INTESTINAL PARASITISM IN WORKING HORSES AND ASSOCIATED ZOONOTIC RISKS IN LOWLANDS OF NEPAL

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ABSTRACT

The presence of intestinal parasites influences equines' well-being and working performance. However, the scenario of parasitism in working horses in the lowlands of Nepal is yet to be explored. The present study aimed to reveal the prevalence and diversity of intestinal parasites (protozoa and helminths) and to list the zoonotic species in working horses in the lowlands of Nepal. Fresh fecal samples (N=102) from horses were collected at two locations (Chitwan and Birgunj) in the lowlands of Terai and were transferred to the research laboratory. Coproscopy was carried out via direct wet mount, formalin ethyl acetate (FEA) sedimentation, saturated salt flotation, and acid-fast staining techniques. Coproscopy revealed an overall prevalence rate of 90.2% (92/102) with 15 known diverse species of parasites (Protozoa: 5 and Helminths: 10) and an unknown coccidian, out of which eight possess zoonotic potential. The prevalence and diversity of intestinal parasites were higher in adult than in young animals (90.7%; 15 spp. vs. 88.9%; 11 spp.) The overall prevalence of helminths was double that of protozoa (89.2% vs. 43.1%). Furthermore, polyparasitism was much more prevalent than monoparasitism (85.3% vs 4.9%). Co-infection with two parasite species (37%) was higher in young horses. In comparison, triplet infection (34%) was higher in adults, and a maximum concurrency of up to six species of parasites at a time was recorded. Following it, the differences in the prevalence rate of parasites based on the predictor of risks, like sex, grazing, domestication type, nature of the floor, and medication practices, were statistically significant. Working horses in the lowlands of Terai harbored a significant variety of intestinal parasites with important prevalence. Since eight of the reported parasitic species were zoonotic, infected horses pose a zoonotic risk to the owners. Therefore, timely deworming, pasture management, and reduction in working pressure are highly recommended.

Keywords *Cryptosporidium;* Strongyle*;* Zoonosis

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INTRODUCTION

Horses (*Equus caballus*)**,** Family: Equidae*,* Taxonomic serial number 180691 (www.itis.gov), are odd-toed domesticated mammals. They are well-known for their sturdy body complexion, highly adaptive running and social behavior **(1)**. Horses have been a part of the Nepalese civilization for many years. Even though multi-purpose animals, horses are mainly used as a means of transportation (horse-driven carts, riding, goods carrying) in the Himalayan, Hilly, and Terai regions of Nepal. According to recent Livestock Statistics data, 54,864 horse heads are found in Nepal, and about 11% of their population is concentrated in the lowlands of Terai and inner Terai **(2)**. The insufficient breeding centers, inffective conservation campaigns, poor nutrition, and modernization are threats for declining of the horse population **(3)**. In this context, the role of gastrointestinal parasites (GIPs) in the health status and survival has not been evaluated and addressed so far. Similar to other terrestrial herbivores, horses are expected to harbor complex macroparasite communities in their GI tract, which is one of the common causes of diseases in equids. Various parasitic diseases, including GIPs have been chronologically reviewed in horses **(4)**. The main GIPs of horses include worms,

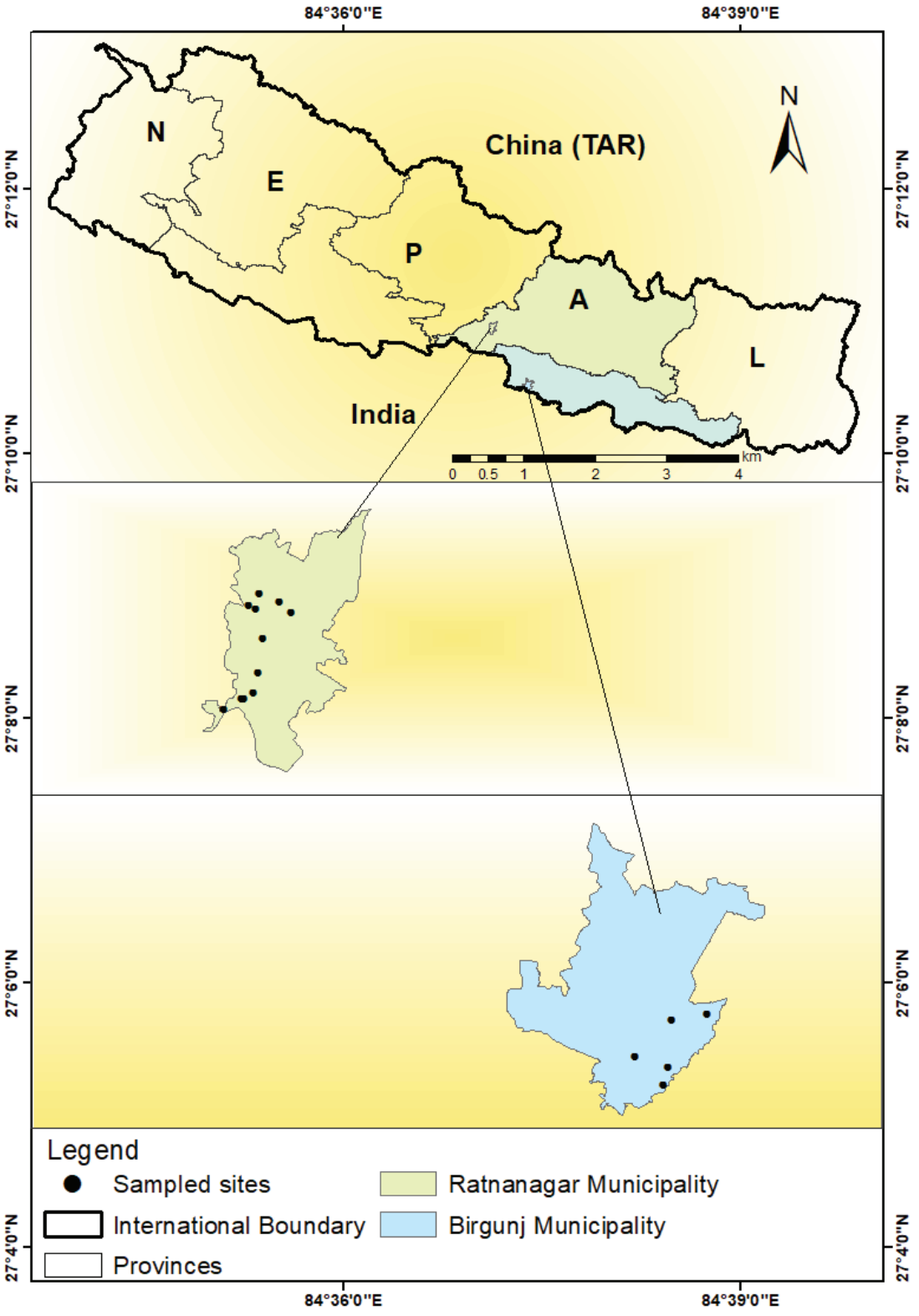
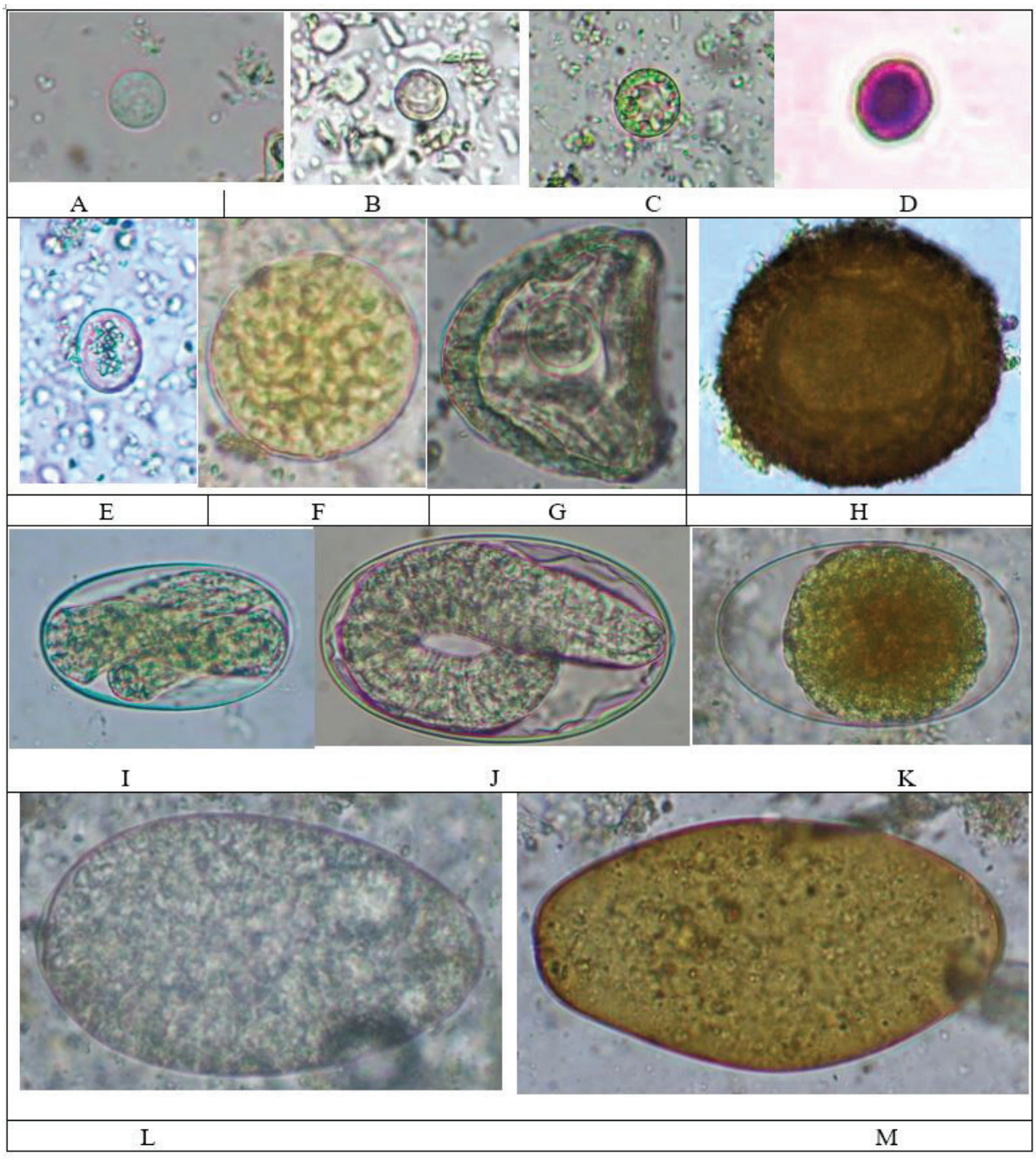


