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Prevalence of Intestinal Parasites in Dogs (*Canis familiaris* Linnaeus, 1758) and Dzos (*Bos grunniens* Linnaeus, 1766) in Upper Humla, Nepal

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Brief Title: Intestinal Parasites of Dogs and Dzos

Abstract

Background: Dogs and dzos are important domestic animals in human communities in high altitude landscapes, and are the potential carriers of different parasites. There is a potential risk of zoonotic parasite transmission between dzos and free-ranging dogs. Therefore, evaluating and managing the parasites could play a role in safeguarding health and overall well-being.

Objectives: The study aimed to investigate the prevalence and diversity of intestinal parasites in free-ranging dogs, owned dogs, and dzos in Upper Humla, Nepal.

Methods: Fecal samples (N=151), including 109 from free-ranging dogs, 12 from owned dogs and 30 from dzos, were collected. Applying direct wet mount and acid-fast staining methods, microscopic examination of fecal samples was carried out.

Results: The overall prevalence of GI parasites was 75.49%, out of which 75.23% were in free-ranging dogs, 66.67% in owned dogs, and 80% in dzos. Nineteen parasite species (18 confirmed) were recorded with nine species in dzos, seven in owned dogs, and seventeen in free-ranging dogs. The triplet infection was higher in free-ranging dogs, duplicate infection was higher in owned dogs, and pentuplet infection was higher in dzos. The dogs and dzos of Upper Humla were commonly infected with *Entamoeba* spp., ascarids, *Cryptosporidium* spp., *Eimeria* spp., and *Taenia* spp.

Conclusion: Intestinal parasites can substantially threaten human populations through zoonotic transmission. Controlling and managing the parasitic infection in dogs and dzos can reduce damage to human health.

Keywords: Agro-pastoralism; Cross-transmission; Gastrointestinal; *Cryptosporidium;* Zoonosis

Introduction

Dogs (*Canis familiaris* Linnaeus, 1758) were first pets domesticated since prehistoric times for purposes (Bradshaw *et al.,* 2017, Freedman *et al.,* 2014). Sequencing of archaic DNA indicates that the dog domestication began in Siberia about 23,000 years ago (Perri *et al.,* 2021). Free-ranging dogs include street dogs, stray dogs, and feral dogs that spend their lives in the wild outside human habitations, hunt with their groups, and breed without disturbances from humans (Boitani & Ciucci, 1995). These free-ranging dogs are opportunistic predators (Sarkar *et al.,* 2023). They may be also threats to wildlife (Thompson, 2013), for possible transmission of various zoonotic diseases (Butler *et al.,* 2004), including several GI parasitic species of zoonotic importance (Thompson, 2013; Khalifa *et al.,* 2023; Sukupayo *et al.,* 2023; Adhikari *et al.,* 2023). The 2024 Worldostats estimates that out of approximately 900 million dog population globally, about 500 million are pet dogs with numerous breeds (Retrieved from: https://worldostats.com/dog-population-by-country-2024/, Retrieved on August 20, 2024). Dzo (*Bos grunniens* Linnaeus, 1766) is a hybrid between the yak [*Bos mutus* (Przewalski, 1883)] and domestic cattle (*Bos taurus* Linnaeus, 1758). Its male form is a Jhoppa, and the female is a Jhuma.

Different types of endoparasites are responsible for causing health issues (Naqid, 2024) (Ismael *et al.,* 2024) (Dalimi & Jaffarian, 2024) and death among pets (Morandi *et al.,* 2020). *A few endoparasites Toxocara canis*, *Ancylostoma* spp., *Giardia duodenalis*, *Cryptosporidium parvum,* and *Toxoplasma gondii are zoonotically important*

*(Oliveira-Sequeira et al., 2002) (*Ahmad *et al., 2020) (*Firooz Jahantigh *et al., 2020) (*David Ola-Fadunsin *et al.,* 2023) (Chamanara *et al.,* 2024). Shelters and scavenging feeding habits were identified as significant contributing factors for occurrence of endoparasite infection (Grandi *et al.,* 2021) (Adhikari *et al.,* 2023).

Dzos are commonly found in a few Hindu-Kush Himalayan (HKH) regions like Nepal, Bhutan, and Tibet. In Humla, dzos are among the most preferred livestock types due to their adaptive nature and multi-purpose uses including for transportation in Upper Humla. Dzos possess frequent interaction with free-ranging dogs. In these contexts, GI parasites can be cross-transmitted among owned dogs, free-ranging dogs, and dzos in high-altitude landscapes. Due to the lack of veterinary health services for timely and routine vaccination of dogs or cattle and the lack of awareness among the local population, there is a high risk of transmitting various GI parasites to nearby wild populations and humans. No studies have been conducted regarding GI parasites of dogs and dzos and their possible impact on zoonosis, although the HKH region is essential in the context of various microspecies on the hosts and environment (Ghimire *et al.,* 2020). Therefore, the current study aims to study the prevalence of GI parasites of dogs and dzos in Namkha Rural Municipality and Simkot Rural Municipality in the Humla district of Nepal.

