoan parasites, whereas *Bunostomum* sp. (29.3%) was the most predominant among the helminthic parasites. A similar study done in Gujarat, India also reported *Buxtonella sulcata* as the most dominant protozoan parasite in the bovines. However, the incidence of both the helminthes-*Cryptosporidium* sp. and *Bunostomum* sp.-was not recorded in the same study (Gunathilaka et al. 2018). A similar study done amongst the dairy cattle in Taiwan has reported Cryptosporidium sp. infection in 32.6% faecal samples and the infection rate is almost the double of the infection rate recorded in our study (Huang et al. 2014). But study of Gunathilaka (2018) in Srilanka has reported 1.2% and Islam et al (2015) in Bangladesh reported 3.9% of *Bunostomum* sp. in cattle which was very low (1.2%) compared to our study. In a study performed in Sri Lanka, *Trichuris* sp. (5.5%) was found as the most frequently discovered helminthic parasite (Gunathilaka et al. 2018), the incidence of which was fairly higher (19%) in the current study. In our study, the prevalence of Strongyle was 15.4%. A similar study carried out in Indonesia reported Strongyle sp. (19.1%) as the most dominant helminth parasite (Nurcahyo et al., 2021). In contrast, its prevalence was very low (4.3%) in a similar study done in Gujarat, India (Maharana et al. 2016). *Buxtonolla* sp. is generally considered as non-pathogenic, but in recent times, there are a few reports which suggest that the parasite can cause diarrhoea (Al-Bakri et al. 2010). The difference in the prevalence of different GI parasites in various studies can be attributed to myriads of factors including climatic condition, altitude, temperature and location (Zajac & Conboy 2012). The incidence of different GI parasites also may vary with the methods of feeding the livestock owner employ (Singh et al. 2012). Altogether, 14 different species of GI parasites were recorded in the current study. In this study, 56.3% livestock had at least two GI parasitic infections. However, the rate is slightly lower than the study done by Squire (75.1%) in Southern Ghana (Squire et al. 2013).

5 | Conclusions

Gastro-intestinal parasites were highly prevalent in the cattle in the study areas, particularly more in low altitude and high temperature areas. Most of the animals had multiple parasitic infections and the range of parasites that infected was also huge. These GI parasites may have economic significance and some are also zoonotic. It is utterly important to further determine the infection dynamics of these parasites to control these parasites effectively. In addition, we recommend the concerned stakeholders to assess the current control strategies in order to improve production of milk, meat and other products from these livestock.

Acknowledgements

We are thankful to people of Chitwan Annapurna Landscape who provide information about their livestock and also provided the permission for the collection of faecal samples. This study was financially supported by the University Grants Commission of Nepal (UGC award no. SRDI-74/75-S&T-07). The authors are indebted to Gobinda Sapkota, former Campus Chief, Professor Daya Ram Poudel, Campus Chief and Mr Ramchandra Piya former head of the Department of Zoology, Birendra Multiple Campus, Bharatpur, Chitwan, Nepal for lending the supportive hands at the time of needs.

Authors' contributions

D. N. D. and J. N. A. conceived the research. D. N. D., J. N. A. and B. P. B. collected samples. D. N. D., B. P. B and S. A. performed laboratory analysis. D. N. D., S. A. and R.S.R. prepared the manuscript. J. N. A. and B. P. B. finalized the manuscript. All authors read and approved the manuscript.

Conflicts of interest

Authors declare no conflict of interest.

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