Fig. 1: Map of study area.

like redworms (*Strongylus* group), threadworms (*Strongyloides* spp.), roundworm (*Parascaris equorum*), tapeworms (*Anoplocephala* spp.), pinworms (*Oxyuris equi*), lungworms (*Dictyocaulus arnfieldi*) and bots (*Gasterophilus intestinalis*) **(5)**. Although each parasite species infects and impacts differently, its burden directly impairs the growth and development, metabolism, nutrition absorption, working and reproductive performances **(6,7)**. Notable pathogenic symptoms of GIPs are the loss of appetite, anemia, rough hair coat, tail rubbing, coughing, debilitation, diarrhea, and various types of

colic leading to the death of these equids **(5,8)**.

Besides, horses are also known to carry zoonotic parasites **(9,10)** and because they fulfill the multipurpose roles as working animals, pets and livestock, their close association with humans increases the risk of transmission of parasitic infections. Interestingly, they naturally harbor more than 56 zoonotic pathogens including parasites, like *Blastocystis*, *Cryptosporidium*, *Entamoeba, Giardia*, *Toxoplasma gondii*, *Echinococcus*, *Fasciola hepatica*, *Trichinella*, and *Trichostrongylus* **(9–11)**, and several studies **(12–15)** claimed for the clinical presence, or associated pathogenesis, as well as the fatal consequences caused by these parasites in humans globally. To date, very few research studies have been carried out in Nepal concerning intestinal parasitism in horses, and these prevalence studies are confined to rural settings in the Himalayan and hilly regions, like Mustang and Rukum districts **(16,17)**. However, the status of parasitism in recently modernized and subtropical to tropical lowlands of Terai and inner Terai with working pressure is yet to be evaluated and discussed. In this research, we aimed to study the prevalence of protozoan and helminth parasite