Materials and Methods

Study area

This study was carried out in the Namkha Rural Municipality and Simkot Rural Municipality in the Humla district of Nepal **(Figure 1)**. Humla district is the second largest in Nepal, covering an area of 5,655 km^2 with a population of 55,394 individuals (NSO, 2022). This region is characterized by its remoteness, rugged terrain, high mountains, and deep valleys so far not connected to the rest of Nepal by road, making it one of Nepal's most isolated and challenging areas to reach (Oli & Zomer, 2011; Lama *et al.,* 2018). The district includes several rivers, including the Karnali River, which flows through Humla from its source in West Tibet. Upper Humla, a district in Nepal, has a harsh climate with snow for up to four months of the year and tropical, wet and dry climate, with most of the rainfall occurring during the monsoon season. With most villages lying at an altitude of 3,000-5,000 meters (9,842-16,404 feet) above sea level, the average altitude is approximately 3,000 meters (9,842 feet) above sea level (https://www.headnepal.org/, Retrieved July 7, 2024). The common leopard, snow leopard, golden jackal, Himalayan black bear, Himalayan tahr, yellow-throated marten, leopard cat, Himalayan langur, Rhesus monkey, chucker partridge, Himalayan griffon, danphe, bearded vulture, rock lizard, and Himalayan pit viper are common wild faunae whereas cattle, goat, sheep, dzo, yak, horse, mule, donkey, and chickens are common domestic faunae in the study areas.

Figure 1. Map of study areas

Sample collection, preservation, and transportation

Before sample collection, a three-day primary field survey was conducted to investigate the study area and familiarize the investigators with the local population, husbandry practices, geographical location, and population status of dogs and dzos. A detailed survey was conducted from October, 21 to October 30, 2022, with the help of trained local field associates. An opportunistic sampling technique was used to collect stool samples as it was challenging for a researcher to create a sampling frame at Upper Humla. A total of 151 fresh fecal samples fallen on the ground just after the defecation were collected opportunistically (Adhikari *et al.,* 2022, 2023) from a total of 121 dogs, which included domestic dogs (n = 12) and free-ranging dogs (n = 109) and dzos (n=30). Initially, the samples were macroscopically examined for blood, mucus, segments of worm, and types of stools' structures. Then, the samples were preserved at 2.5% potassium dichromate solution in 20 milliliters (mL) sterile vials. A unique identification code was written on the vial of each fecal sample, and finally, these samples were then transported to the research laboratory in Kathmandu, Nepal.

Laboratory processing and examination

The fecal samples were analyzed using previously explained methods (Adhikari *et al.,* 2022, 2023, 2024) (Thapa Magar & Ghimire, 2024). These methods included direct wet mount techniques with or without using iodine staining and modified acid-fast staining techniques. A solution of iodine, which consisted of 10 grams of potassium iodide dissolved in 100 mL

of distilled water along with 5 grams of iodine crystals, was utilized for the wet mount method (Zajac *et al.,* 2021).

Approximately 2 grams of a few stool samples were stirred/mixed carefully. A single drop of each sample was put on a clean and dry glass slide with or without Gram's iodine stain. The sample was covered with a coverslip and observed under a microscope at magnifications of 10X and 40X (Adhikari *et al.,* 2023, 2024) (Thapa Magar & Ghimire, 2024). The same sample was observed without using an iodine solution in the same magnifications.

The acid-fast staining test was performed mainly to identify *Cryptosporidium* spp.; therefore, a stool smear was prepared, air dried and heat fixed. The smear was covered with carbol fuchsin (**a mixture of phenol and basic fuchsin). The slide was placed over steam. After 5-10 minutes, the slide was cooled and gently rinsed with distilled water. Two to three drops of acid alcohol were put on the slide, and rinsed with water. A drop of methylene blue was added to the slide for one minute and rinsed with water. Then, the slide was air-dried and observed under a compound microscope at magnification of 100X in immersion oil (Ghimire & Bhattarai, 2019) (Adhikari & Ghimire, 2021) (Adhikari *et al.,* 2020, 2022, 2023).**

Parasite identification

The microscopic images of the detected parasitic stages, such as eggs, cysts, trophozoites, and oocysts, were captured using a camera built-in mobile (iphone 11promax). Their identification and taxonomic position were confirmed using literature and online sources

(Soulsby, 2012) (Zajac *et al.*, 2021) (Adhikari *et al.*, 2023) (Sukupayo *et al.*, 2023). *Entamoeba* spp. were defined by the smaller cysts with one to four nuclei. But *Entamoeba coli* were confirmed by the larger cysts with eight nuclei.

Data analysis

Data were tabulated, edited, and expressed in Microsoft Excel 2010. The prevalence rates of parasites were calculated in each host, and their statistical significance was tested using *p*-values obtained from the chi-square tests and nonparametric correlation (Spearman, r). A *p*-value less than 0.05 (95% confidence interval) was considered statistically significant when comparing the prevalence rates among different subjects and variables. The intensity of each infection was calculated depending on the observation of cyst/oocyst/trophozoite/egg of parasite after direct wet mount. Therefore, the following symbolic indications were used for evaluation of the intensity of particular parasitic species:

+1-3 per field; ++: 4-10 per field; +++: 11 or more (X400 field for protozoa)

+1-3 per field; ++: 4-10 per field; +++: 11 or more (X100 field for helminths)

Results

In this study, out of 151 fecal samples, 114 (75.49%) were positive for GI parasites with rates of 66.67% (8/12) in owned dogs, 75.23% (82/109) in free-ranging dogs, and 80% (24/30) in dzos. Nineteen different parasitic species were recorded in this study with nine species in

dzos, seven in owned dogs, and seventeen in free-ranging dogs **(Figure 2) (Table 1)**. The prevalence of *Cryptosporidium* spp. was the highest in all three hosts. Similarly, GI parasites shared by three types of hosts were analyzed. These hosts shared *Cryptosporidium* spp., ascarid, *Entamoeba* spp., *Eimeria* spp., and *Taenia* spp. Interestingly, Blastocystis sp. was shared by only owned dogs and dzos, but *Cyclospora* sp. and *Neospora caninum* and unknown coccidia were shared by stray dogs and dzos **(Table 1)**.

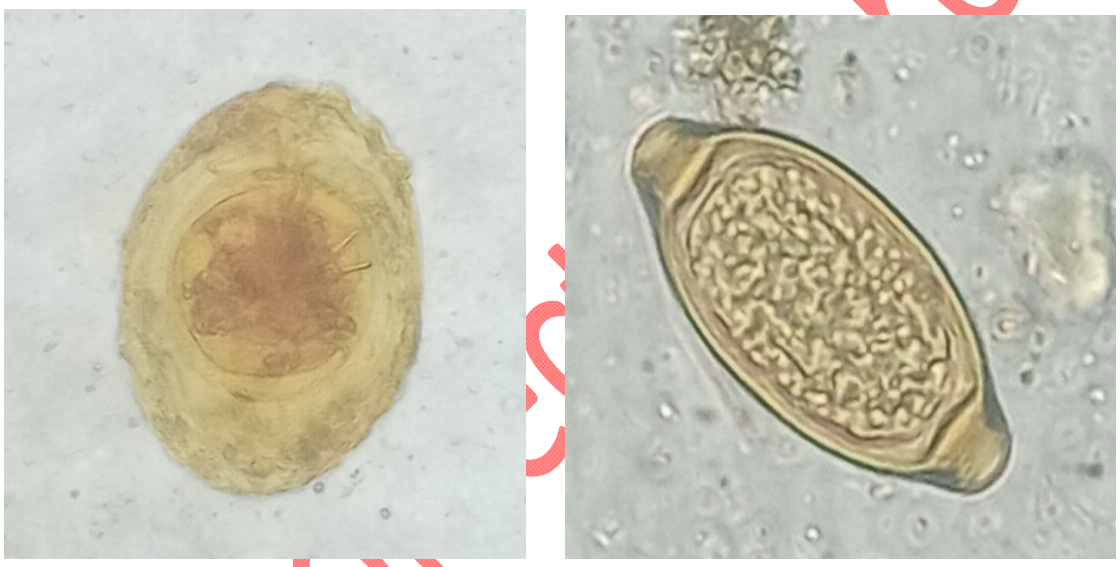

Figure 2A. Egg of *Hymenolepis nana* (Iodine staining, X400) **Figure 2B.** Egg of *Trichuris* sp. (Iodine staining, X400)