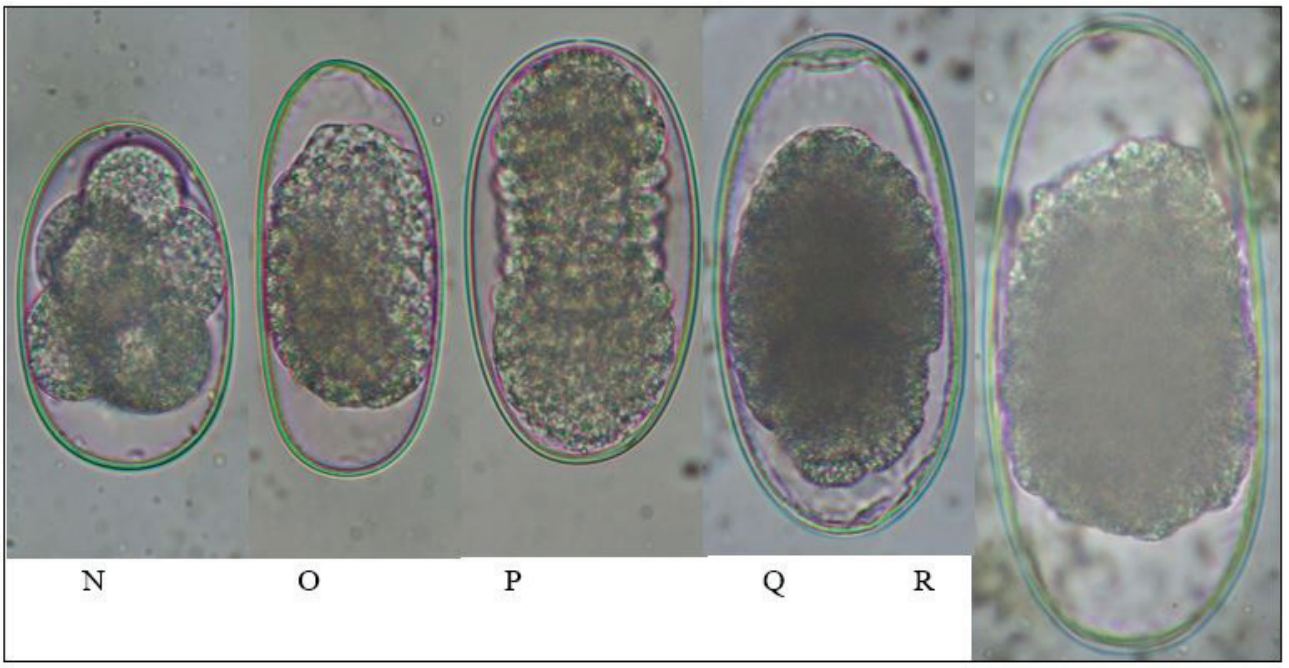


Fig. 2: Gastrointestinal parasites identified in working horses under varying magnification of objective lens (A) Cyst of *Entamoeba* sp. (10x10 µm), 40x after FEA sedimentation technique. (B) Cyst of *Iodamoeba* sp. (12x12), 40x after direct wet mount technique. (C) *Blastocystis* sp. (18x17µm), 40x after direct wet mount technique. (D) *Cryptosporidium* sp. (6x5 µm), 100x after acid fast staining technique. (E) Oocyst of unknown coccidian (21x17 µm), 40x after direct wet mount technique. (F) Cyst of *Balantidium coli* (46x45 µm), 40x after direct wet mount technique. (G) Egg of *Anoplocephala perfoliata* (68x59 µm), 40x after flotation technique. (H) Egg of *Parascaris equorum* (85x79 µm), 40x after flotation technique. (I) Egg of *Strongyloides* sp. (64x37 µm), 40x after flotation technique. (J) Egg of *Dictyocaulus arnfieldi* (98x58 µm), 40x after FEA sedimentation. (K) Egg of Strongyle (1) (91x56 µm), 40x after flotation technique. (L) Egg of *Gastrodiscus* sp. (140x81 µm), 40x after direct wet mount technique. (M) Eggs of *Fasciola* sp. (148x80 µm), 40x after direct wet mount technique. (N) Egg of Strongyle (2) (79x48 µm), 40x after flotation technique. (O) Egg of *Trichostrongylus* sp. (94x43 µm), 40x after FEA sedimentation technique. (P) Egg of Strongyle (3) (100x55 µm), 40x after FEA sedimentation technique. (Q) Egg of Strongyle (4) (124x62 µm), 40x after FEA sedimentation technique. (R) Egg of *Nematodirus* sp. (152x68 µm), 40x after FEA sedimentation technique.

species in working horses at two different locations in the lowlands of Nepal and their possible zoonotic significance and risk factors for the human hosts.

MATERIALS AND METHODS

Study area

The study was conducted within Ratnanagar Municipality (27.62°N, 84.51°E) and Birgunj Metropolitan City (27.0°N, 84.52°E) in the lowlands of Nepal **(Fig. 1)**. Ratnanagar Municipality lies in eastern Chitwan in Central Nepal and has a reasonably subtropical to tropical climate (maximum average temperature 21–35 °C and minimum average temperature 10-24 °C) **(18)**. Chitwan has a low population of horses, and most of them are found in the Sauraha area, the main entrance to Chitwan National Park (CNP). Horses are used for pulling carts (*Tanga* & *Baggi*) carrying local and international tourists from the east-west highway (Sauraha Chowk) to the CNP entry point. Similarly, Birgunj Metropolitan City lies in southern Terai in the Parsa district and is also known as the Gateway of Nepal. It has a sub-tropical monsoon climate with a very hot and humid summer (maximum average temperature 21.8–35.8 °C and minimum average temperature 9.9-25.8 °C (Retrieved on 18th June 2023 from https://en.climate-data.org/asia/nepal/central-development-region/birganj-47722/). The horse carts have historically been the popular mode of transportation for the local people and outsiders during short visits or festive ceremonies in Terai. Therefore, despite modernization, the use of carts is

a popular means for carrying people and goods from Birgunj to Raxaul, a border region in India and nearby locations or vice versa.

Sample collection, preservation, and examination

The fecal samples were collected from November 9, 2020 to April 15, 2021. All horses studied were the indigenous breed (mainly Terai Pony) working horses aged three months to 21 years. Based on their age, sampled horses were classified into two groups: young (≤3 years) and adults (> 3 years). A total of 102 fresh fecal samples (25 from Chitwan and 77 from Birgunj) were collected via non-invasive technique immediately, right after the defecation. The samples were preserved in 2.5% potassium dichromate solution in screw-capped disinfected vials and then transported to the research laboratories (Nepal Academy of Science and Technology, Lalitpur). Finally, the samples were stockpiled at 4°C in a refrigerator before the laboratory investigation.

Laboratory processing

The coproscopy was carried out following four different standard Ova and Parasite examination techniques, including direct wet mount, formalin-ethyl acetate (FEA) sedimentation, saturated salt (45% w/v NaCl) flotation, and acid-fast staining techniques based on the procedure previously documented in the literature **(19–23)**. To prepare a direct wet smear, a small amount of the sample was taken in a glass slide, mixed with 1-2 drops of normal saline or iodine, and then observed under the microscope (40x).

For FEA sedimentation, 2g of feces were mixed in 12 ml of normal saline (0.9%), filtered using a tea strainer into a conical centrifuge tube, and subsequently centrifuged with normal saline and the mixture of 4 ml of ethyl acetate and 10 ml of 10% formalin at (1200 rpm for 5 mins). The supernatant and debris plug were discarded, and a single drop of sediment was observed under the microscope (40x).

Similarly, the sediment obtained after FEA sedimentation was used for smear preparation for acid-fast staining. After air-drying, the smear was fixed in absolute methanol for 2 min, flooded with carbol fuchsin stain for 15 min, destained with acid alcohol for one min, counterstained with malachite green for another minute and rinsed with tap water. Finally, the smear was air-dried and examined under the microscope (100 x) with immersion oil.

For the flotation technique, the sediment obtained after the first centrifugation was added to the saturated salt solution and centrifuged at (1200 rpm for 5 min). Without discarding the supernatant, flotation media was added further to fill the tube, and a coverslip was placed on its mouth. Finally, after 10 mins, the coverslip was picked up carefully and placed on a glass slide for microscopic examination (40x).

Estimation of parasitic burden/severity of infection

A 2-Cell McMaster Counting Slide (Hawksley and Sons Ltd) was used to estimate the burden of helminthic infection. It was quantified by counting the number of eggs released per g of faeces (epg), according to manufacturer's instructions and as previously explained (24,25).

Parasitic identification

A light microscope (B-383PLi, OPTIKA) and ImageJ (National Institute of Health) software for microscopy and photo micrometry, respectively, were used. The identification of parasitic stages like trophozoites, cysts, oocysts, eggs, and larvae was carried out as previously explained **(25–27)**.

Data analysis

The overall prevalence of parasites and individual parasites prevalence was calculated by dividing total positive samples or positively specified parasites by the total number of samples and then multiplying it by 100. Data were analyzed using GraphPad Prism (Prism 5 for Windows v.5). Chi-square and Fisher exact tests were used to evaluate the significance level between any variables. The p values less than 0.05 were considered statistically significant (95% confidence level).

$$\text{Prevalence (\%)} = \frac{\text{Total Positive Samples}}{\text{Total number of samples}} \text{ X 100}$$

RESULTS

The coproscopy carried out in all the fecal samples (N=102) collected from horses revealed that 92 (90.2%) horses were positive for at least one species of GIP. Geographically, the prevalence of GIPs was

higher in Birgunj compared to Ratnanagar (76, 98.7% vs 16, 64%).

Out of 16 different species of GIPs (Protozoa: 6 and Helminths: 10), eight species (*Entamoeba* sp. *Cryptosporidium* sp., *Balantidium coli*, *Blastocystis* sp., *Iodamoeba* sp., *Strongyloides* sp., *Trichostrongylus* sp., and *Fasciola* sp.) were zoonotically significant according to literature data **(Fig. 2)**. Strongylid species (74.5%) were most prevalent, followed by *Parascaris equorum* (31.4%), while the prevalence of *Oxyruis equi* (1.9%) was the least **(Table 1)**. The overall prevalence and diversity of helminths were higher compared to protozoa (89.2% versus 43.1%) (Trematoda: 2, Cestoda: 1, Nematoda: 7, and Protozoa: 6). Similar results was also obtained for the prevalence of helminths and protozoa in the young (85.2% vs 51.9%) and adult (90.7% vs 40%) population. We reported five morphotypes of strongylid eggs with size ranges (77–127 µm x 39–62 µm). In addition, two morphotypes of unknown coccidian oocysts were identified (size range: 21–22 µm x 16–20 µm). Considering the age, adult horses had a higher prevalence (90.7% versus 88.9%) as well as greater diversity (15 spp. versus 11 spp.) of GIPs as compared to young, without statistical significance ($p>0.05$). Except for the common strongyle, the prevalence of *Entamoeba* sp., *Parascaris equorum,* and *Strongyloides* sp. was higher in the young animals. At the same time, adults were highly dominated by *Entamoeba* sp., *Anoplocephala perfoliata, Nematodirus* sp., *Fasciola* sp., and *Gastrodiscus* sp. Considering the zoonotic GIPs, *Entamoeba* sp. and *Strongyloides* sp. were prevalent in the young, while adults had a leading prevalence of *Blastocystis* sp., *B. coli*, *Cryptosporidium* sp., *Iodamoeba* sp., *Trichostrongylus* sp., and *Fasciola* sp. **(Table 1)**.