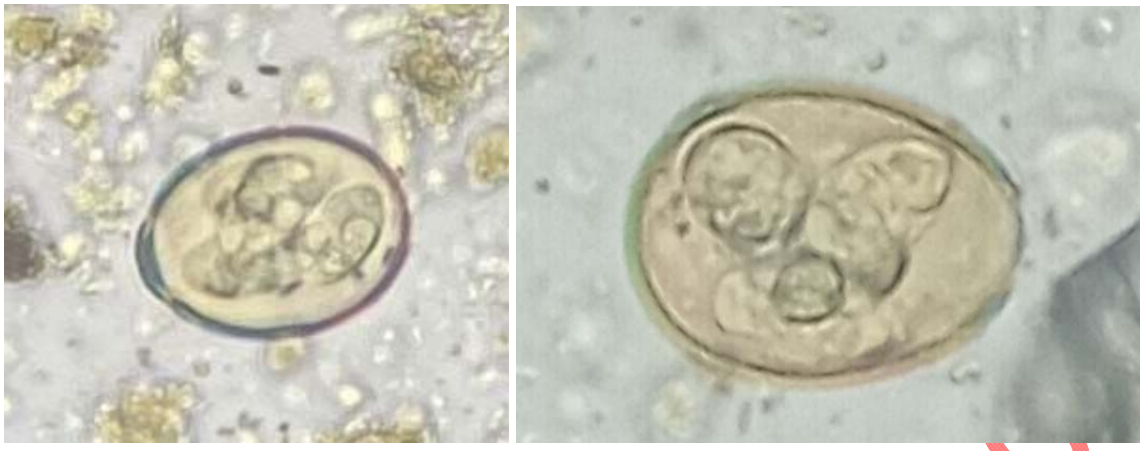

Figure 2C. Oocysts of *Eimeria* spp. (Iodine staining, X400)

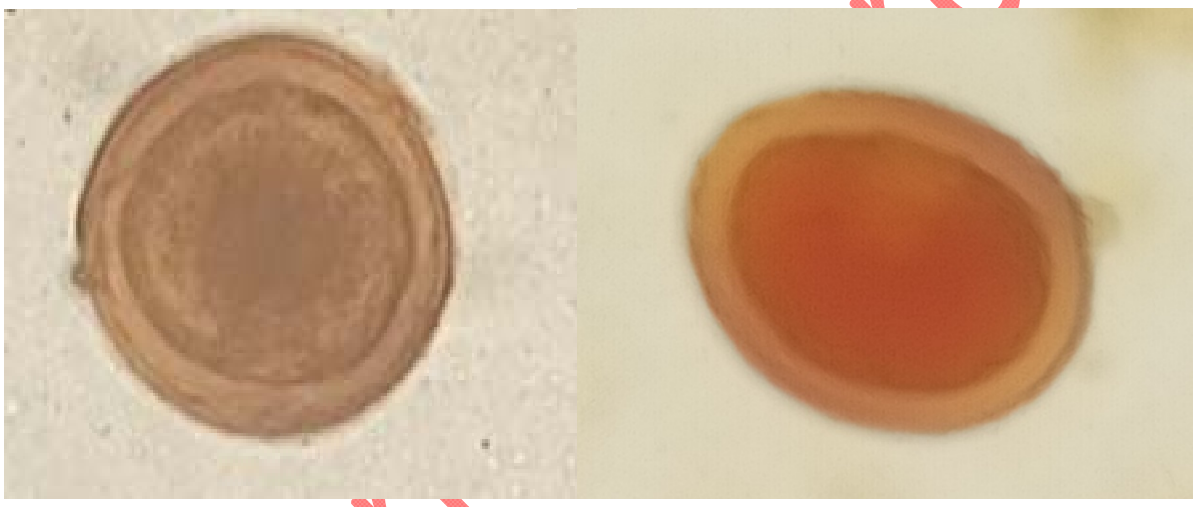

Figure 2D. Eggs of Ascarid (Iodine staining, X400).