Moreover, the concurrency of infection revealed that most horses (85.3%, 87/102) were co-infected with one or more GIPs. In comparison, very few (4.9%, 5/102) of the horses were infected with a single parasite species. Young animals were reported to have a concurrency of up to four different parasite species, while in adults up to six parasites at a time were detected **(Table 2)**. In the same way, young horses were excreting a higher count of *Parascaris equorum* (400–2800 epg) and *Strongyloides* sp. (200–1800 epg), while the adults were excreting more strongyle (200–2200 epg), *Dictyocaulus arnfieldi* (100–1100 epg), *Trichostrongylus* sp. (200–1400 epg), and *Nematodirus* sp. (100–500 epg).

Furthermore, females had a significantly higher prevalence of intestinal parasites than males (97.5% versus 61.9%) ($p<0.05$) **(Table 3)**. Further analysis revealed that horses with grazing opportunities, easy access to outdoor water sources, mixed domestication with other animals, living in muddy stables, and those with unknown or more significant than one-year early history of medication (deworming) were highly infected with intestinal parasites **(Table 3)**.

DISCUSSION

In the present study, the overall prevalence rate of intestinal parasites in horses was 90.2% (92/102). This rate was lower than the findings from Rome (100%; n=10) and Libya (98%; n=50) **(28)** but higher than other studies from Nepal (81.9 % - 84.76 %; n=105 **(16,17)**, India (84%; n=100) **(29)**, Ethiopia (63.73% - 73.81%; n= 102-317) **(30,31)**, and Colombia (87.5%; n=1004) **(32)**. The results might be associated with methodological tools and techniques during sample collection, laboratory techniques, geo-climatic conditions, horse rearing, and health management practices. The subtropical-to-tropical geo-climatic condition, traditional rearing practices, irregular deworming and other veterinary checkups, and repeated animal exposure to contaminated water bodies and grazing sites are the underlying causes for the current high prevalence rates.

The prevalence and diversity of helminths were higher than the protozoa, which agreed with the Libya report **(28)**. Strongylids (74.5%) were the most dominant GIPs in the current equines. The rate was lower than reported in Rome (100%) **(33)** and Colombia (86.4%-89.4%) **(32)** but higher than reported in India (52.38%) **(29)** and Nepal (68.57%) **(16)**. Based on morphology, equine strongylids include Strongylinae (large strongyles) and Cyathostominae (small strongyles) **(26)**, and interestingly, they constituted more than 75% of the total parasites infecting horses globally **(26)**. In this study, the larval culture to distinguish the species of strongylids was not performed; therefore, all the egg morphologies were considered “strongyle”. Notably, horses never develop complete immunity against the strongylids;

Table 1. GIPs of horses (N) = 102 from horses in lowland of Nepal. * p<0.05 (Fisher Exact Test), while comparing the rates between young and adult animals. RR: Relative Risk, CI: Confidence Interval.

Parasites	Young (N1=27)	Adults (N2= 75)	Total Prevalence	RR (95%CI)	OR (95%CI)	Zoonotic importance
Sarcodina						
Entamoeba sp.[ns]	12 (44.4%)	26 (34.7%)	38 (37.3%)	1.29 (0.91 to 1.81)	1.52 (0.86 to 2.69)	Yes
Iodamoeba sp.[ns]	1 (3.7%)	2 (2.7%)	3 (2.9%)	1.33 (0.31 to 5.81)	1.35 (0.29 to 6.18)	Yes
Blastocystis sp.[ns]	1 (3.7%)	3 (4%)	4 (3.9%)	1.0 (0.26 to 3.89)	1.0 (0.24 to 4.12)	Yes
Coccidia						
Cryptosporidium sp.[ns]	5 (18.5%)	19 (25.3%)	24 (23.5%)	0.73 (0.43 to 1.23	0.67 (0.34 to 1.31)	Yes
Unknown coccidia[ns]	0 (0%)	2 (2.7%)	2 (1.9%)	-	-	Unknown
Ciliate						
Balantidium coli[ns]	3 (11.1%)	7 (9.3%)	10 (9.8%)	1.22 (0.53 to 2.82)	1.25 (0.49 to 3.16)	Yes
Total Protozoa[ns]	**14 (51.9%)**	**30 (40%)**	**44 (43.1%)**	**1.30 (0.96 to 1.76)**	**1.63 (0.93 to 2.85)**	
Nematoda						
*Parascaris equorum**	13 (48.1%)	19 (25.3%)	32 (31.4%)	1.92 (1.29 to 2.85)	2.77 (1.52 to 5.04)	No
Strongyle[ns]	12 (44.5%)	64 (85.3%)	76 (74.5%)	0.90 (0.67 to 1.20)	0.82 (0.47 to 1.43)	Zoonotic?
Strongyloides sp.*	11 (40.7%)	17 (22.7%)	28 (27.5%)	1.78 (1.16 to 2.74)	2.33 (1.26 to 4.29)	Yes
Trichostrongylus sp.*	2 (7.4%)	14 (17.3%)	16 (15.7%)	0.41 (0.18 to 0.95)	0.37 (0.15 to 0.93)	Yes
Oxyuris equi[ns]	1 (3.7%)	1 (1.3%)	2 (1.9%)	4.0 (0.45 to 35.18)	4.13 (0.45 to 37.59)	No
*Dictyocaulus arnfieldi**	2 (7.4%)	12 (16%)	14 (13.7%	0.41 (0.18 to 0.95)	0.37 (0.15 to 0.93)	No
Nematodirus sp.	0	5 (6.7%)	5 (4.9%)			No
Trematoda						
Fasciola sp.	0	14 (18.7%)	14 (13.7%)			Yes
Gastrodiscus sp.	0	18 (24%)	18 (17.6%)			No
Cestoda						
Anoplocephala perfoliata	0	21 (28%)	21 (20.6%)			No
Total helminths[ns]	**23 (85.2%)**	**68 (90.7%)**	**91 (89.2%)**	**0.93 (0.84 – 1.04)**	**0.56 (0.23 to 1.35)**	
Overall Total[ns]	**24 (88.9%)**	**68 (90.7%)**	**92 (90.2%)**	**0.98 (0.89 to 1.07)**	**0.80 (0.32 to 2.03)**	

Table 2. Assessment of risk factors among the horse population (N=102) p-values (Chi-Square Test/Fisher Exact Test. Risk Ratio: RR, Odds Ratio: OR, CI: Confidence Interval. *: <0.05, ns: not significant.

Potential Risk	Categories	Total individual	Prevalence Rate	RR (95%CI)	OR (95%CI)
Age^{ns}	≤3 years	27	25 (92.6%)	1.05 (0.96 to 1.14)	1.64 (0.61 to 4.43)
	>3 years	75	67 (89.33%)		
Sex*	Male	21	13 (61.9%)	0.63 (0.54 to 0.74)	0.03 (0.01 to 0.14)
	Female	81	79 (97.5%)		
Grazing*	Grazers	72	70 (97.2%)	1.33 (1.17 to 1.50)	11.96 (3.49 to 40.96)
	Non-grazers	30	22 (73.3%)		
Drinking water sources*	Indoor	54	46 (85.2%)	0.89 (0.81 to 0.97)	0.24 (0.08 to 0.74)
	Excess to outdoors (rivers)	48	46 (95.8%)		
Domestication Practices*	Single	33	27 (81.8%)	-	-
	Multiple	42	38 (90.5%)		
	Mixed (with livestock)	27	27 (100%)		
Stable (Floor) *	Muddy	33	32 (97%)	-	-
	Wooden	44	41 (93.2%)		
	Cemented	25	19 (76%)		
Medication/deworming*	≤6 months	18	10 (55.6%)	-	-
	7–12 months	32	30 (93.8%)		
	>12 months/ unknown history	52	52 (100%)		

thus, they can be recovered from well-managed and aged adults **(34)**. Interestingly, five adult horses were positive for *Nematodirus* sp., which usually impacts the hosts during polyparasitism **(35)**. Another nematode was *Parascaris equorum* (31.4%), which was also reported from Nepal (10.47% – 14.28%) **(16,17)** and India (10.71%) **(29)**. Unlike young and aging horses, it seldom occurs and impacts adults **(36)**. Working equines under tropical conditions repeatedly harbor huge ascarid burdens irrespective of age **(37)**. Similarly, the prevalence of *Dictyocaulus arnfieldi* was 13.7% recorded from Nepal (8.57%) **(17)**, Ethiopia (22.7%) **(38)**, and Iran (15.71%) **(39)**. It causes severe coughing symptoms, pneumonia, emphysema, and pulmonary edema **(40)**, and increases the secondary infection by respiratory viruses like influenza **(39)**. Another nematode, *Strongyloides* sp. (27.5%), was detected in horses similar to previous records from Nepal (23.8%) **(16)** and Ethiopia (19.6%) **(30)**. These species are infected via transmammary, percutaneous, or soil/fecal-oral routes **(41,42)**. Although *Strongyloides westeri* infect

Table 3. Concurrency of GIPs in young and adult horses population (N=102). The p-values were calculated using Fisher Exact Test. Risk Ratio: RR, Odds Ratio: OR, CI: Confidence Interval. *: <0.05, ns: not significant.

Concurrency	Young (N1=27)	Adults (N2= 75)	Total Prevalence	RR (95%CI)	OR (95%CI)
Single[ns]	3 (8.1%)	2 (2.7%)	5 (4.9%)	2.69 (0.74 to 9.86)	2.84 (0.73 to 11.05)
Double*	10 (37%)	7 (9.3%)	17 (16.7%)	4.11 (2.09 to 8.07)	5.94 (2.68 to 13.17)
Triple[ns]	8 (29.6%)	25 (33.3%)	33 (32.4%)	0.91 (0.60 to 1.37)	0.87 (0.48 to 1.58)
Quadruple*	3 (11.1%)	18 (24%)	21 (20.6%)	0.46 (0.24 to 0.88)	0.39 (0.18 to 0.85)
Pentuple[na]	0 (0%)	11 (14.7%)	11(10.7%)	-	-
Hexuple[na]	0 (0%)	5 (6.7%%)	5 (4.9%)	-	-

horses **(43)**, further molecular taxonomy would confirm the species of the current study.

Notably, *Anoplocepha perfoliata* (20.6%) is the only cestode reported in this study. It was also reported from Libya **(28)** and Ethiopia (15.7%) **(30)**. Its infection occurs via the oribatid mite ingestion while grazing on the contaminated pasture **(44)**. In the context of trematodes, *Gastrodiscus* sp. (17.6%), a large intestinal amphistome, was also reported from Nepal (6.67%) **(17)** and India (7.14%) **(29)**. Another trematode *Fasciola* sp. (13.7%), was also reported from the UK (2.2%) **(45)**, indicating the availability of intermediate freshwater Planorbid and Lymnaeid snail hosts for both trematodes in these geographies. Although equine fascioliasis has received minimal interest, the role of donkeys and horses as a reservoir of human fascioliasis in endemic areas has been highlighted in recent studies with its prevalence of 24.4%–39.5% **(11)**.

Considering protozoa, *Entamoeba* sp. (37.3%), the dominant species, was also recorded in China (78.1%) **(10)** and Nepal (3.80%) **(17)**. Equines are reported to have *E. moshkovskii*, *E. histolytica*, *E. ecuadoriensis*, *E. bovis, E. sp. RL4, E. sp. RL9* **(10)** *that can spread via* fecal-oral ingestion **(46)**. Two zoonotic strains, like *E. moshkovskii* and *E. histolytica* **(7)**, are critical for the owners and farmers. Notably, a higher prevalence of *Entamoeba* spp. in domestic buffaloes **(47)** and genetically confirmed *E. bovis* from wild water buffaloes **(48)** are circulating in the same ecological niches.

For the first time, other protozoa like *Iodamoeba* sp. (2.9%) and *Blastocystis* sp. (3.9%) have been reported from horses in Nepal. Even though *Iodamoeba* sp. commonly infects humans, non-human primates, and swine, its occurrence in horses might be interesting. Hypothetically, we assumed its acquisition via cross-transmission from either humans or pigs because this parasite has already been reported in these hosts in the same geography **(15,24)**. The presence of *Blastocystis* sp. (3.9%) was reported from Colombia (43.8%) and Thailand (12.5%). To date, 12 *Blastocystis* subtypes have been confirmed in horses, out of which seven have already been reported in humans and are potentially zoonotic **(49)**. Another zoonotic parasite *Cryptosporidium* sp. (23.5%), was reported in the current horses. Similar reports are present in Libya (33%) **(28)** and Italy (37.8%) **(50)**. Studies reported *C. parvum*, *C. hominis*, *C. muris*, *C.* horse genotype, *C. tyzzeri, C. erinacei,* and *C. andersoni* in horses **(51–54)** and critically, the first two species can cause about 90% of human cryptosporidiosis **(12)** and human cases of *C. andersoni* and *C.* horse genotypes have also been recorded **(12,55,56)**. This indicates an immediate need of molecular studies for species identification in the horse population in Nepal.

Under mixed domestication with livestock, cross-transmission of GIPs between young horses (foals) and calves exists, as evidenced by *C. parvum* cattle 211 genotypes from a severe outbreak of neonatal foal diarrhea in New Zealand **(57)**. Similarly, upon usual grazing, horses share the same pastureland, which has previously been grazed by cattle, calves, or wild ruminants in the periphery of CNP in the

study area. Grazing in common pastureland is the source of flukes, tapeworms, and strongyles in horses previously **(39,58).** However, literature claims the dilution effect is a co-grazing benefit, which occurs through the declination of parasite burdens in horses in a mixed horse-cattle grazing system **(59)**. Likewise, plant nut-/water-borne parasites like *Fasciola* sp. and *Gastrodiscus* sp. are acquired via the consumption of water from contaminated water bodies and rice straw, which contain infective metacercaria larvae of flukes **(60)**.

In the same way, a muddy floor is an essential source as larvae and eggs remain viable due to humidity, and survival is warranted even if the floor is wooden and with crevices on it. Soil-transmitted helminths (STH) are more prevalent in buffaloes **(47)** and pigs **(24)** raised on muddy floors. Interestingly, contamination with STH in and around stable (shed) was almost 50% **(61)**, indicating the need for mud management for parasite control on horse farms. Interestingly, 100% of the horses out of 52 without any known history or more than 1 year of deworming were positive compared to the horses with medication before six months. Regular and frequent deworming can reduce the rate of *Strongylus vulgaris* **(58,62)**. However, few parasites, especially the Cyathostomins are anthelmintic-resistant **(63,64),** suggesting the possibility that there are still positive cases in the current equines despite the medication.