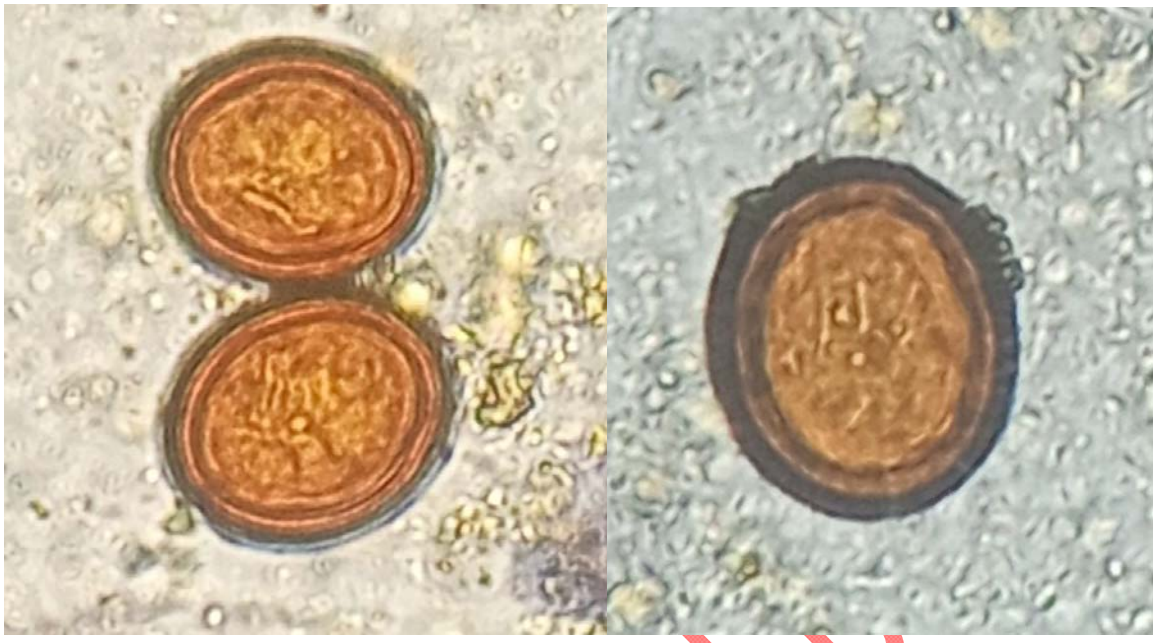

Figure 2E. Eggs of Tapeworm (Iodine staining, X400)

Figure 2. Representative parasites in the fecal samples of dogs and dzos

Table 1. Prevalence of GI parasites in domestic dogs, stray dogs, and dzos. $p<0.0001$ (Chi-square tests) while comparing the rates of GI parasites in particular host or among domestic dogs, stray dogs, and dzos.

Parasites	**Domestic dogs (*p* <0.0001)**		**Stray dogs (*p* <0.0001)**		**Dzos (*p*<0.0001)**	
	Positive	**%**	**Positive**	**%**	**Positive**	**%**
Cryptosporidium spp.	8	66.67	64	58.72	19	63.33

Ascarid	4	33.33	37	33.94	17	56.67
Entamoeba spp.	4	33.33	23	21	17	56.67
E. coli	0	0.00	19	17.43	0	0.00
Eimeria spp.	2	16.67	13	11.92	14	46.67
Balantidium coli	0	0.00	11	10.1	0	0.00
Sarcocystis spp.	0	0.00	9	8.26	0	0.00
Taenia spp.	1	8.33	8	7.33	6	20
Neospora caninum	1	8.33	8	7.33	0	0.00
Acanthocephalid	0	0.00	4	3.67	0	0.00
Isospora spp.	0	0.00	3	2.75	0	0.00
Strongyloides sp.	0	0.00	2	1.83	0	0.00
Trichuris sp.	0	0.00	2	1.83	0	0.00
Cyclospora sp.	0	0.00	1	0.92	7	23.33
Ancylostoma	0	0.00	1	0.92	0	0.00
Hymenolepis nana	0	0.00	1	0.92	0	0.00
Blastocystis sp.	2	16.67	0	0	1	3.33
Giardia sp.	0	0.00	0	0	1	3.33
Unknown coccidia	0	0.00	1	0.92	3	10.00

In owned dogs, ascarids and *Entamoeba* spp. showed the highest prevalence of low intensity. Interestingly, *Cryptosporidium* spp. showed the highest prevalence of both moderate and high intensities. In stray dogs, ascarids showed the highest prevalence of low intensity, whereas *Cryptosporidium* spp. showed the highest prevalence of either moderate or high intensity. In dzos, *Entamoeba* spp. showed the highest low intensity, whereas *Cryptosporidium* spp. had the highest moderate intensity, and *Taenia* spp. had the highest high intensity **(Table 2)**. Data were analyzed using nonparametric correlation (Spearman, r) between any two selected intensity sets for all total GI parasitic species to calculate r with 95% confidence interval (CI) and Gaussian Approximation p values (Two-tailed). In the domestic dogs, comparing between ++ and +++ intensities resulted in the r of 1.000 ($p<0.0001$, 95% CI=1.000 to 1.000). In stray dogs, r was 0.4998 ($p<0.05$ and 95% CI=0.04437 to 0.7832) when compared between + and ++ intensities. Similar results were obtained after comparing between ++ and +++ intensities that generated r of 0.9899 (95% CI=0.9732 to 0.9962, $p<0.0001$). In dzos, comparison between ++ and +++ intensities generated r of 1.000 (95% CI=1.000 to 1.000, $p<0.0001$). Interestingly, while comparing between total intensities between ++ and +++, r was 0.7265 and 95% CI of 0.3941 to 0.8908 with $p<0.0005$ **(Table 2)**.