As expected, parasitism impairs the well-being of horses, and our finding of 8 zoonotic parasite species in them is a serious public health concern. Under favorable circumstances, these pathogens can be transmitted between horses and humans, and cause GI illness, indicating that humans with occupational or recreational exposure to horses are at the topmost zoonotic risk. However, due to lack of knowledge among health professionals, underreporting of zoonosis between humans and horses is usual **(24)**. Thus, further *in vivo* studies regarding the pathologic impact of zoonotic infections and the associated risk factors need to be carried out.

In this study, polyparasitism was dominant over monoparasitism. It is difficult to explain the effects of multiple infections as each co-infecting member induces various responses **(65)**. These responses may be positive, negative, or neutral **(66)**. For example, co-infection of strongyles and protozoa in Welsh ponies contributed to each other's success **(67)**. However, higher epg of Cyathostomin egg was associated with richer gut microbiota **(68)**. In Australian horses, positive interaction among co-infecting strongyloid nematodes at high intensity has been reported **(69)**. In other hosts like human, buffalo claves, wild water buffaloes, wild rabbit co-infection with either helminths-helminths, or protozoan-helminths, or microparasite-helminth were associated with infection severity and several pathological consequences **(70–73)**. These consequences and intestinal parasitism are enhanced by partial or complete starvation and malnutrition **(74)** . Shedding eggs by different nematodes showed various results in young and adult horses. For example, *Parascaris equorum* showed the highest prevalence among young and strongyles showed the highest prevalence among adults, indicating differences between young and adult GI anatomy and physiology or other unknown factors. Most horses in the current study were working types; thus, high horse mobility may be associated with high egg shedding by many helminths, including *Parascaris* sp. **(75)**. Furthermore, feed deprivation or malnutrition not only causes the declination of energy, stamina, and focus by adding strain but also leads to intestinal ulcers resulting from the repeated exposure of intestinal mucosa to high acidity **(74,76)**. However, further equine immunology before and after carting would correctly answer this hypothesis.

Moreover, the current result suggests a greater possibility that a higher load of strongyles, as suggested by fecal epg count and prevalence of *Entamoeba* spp., including other species in the current equines, may be associated to malnutrition and stress. Also, seasonal and other climate factors may determine the epg counts **(75)**, while the current study lacked a seasonal context. In order to characterize the epidemiology of intestinal parasites, the One Health Approach should be applied including all associated sources of parasite infection in man and domestic animals, other than single equid hosts, like grazing, soil, and water sources. Another limitation of the current study is the lack of histopathological tests. Further studies must confirm the underlying association of polyparasitism in these hosts.

CONCLUSION AND RECOMMENDATION

The existing horse population in lowland touristic areas of Nepal possesses a high prevalence and diversity of GIPs. These GIPs vary with age and sex. The grazing opportunity, access to outdoor water bodies, mixed domestication, muddy stable, and irregular deworming practices are the major contributing factors. GIPs in horses may have the potential of public health significant zoonoses, suggesting the necessity of the One Health Approach in parasite research. The latter means expanding the range of hosts as well as the nearby environment, including grazing ecosystem, soil, water sources, and stable. In this context, *in vivo* studies of equine parasites using different mammalian models will be helpful in identifying their zoonotic potentialities in humans. While stressed and malnourished equines may carry higher loads of parasites, the current study concludes that regular deworming, pasture management, improved and timely feeding, reduced strain and optimal rest can reduce GIPs. These measures, along with molecular taxonomy, will support the effective therapeutic and efficient management of the equine industry in Nepal.

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REFERENCES

1. Torres Borda L, Auer U, Jenner F. Equine Social Behaviour: Love, War and Tolerance. Animals. 2022;13(9):1473.
2. MoAD. LIVESTOCK STATISTICS OF NEPAL 2077/78 (2020/21) [Internet]. 2022. Available from: Livestock_Statistics_of_Nepal_2077_78_1659524236-1669717523.pdf
3. Bhatta BR, Kafle A, Shrestha S, Kaphle K. Horse Breeds of Nepal. Int J Zool Anim Biol [Internet]. 2020 [cited 2023 Aug 17];3(2). Available from: https://medwinpublishers.com/IZAB/horse-breeds-of-nepal.pdf
4. Sazmand A, Bahari A, Papi S, Otranto D. Parasitic diseases of equids in Iran (1931–2020): a literature review. Parasit Vectors [Internet]. 2020 Dec [cited 2023 Oct 10];13(1):586. Available from: https://parasitesandvectors.biomedcentral.com/articles/10.1186/s13071-020-04472-w
5. Machtinger ET, Weeks ENI, Geden CJ, Lacher E. Pests and parasites of horses [Internet]. Brill | Wageningen Academic; 2022 [cited 2023 Oct 5]. Available from: https://brill.com/view/title/68663
6. Tyrnenopoulou P, Boufis PT, Fthenakis GC, Papadopoulos E. Interactions between Parasitic Infections and Reproductive Efficiency in Horses. Parasitologia. 2021;1(3):148–57.
7. Zhang Y, Choi SH, Nogoy KM, Liang S. The development of the gastrointestinal tract microbiota and intervention in neonatal ruminants. Animal. 2021;15(8):100316.
8. Mohd Rajdi NZI, Mohamad MA, Tan LP, Choong SS, Reduan MFH, Hamdan RH, et al. First case report on the occurrence of Trypanosoma evansi in a Siam B Mare in Kelantan, Malaysia. Vet Med Sci. 2021;7(2):303–9.
9. Sack A, Oladunni FS, Gonchigoo B, Chambers TM, Gray GC. Zoonotic Diseases from Horses: A Systematic Review. Vector-Borne Zoonotic Dis [Internet]. 2020 Jul 1 [cited 2024 Jan 6];20(7):484–95. Available from: https://www.liebertpub.com/doi/10.1089/vbz.2019.2541
10. Ai S, Zhang Z, Wang X, Zhang Q, Yin W, Duan Z. The first survey and molecular identification of *Entamoeba* spp. in farm animals on Qinghai-Tibetan Plateau of China. Comp Immunol Microbiol Infect Dis. 2021;75:101607.
11. y Sierra RM, Neira G, Bargues MD, Cuervo PF, Artigas P, Logarzo L, et al. Equines as reservoirs of human fascioliasis: transmission capacity, epidemiology and pathogenicity in *Fasciola hepatica*-infected mules. J Helminthol. 2020;94:e189.
12. Leoni F, Amar C, Nichols G, Pedraza-Diaz S, McLauchlin J. Genetic analysis of *Cryptosporidium* from 2414 humans with diarrhoea in England between 1985 and 2000. J Med Microbiol. 2006;55(6):703–7.
13. Torgerson PR, Devleesschauwer B, Praet N, Speybroeck N, Willingham AL, Kasuga F, et al. World Health Organization Estimates of the Global and Regional Disease Burden of 11 Foodborne Parasitic Diseases, 2010: A Data Synthesis. PLoS Med. 2015 Dec;12(12):e1001920.
14. Shirley DAT, Farr L, Watanabe K, Moonah S. A Review of the Global Burden, New Diagnostics, and Current Therapeutics for Amebiasis. Open Forum Infect Dis. 2018 Jul;5(7):ofy161.
15. Adhikari RB, Parajuli RP, Maharjan M, Ghimire TR. Prevalence and risk factors of gastrointestinal parasites in the Chepangs in Nepal. Ann Parasitol [Internet]. 2021 [cited 2023 Aug 9];(67(3)):387–405. Available from: https://doi.org/10.17420/ap6703.353
16. Devkota RP, Subedi JR, Wagley K. Prevalence of gastrointestinal parasites in equines of Mustang District, Nepal. Biodiversitas J Biol Divers [Internet]. 2021 Sep 8 [cited 2023 Aug 17];22(9). Available from: https://smujo.id/biodiv/article/view/8962
17. Oli N, Subedi JR. Prevalence of Gastro-Intestinal Parasites of Horse (Equus Caballus Linnaeus, 1758) In Seven Village Development Committee of Rukum District, Nepal. J Inst Sci Technol [Internet]. 2018 Apr 9 [cited 2023 Aug 17];22(2):70–5. Available from: https://www.nepjol.info/index.php/JIST/article/view/19596
18. Adhikari RB, Adhikari Dhakal M, Ale PB, Regmi GR, Ghimire TR. Survey on the prevalence of intestinal parasites in domestic cats (*Felis catus* Linnaeus, 1758) in central Nepal. Vet Med Sci [Internet]. 2022 Nov[cited 2023 Aug 9];9(2):559–71. Available from: https://onlinelibrary.wiley.com/doi/10.1002/