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Table 2. Intensity of intestinal parasites (%) in domestic dogs, stray dogs, and dzos.

Parasitic species	**Domestic dogs** (++ vs +++: p<0.0001, 95% CI=1.000 – 1.000)			**Stray dogs** (+vs++: p<0.05, 95% CI=0.044 – 0.783)(++vs+++: p<0.0001, 95% CI=0.9732 – 0.9962)			**Dzos** (++ vs +++: p<0.0001, 95% CI=1.000 – 1.000)			**Total (** ++ vs+++: p<0.0005, 95% CI=0.394 – 0.891**)**		
	+	++	+++	+	++	+++	+	++	+++	+	++	+++
Protozoa												
Cryptosporidium spp.	3(25)	1(8.33)	4(33.33)	20(18.34)	14(12.84)	30(27.52)	3(10)	6(30)	10(33.34)	26 (17.21)	21 (13.90)	44 (29.13)
Entamoeba spp.	4(33.33)	0(0.00)	0(0.00)	23(21.10)	0(0.00)	0(0.00)	17(56.67)	0(0.00)	0(0.00)	44(29.13)	0(0.00)	0(0.00)
Entamoeba coli	0(0.00)	0(0.00)	0(0.00)	19(17.43)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	19(17.43)	0(0.00)	0(0.00)
Eimeria spp.	2(16.67)	0(0.00)	0(0.00)	13(11.92)	0(0.00)	0(0.00)	14(46.67)	0(0.00)	0(0.00)	29(19.20)	0(0.00)	0(0.00)
Balantidium coli	0.00	0(0.00)	0(0.00)	11(10.1)	0(0.00)	0(0.00)	0.00	0(0.00)	0(0.00)	11(7.28)	0(0.00)	0(0.00)
Sarcocystis spp.	1(8.33)	0(0.00)	0(0.00)	9(8.26)	0(0.00)	0(0.00)	0.00	0(0.00)	0(0.00)	9(5.96)	0(0.00)	0(0.00)
Neospora caninum	1 (8.33)	0(0.00)	0(0.00)	8(7.34)	0(0.00)	0(0.00)	0.00	0(0.00)	0(0.00)	9(5.96)	0(0.00)	0(0.00)
Cyclospora sp.	0(0.00)	0(0.00)	0(0.00)	1(0.92)	0(0.00)	0(0.00)	7(23.33)	0(0.00)	0(0.00)	8(5.29)	0(0.00)	0(0.00)
Isospora spp.	0(0.00)	0(0.00)	0(0.00)	3(2.75)	0(0.00)	0(0.00)	0.00	0(0.00)	0(0.00)	3(1.98)	0(0.00)	0(0.00)
Blastocystis sp.	2(16.67)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(3.33)	0(0.00)	0(0.00)	3(1.98)	0(0.00)	0(0.00)
Giardia sp.	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(3.33)	0(0.00)	0(0.00)	1(0.66)	0(0.00)	0(0.00)
Unknown coccidia	0(0.00)	0(0.00)	0(0.00)	1(0.92)	0(0.00)	0(0.00)	3(10)	0(0.00)	0(0.00)	4(2.65)	0(0.00)	0(0.00)
Nematoda												
Ascarid	4(33.33)	0(0.00)	0(0.00)	35(32.11)	2(1.83)	0.00	17(56.67)	0(0.00)	0(0.00)	56(37.08)	2(1.83)	0.00
Ancylostoma	0(0.00)	0(0.00)	0(0.00)	1(0.92)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.66)	0(0.00)	0(0.00)
Strongyloides sp.	0(0.00)	0(0.00)	0(0.00)	2(1.84)	0(0.00)	0(0.00)	0.00	0(0.00)	0(0.00)	2(1.32)	0(0.00)	0(0.00)
Trichuris sp.	0(0.00)	0(0.00)	0(0.00)	2(1.84)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(1.32)	0(0.00)	0(0.00)
Cestoda												
Taenia spp.	1(8.33)	0(0.00)	0(0.00)	8(7.34)	0(0.00)	0(0.00)	6(20)	0(0.00)	0(0.00)	15(9.93)	0(0.00)	0(0.00)
Hymenolepis nana	0(0.00)	0(0.00)	0(0.00)	1(0.92)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(0.66)	0(0.00)	0(0.00)
Acanthocephala												
Acanthocephalid	0(0.00)	0(0.00)	0(0.00)	4(3.67)	0(0.00)	0(0.00)	0.00	0(0.00)	0(0.00)	4(2.64)	0(0.00)	0(0.00)

The current study also analyzed the concomitance of GI parasites in these hosts. Mixed infection of up to five different GI parasites was present in owned dogs, whereas seven GI parasites were present in free-ranging dogs or dzos, indicating a high concurrence of GI parasites **(Table 3)**.

Table 3. Co-infection of intestinal parasites in domestic dogs, stray dogs, and dzos. $p<0.0001$ while comparing the rates of co-infection in single host or among domestic dogs, stray dogs, and dzos.

Parasites	**Domestic dogs ($p<0.0001$)**		**Stray dogs ($p<0.0001$)**		**Dzos ($p<0.0001$)**	
	n	**%**	**n**	**%**	**n**	**%**
Single	1	8.33	17	15.60	2	6.67
Double	3	25	22	20.18	5	16.67
Triple	1	8.33	25	22.93	1	3.33
Quadruple	2	16.67	12	11	6	20
Pentuple	1	8.33	4	3.67	7	23.33
Sextuple	0	0	1	1.22	2	6.67
Septuple	0	0	1	1.22	1	3.33

Discussion

The current research was the first to record the prevalence of various GI parasites in dogs and dzos in the complex landscapes of Upper Humla, one of the best representative areas of HKH. The rates of GI parasites in owned dogs in other geographical regions are different; for example, Brazil (11.3%, n=400) (Arruda *et al.,* 2021), Spain (48.8%, n=252) (Mateo *et al.,* 2023), Kenya (65%, n=100) (Mulinge *et al.* 2020), the high mountain areas of Columbia (43.9%, n=41) (Peña-Quistial *et al.,* 2020), and Algeria (61.07%, n=131) (Ziam *et al.,* 2022). Similarly, prevalence of GI parasites in free-ranging dogs are variable; for example, Bangladesh (95%; n=60) (Das *et al.,* 2012), India (99%; n=101) (Traub *et al.,* 2014) and (90.7%; n = 108) (Sudan *et al.,* 2015), Nepal (95.7%; n=332) (Adhikari *et al.,* 2023), South Africa (82.5%; n=240) (Mukaratirwa & Singh, 2010), and Vietnam (55.5%, n=200) (Ng-Nguyen *et al.,* 2015). The prevalence of GI parasitic rates in dzos has not been reported elsewhere; however, similar species, yaks, have been studied. For example, the GI parasitic rates in yaks ranged from 1.3%-82.5% (n=40-733) in China (Qin *et al.,* 2019; Chen *et al.,* 2022).

It is hard to explain such discrepancies in the prevalence rates among different hosts. However, many factors such as geographical and environmental variations, types of hosts, their breeds and characteristics, history of antiparasitic treatment and its routine strategies, study design, samples, sample collection techniques, sampling types and design, microscopic

assay tools and techniques, and other unknown variables can play a role (Soulsby, 2012) (Uiterwijk *et al.,* 2019) (Adhikari *et al.,* 2023, 2024). Among these variables, coproscopic techniques have a direct role in parasite detection efficiency. For instance, the employment of different coproscopic techniques can produce a variety of results (Kotwa *et al.*, 2021). In this context, the direct wet mount technique may be less specific; however, the modified Ziehl-Neelsen technique produces a high specificity result for coccidian parasites. Even, the modified Ziehl-Neelsen technique is less sensitive compared to ELISA and PCR techniques; the former is more advantageous as it indicates only the active *Cryptosporidium* infection. Therefore, in the absence of gold-standard techniques, staining methods will be the best option for *Cryptosporidium* detection.

Several underlying causes of high prevalence and parasite loads in the currently studied population exist. First, Upper Humla is a rural area with limited access to healthcare facilities, including veterinary care. This lack of infrastructure for regular medical care and regular and timely deworming for humans, livestock, and dogs, could contribute to the spread of disease among these hosts. In contrast to developed countries where authorities and people engage in preventive measures and seek veterinary care when needed, this is usually neglected in poor and less developed areas. Dzos' feces are useful to make fuel and manure. Dogs are free-ranging, and with frequent movement of people and livestock in these municipalities, dogs represent excellent health threats. This is also evidenced by the presence of seventeen species of GI parasites in free-ranging dogs compared to owned dogs and dzos which had seven and nine GI parasites in the current study.