vms3.999
19. Adhikari RB, Ale PB, Adhikari Dhakal M, Ghimire TR. Prevalence and diversity of intestinal parasites in household and temple pigeons (*Columba livia*) in central Nepal. Vet Med Sci [Internet]. 2022 Mar [cited 2023 Aug 9];8(4):1528–38. Available from: https://onlinelibrary.wiley.com/doi/10.1002/vms3.792
20. Adhikari RB, Adhikari Dhakal M, Ghimire TR. Prevalence of intestinal parasites in street dogs (*Canis lupus familiaris*) with highlights on zoonosis in Lalitpur, Nepal. Vet Med Sci [Internet]. 2023 Sep 5 [cited 2023 Sep 18];vms3.1258. Available from: https://onlinelibrary.wiley.com/doi/10.1002/vms3.1258
21. Adhikari RB, Maharjan M, Ghimire TR. Prevalence of Gastrointestinal Parasites in the Frugivorous and the Insectivorous Bats in Southcentral Nepal. Serrano Ferron E, editor. J Parasitol Res [Internet]. 2020 Dec 12 [cited 2023 Aug 9];2020:1–12. Available from: https://www.hindawi.com/journals/jpr/2020/8880033/
22. Ghimire TR, Adhikari RB, Bhattarai N. Diversity and prevalence of *Eimeria* species in goats of Nepal. J Hell Vet Med Soc. 2021;72(4):3299–306.
23. Sapkota B, Adhikari RB, Regmi GR, Bhattarai BP, Ghimire TR. Diversity and prevalence of gut parasites in urban macaques. Appl Sci Technol Ann [Internet]. 2020 Jun 30 [cited 2023 Aug 9];1(1):34–41. Available from: https://www.nepjol.info/index.php/asta/article/view/30270
24. Adhikari RB, Adhikari Dhakal M, Thapa S, Ghimire TR. Gastrointestinal parasites of indigenous pigs (*Sus domesticus*) in south-central Nepal. Vet Med Sci [Internet]. 2021 May [cited 2023 Aug 17];7(5):1820–30. Available from: https://onlinelibrary.wiley.com/doi/10.1002/vms3.536
25. Soulsby E.J. Helminths, arthropods and protozoa of domestic animals. 7th ed. New Delhi, India: East-West Press Private, Limited2012; 2012.
26. Lichtenfels JR, Kharchenko VA, Dvojnos GM. Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). Vet Parasitol. 2008;156(1–2):4–161.
27. Zajac AM, Conboy GA, Little SE, Reichard MV. Veterinary clinical parasitology. John Wiley & Sons; 2021.
28. Elmajdoub LO, Mosaab O, Alsaghir OA, Shimaa SS. Investigation and Prevalence of Gastrointestinal Parasites of Equestrian Clubs Horses in Misurata, Libya. Eur J Biol Biotechnol. 2022;3(6):5–9.
29. Adeppa J, Ananda KJ, Krishna Murthy CM, Satheesha GM. Incidence of gastro-intestinal parasites in horses of Shimoga region, Karnataka state. J Parasit Dis. 2016;40(3):919–21.
30. Mathewos M, Girma D, Fesseha H, Yirgalem M, Eshetu E. Prevalence of gastrointestinal helminthiasis in horses and donkeys of Hawassa District, Southern Ethiopia. Vet Med Int. 2021;2021.
31. Tedla M, Abichu B. Cross-sectional study on gastro-intestinal parasites of equids in South-western Ethiopia. Parasite Epidemiol Control [Internet]. 2018 Nov [cited 2023 Aug 18];3(4):e00076. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2405673118300060
32. Ramírez-Hernández A, Polo G, Robayo-Sánchez LN, Cruz-Maldonado OA, Imbacuán-Pantoja WO, Cortés-Vecino JA. Gastrointestinal and pulmonary parasites of working horses from Colombia. Vet Parasitol Reg Stud Rep. 2019;17:100296.
33. Anuțescu SM, Buzatu MC, gruianu A, Bellaw J, Mitrea IL, Ioniță M. Use of larval cultures to investigate the structure of strongyle populations in working horses, Romania: preliminary data. AgroLife Scientific Journal. 2016;5(1):9–13.
34. Nielsen MK, Reinemeyer CR. Handbook of equine parasite control. John Wiley & Sons; 2018.
35. Gelberg HB. Alimentary system and the peritoneum, omentum, mesentery, and peritoneal cavity. Pathol Basis Vet Dis. 2017;324.
36. Nielsen MK. Evidence-based considerations for control of *Parascaris* spp. infections in horses. Equine Vet Educ. 2016;28(4):224–31.
37. Getachew AM, Innocent GT, Trawford AF, Feseha G, Reid SJW, Love S. Equine parascarosis under the tropical weather conditions of Ethiopia: a coprological and postmortem study. Vet Rec. 2008;162(6):177–80.
38. Abdulkadir K, Ibrahim N, Deneke Y. Prevalence of equine lungworm and associated risk factors in Sudie district, Oromia region, south eastern Ethiopia. Afr J Agric Res. 2017;12(18):1526–31.
39. Saadi A, Tavassoli M, Dalir-Naghadeh B, Samiei A. A survey of *Dictyocaulus arnfieldi* (Nematoda) infections in equids in Urmia region, Iran. Ann Parasitol. 2018;64(3).
40. Ibrahim N. Equine lung worm: A systematic review. Glob J Med Res. 2017;17:19–25.
41. Dewes HF, Townsend KG. Further observations on *Strongyloides westeri* dermatitis: recovery of larvae from soil and bedding, and survival in treated sites. N Z Vet J. 1990;38(1):34–7.
42. Thamsborg SM, Ketzis J, Horii Y, Matthews JB. *Strongyloides* spp. infections of veterinary importance. Parasitology. 2017;144(3):274–84.
43. Abbas G, Ghafar A, Koehler AV, Bauquier J, Wilkes EJ, Jacobson C, et al. Molecular detection of *Strongyloides* sp. in Australian Thoroughbred foals. Parasit Vectors. 2021;14:1–7.
44. Roelfstra L, Betschart B, Pfister K. A study on the seasonal epidemiology of *Anoplocephala* spp.-infection in horses and the appropriate treatment using a praziquantel gel (Droncit 9% oral gel). Berl Munch Tierarztl Wochenschr. 2006;119(7–8):312–5.
45. Howell AK, Malalana F, Beesley NJ, Hodgkinson JE, Rhodes H, Sekiya M, et al. *Fasciola hepatica* in UK horses. Equine Vet J. 2020;52(2):194–9.
46. Matsubayashi M, Matsuura Y, Nukata S, Daizi Y, Shibahara T, Teramoto I, et al. First detection and molecular identification of *Entamoeba bovis* from Japanese cattle. Parasitol Res. 2018;117:339–42.
47. Adhikari RB, Adhikari Dhakal M, Ghimire TR. Prevalence and diversity of gastrointestinal parasites in domestic buffaloes (*Bubalus bubalis* Linnaeus, 1758) reared under captive and semi-captive conditions in Ratnanagar, Chitwan, Nepal. Ann Parasitol. 2022;68(4):701–13.
48. Aryal M, Adhikari RB, Kandel P, Ghimire TR, Khadka D, Maharjan J, et al. First report on the molecular detection of *Entamoeba bovis* from the endangered wild water buffalo (*Bubalus arnee*) in Nepal. Vet Med Sci [Internet]. 2021 Dec [cited 2023 Aug 9];8(2):799–807. Available from: https://onlinelibrary.wiley.com/doi/10.1002/vms3.697
49. Baek S, Maloney JG, Molokin A, George NS, Cortés Vecino JA, Santin M. Diversity of *Blastocystis* subtypes in horses in Colombia and identification of two new subtypes. Microorganisms. 2022;10(9):1693.
50. Galuppi R, Piva S, Castagnetti C, Iacono E, Tanel S, Pallaver F, et al. Epidemiological survey on *Cryptosporidium* in an Equine Perinatology Unit. Vet Parasitol [Internet]. 2015 Mar [cited 2023 Aug 9];210(1–2):10–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304401715001314
51. Inácio SV, Widmer G, Oliveira BCM, Gomes JF, Falcao AX, Ferrari ED, et al. Review: Equine cryptosporidiosis–cosmopolitan occurrence. Anim Husb Dairy Vet Sci. 2018;2:1–7.
52. Jian F, Liu A, Wang R, Zhang S, Qi M, Zhao W, et al. Common occurrence of *Cryptosporidium hominis* in horses and donkeys. Infect Genet Evol. 2016;43:261–6.