Secondly, both Namkha and Simkot areas have a lack of management of owned dogs and free-ranging dogs, without proper sewage and fecal management, allowing dogs to pose a

294 great health threat to people and livestock. Thus, free-ranging dogs are important bridge hosts
295 in the current study sites among domestic pets, livestock and the nearby wild faunae. For
296 example, free-ranging dogs are usually in contact with wildlife, including jackals, foxes, and
297 wild cats such as snow leopards. In addition, they are also in contact with pet dogs. Although
298 owned dogs are often confined to homes during the day, they are free to roam at night.
299 During nocturnal excursions, they may encounter free-ranging dogs, potentially spreading
300 parasites between these two rival groups. Existence of a domestic and sylvatic cycle of
301 tapeworms and other infestations is related to wild carnivores (Abdybekova *et al.,* 2012)
302 (Jannat *et al.,* 2020), indicating a critical zoonotic risk factor.

303 It is important that such sharing of common niches and direct contact in the grazing
304 environments of livestock like dzos can result in the spread of parasites. The roles of dzos in
305 GI parasite spread must be addressed in this setting. Although the dried form of dung is
306 usually used as fuel and manure, their handling during drying processes or distribution in the
307 fields may pose possible GI parasite transmission. For example, the shared GI parasites
308 recorded in free-ranging dogs and owned dogs were *Entamoeba* spp., ascarid worms,
309 *Cryptosporidium* spp., *Eimeria* spp., *Taenia* spp., and *Neospora caninum* in this study.
310 Moreover, dzos have shared these parasites, indicating a route of GI parasite transmission
311 exists in the study area.

312 *Cryptosporidium* spp. are major coccidian parasites up to three mixed species of other
313 GI parasites shared by three hosts. It suggests that these coccidian species infect all three
314 hosts with high intensity. The presence of 12 species of *Cryptosporidium* spp.
315 species/genotypes in yaks have been reviewed, for example, *C. parvum*, *C. hominis*, *C.*
316 *ubiquitum*, *C. bovis*, *C. ryanae*, *C. baileyi*, *C. andersoni*, *C. canis*, *C. struthionis*, and *C.*

317 *xiaoi* with former three species with zoonotic potential (Ryan *et al.,* 2021; Geng *et al.,* 2021).
318 In these contexts, these coccidia can be zoonotic sources for nearby hosts via rainfall-
319 mediated transportation of infected dung in high altitudes.

320 Regarding mixed infection, the current dzo population had a higher GI parasitic load.
321 For example, pentuplet parasitic infection was higher than quadruplet, duplet, triplet,
322 sextuplet and septuplet GI parasitic infections. Although the pathogenic consequences of this
323 heavy infestation of GI parasites cannot be ignored, multiple coinfections may also lead to
324 positive, negative, or null effects. The effect may mainly occur by altering host susceptibility
325 by one parasite to other parasitic species (Viney & Graham, 2013). For example, helminth
326 infections can downregulate host resistance to *Mycobacterium tuberculosis*, HIV, and
327 *Plasmodium* spp. (Salgame *et al.,* 2013) as well as to vaccine immunity for BCG (Elias *et al.,*
328 2001) and TT (Sabin *et al.,* 1996). However, polyparasitized cats contained
329 reduced *Toxocara* loads (Serrano & Millan, 2014) indicating negative interactions by mixed
330 infections. In null effects, there will be no effect of one parasite to others. Further studies
331 should be conducted on how this coinfection affects the dzos and associated interacting hosts
332 at high altitudes in the future. Notably, out of 1415 human pathogens, 62% have been
333 indicated as of the zoonotic origin (Billinis, 2013) and wild animals act as principal reservoirs
334 for many zoonoses to domestic animals and humans (Thompson, 2013). Therefore,
335 understanding the interactions of whole intestinal pathogens in those population, including
336 feral dogs and domestic animals like dzos, will provide guideline of management of spillover
337 mechanisms.
338

Conclusions

This is the first study documenting GI parasites in dogs and dzos in Humla. This microscopic study documented intestinal parasites in dogs and dzos in the high altitudes of Nepal, representing HKH regions predominant for GI parasitic infections. The hosts documented in this study shared GI parasites, including *Entamoeba* spp., ascarid worms, *Cryptosporidium* sp., *Eimeria* spp., and *Taenia* spp. These GI parasites are zoonotically critical and may be transmitted among the associated human population, although further studies on how these GI parasites exist, survive, spread, and cause pathologies, and their epidemiology in complex landscapes such as the current study area of HKH should be investigated in future.

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