53. Li XM, Geng HL, Wei YJ, Yan WL, Liu J, Wei XY, et al. Global prevalence and risk factors of *Cryptosporidium* infection in *Equus*: A systematic review and meta-analysis. Front Cell Infect Microbiol. 2022;12:1774.
54. Liu A, Zhang J, Zhao J, Zhao W, Wang R, Zhang L. The first report of *Cryptosporidium andersoni* in horses with diarrhea and multilocus subtype analysis. Parasit Vectors. 2015;8:1–4.
55. Robinson G, Elwin K, Chalmers RM. Unusual *Cryptosporidium* genotypes in human cases of diarrhea. Emerg Infect Dis. 2008;14(11):1800.
56. Zajączkowska Ż, Brutovská AB, Akutko K, McEvoy J, Sak B, Hendrich AB, et al. Horse-specific *Cryptosporidium* genotype in human with Crohn's disease and arthritis. Emerg Infect Dis. 2022;28(6):1289.
57. Grinberg A, Oliver L, Learmonth JJ, Leyland M, Roe W, Pomroy WE. Identification of *Cryptosporidium parvum* 'cattle' genotype from a severe outbreak of neonatal foal diarrhoea. Vet Rec. 2003;153(20):628–31.
58. Tydén E, Jansson A, Ringmark S. Parasites in horses kept in a 2.5 year-round grazing system in nordic conditions without supplementary feeding. Animals. 2019;9(12):1156.
59. Forteau L, Dumont B, Sallé G, Bigot G, Fleurance G. Horses grazing with cattle have reduced strongyle egg count due to the dilution effect and increased reliance on macrocyclic lactones in mixed farms. Animal [Internet]. 2019 Nov [cited 2023 Oct 10];14(5):1076–82. Available from: https://linkinghub. elsevier.com/retrieve/pii/S1751731119002738
60. Mahato SN, Harrison LJS. Control of fasciolosis in stall-fed buffaloes by managing the feeding of rice straw. Trop Anim Health Prod. 2005;37:285–91.
61. Mahartina D, Hastutiek P, Mulyati S, Hambal M. Identification and Distribution of Soil Transmitted Helminths around The Shed and Grazing Fields of Madura Cattle in Sub-District of Geger, Bangkalan Regency. J Parasites Sci. 2020;4(2).
62. Kuzmina TA, Dzeverin I, Kharchenko VA. Strongylids in domestic horses: Influence of horse age, breed and deworming programs on the strongyle parasite community. Vet Parasitol. 2016;227:56–63.
63. Seyoum Z, Zewdu A, Dagnachew S, Bogale B. Anthelmintic resistance of strongyle nematodes to ivermectin and fenbendazole on cart horses in Gondar, Northwest Ethiopia. BioMed Res Int. 2017;2017.
64. Traversa D, von Samson-Himmelstjerna G, Demeler J, Milillo P, Schürmann S, Barnes H, et al. Anthelmintic resistance in cyathostomin populations from horse yards in Italy, United Kingdom and Germany. In: Parasites & Vectors. BioMed Central; 2009. p. 1–7.
65. Supali T, Verweij JJ, Wiria AE, Djuardi Y, Hamid F, Kaisar MM, et al. Polyparasitism and its impact on the immune system. Int J Parasitol. 2010;40(10):1171–6.
66. Hoarau AOG, Mavingui P, Lebarbenchon C. Coinfections in wildlife: Focus on a neglected aspect of infectious disease epidemiology. Knoll LJ, editor. PLOS Pathogen [Internet]. 2020 Sep 3 [cited 2023 Aug 17];16(9):e1008790. Available from: https://dx.plos.org/10.1371/journal.ppat.1008790
67. Clark A, Sallé G, Ballan V, Reigner F, Meynadier A, Cortet J, et al. Strongyle infection and gut microbiota: profiling of resistant and susceptible horses over a grazing season. Front Physiol. 2018;9:272.
68. Boisseau M, Dhorne-Pollet S, Bars-Cortina D, Courtot É, Serreau D, Annonay G, et al. Species interactions, stability, and resilience of the gut microbiota-helminth assemblage in horses. Iscience. 2023;26(2).
69. Bucknell D, Hoste H, Gasser RB, Beveridge I. The structure of the community of strongyloid nematodes of domestic equids. J Helminthol. 1996;70(3):185–92.
70. Adhikari RB, Ghimire TR. A case study of multiple parasitisms in a calf buffalo (*Bubalus bubalis*). Agric Sci Dig - Res J [Internet]. 2021 Jan 22 [cited 2023 Aug 9];(Of). Available from: http://arccjournals.com/journal/agricultural-science-digest/D-5172
71. Ezeamama AE, McGarvey ST, Acosta LP, Zierler S, Manalo DL, Wu HW, et al. The synergistic effect of concomitant Schistosomiasis, Hookworm, and *Trichuris* Infections on Children's Anemia Burden. Brooker S, editor. PLoS Negl Trop Dis [Internet]. 2008 Jun 4 [cited 2023 Oct 7];2(6):e245. Available from: https://dx.plos.org/10.1371/journal.pntd.0000245
72. Jolles AE, Ezenwa VO, Etienne RS, Turner WC, Olff H. Interactions between macroparasites and microparasites drive infection patterns in freeranging African buffalo. Ecology [Internet]. 2008 Aug [cited 2023 Oct 7];89(8):2239–50. Available from: http://doi.wiley.com/10.1890/07-0995.1
73. Lello J, Boag B, Hudson P. The effect of single and concomitant pathogen infections on condition and fecundity of the wild rabbit (*Oryctolagus cuniculus*). Int J Parasitol [Internet]. 2005 Jul [cited 2023 Oct 7];35(14):1509–15. Available from: https://linkinghub. elsevier.com/retrieve/pii/S002075190500204
74. Kronfeld DS. Starvation and malnutrition of horses: recognition and treatment. J Equine Vet Sci [Internet]. 1993 May [cited 2023 Oct 5];13(5):298–304. Available from: https://linkinghub.elsevier.com/retrieve/pii/S073708060780253X
75. Hautala K, Näreaho A, Kauppinen O, Nielsen MK, Sukura A, Rajala-Schultz PJ. Risk factors for equine intestinal parasite infections and reduced efficacy of pyrantel embonate against Parascaris sp. Vet Parasitol [Internet]. 2019 Sep [cited 2023 Oct 10];273:52–9. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304401719301864
76. Andrews FM. Equine Gastric Ulcer Syndrome. AAEP. [Internet]. 2019 [cited 2023 Oct 5]. Available from: https://aaep.org/horsehealth/equine-gastric-ulcer-syndrome