

Prevalence of Gastrointestinal Parasites in Free-Range Yaks of North-Western and Central Bhutan

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Abstract

In ruminants, including yaks, gastrointestinal (GI) diseases pose significant challenges to the health and productivity of animals, leading to substantial economic losses for farmers, especially in areas with poor grazing conditions. This cross-sectional study aimed to determine GI parasites in free-range yaks from Laya (west) and Saephu (central) block of Bhutan during winter. A total of 166 fresh faecal samples were collected from yaks (west, 80; central, 86) across 28 herds (14 from each region). These samples were analysed to examine the presence of GI parasites, followed by face-to-face interviews with herders related to animal health care practices and services. The study found that the majority of the faecal samples from yaks (west, 96.25%; central, 97.67%) were infected with GI parasites. Three GI parasites (*Strongyloides* sp., coccidia and *Trichuris* sp.) were detected in the faecal samples of the yak in both the regions and two more GI parasites (*Strongyles* sp. and *Balantidium coli*) were also detected in yaks of the central region. More than half of the faecal samples (west, 82.89%; central, 75.0%) showed multiple parasite infections. The burden of GI parasites in the faecal samples of yaks in the central region (median, 900 epg) was significantly higher than the yaks in the west (median, 500 epg). Moreover, a negative relationship between burden of GI parasites and frequency of deworming could effectively control GI parasites burdens in yaks.

Keywords: gastrointestinal parasites, burden, yak, free range, Bhutan

Introduction

Yak (*Bos grunniens*) is concentrated in the high altitudes of Eurasia ranging from 2500 to 5000 meters above sea level (masl) of Afghanistan, Bhutan, China, India, and Nepal, which are harsh for agricultural farming. In these conditions, millions of pastoralists rely on yaks for all kinds of products and services

(food, draught, fur and hides, manure, fuel) to sustain their livelihoods (Derville & Bonnemaire, 2010; Dorji et al., 2020). Bhutan has yak-based transhumant communities; however, these communities are under pressure due to several factors, such as forage and labour shortage, yak mortality, and limited markets to sell the yak products (Dorji et al., 2020). These transhumant communities migrate with their yaks between summer (5000 masl) and winter (2500 masl) rangelands to avoid harsh weather conditions and forage shortages.

Yak mortality is one of the main concerns to yak-based communities because yak is their main source of livelihood. Previous re-

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searchers have identified several contributors to yak mortality, including wild predators (Sangay & Vernes, 2008), water poisoning, forage shortage and accidents (Dorji et al., 2020). However, GI parasites have not been thoroughly examined. In Bhutan, several factors likely promote the transmission of GI parasites among yaks and other ruminants. These factors include the free-range and migratory rearing systems of yaks, which put them at high risk to GI parasites, especially when they interact with other infected ruminants (Pal et al., 2015; Angell et al., 2018). In fact, most Bhutanese cattle farmers allow their animals to graze freely rangelands or nearby forests, potentially favouring the transmission of GI parasites among the animals (Tshering & Dorji, 2013). Besides, yak herds are often dispersed, underprivileged, and located in the most remote parts of the country, where herders may have limited access to animal health care services (Derville & Bonnemaire, 2010). Consequently, infected animals may be left unattended, leading to further spread of parasites to healthy yaks and other animals. Furthermore, herders may be unaware of the impact of GI parasites have on their livestock, resulting in infested yaks being unattended (Shrestha & Bindari, 2011).

Yaks infected with the GI parasites can cause substantial losses to yak-based families, discouraging them from continuing yak farming. The decline in the number of yak-based families has become a concern to the Bhutanese government. While there are report of the prevalence of the GI parasites infection in yaks from India Himalayan (RangaRao et al., 1994; Bandyopadhyay et al., 2010; Bam et al., 2012), Nepal (Acharya et al., 2016; Angell et al., 2018) and China (Qin et al., 2019), no studies have been conducted, nor evidence collected to estimate prevalence of the GI parasites in yaks of Bhutan. The GI parasites infect animals of all ages, potentially leading to high morbidity and mortality of animals (Acharya et al., 2016; Qin et al., 2019) and economic losses for farmers, especially in areas with poor grazing con-

ditions. Therefore, this study aims to explore and assess the prevalence of GI parasites in free-range yaks in Bhutan, which can guide animal deworming efforts.

Materials and Method

Study area

The study areas included two blocks, Laya in the Gasa district (28°04'00.00 N, 89°41'00.00 E) and Saephu in the Wangdue Phodrang district (27°29'10.14 N, and 89°53'56.94 E), both located over 3000 meters above sea level. The average maximum summer temperature is 17°C, while the average minimum winter temperature is 10°C. In Bhutan, blocks are local administrative units consisting of several villages. Both of these blocks are located within declared protected areas, with Laya in Jigme Dorji Wangchuck National Park and Saephu in Wangchuck Centennial National Park.

Yak herds in these regions are typically about 1 – 10 days walk from the permanent settlement (village). Laya (western region) and Saephu (central region) (Figure 1) were purposefully selected for the study because of differences in livestock grazing practices. For example, yaks of Saephu shared common rangelands with cattle and other ruminants in winter, unlike in Laya. Furthermore, Saephu is connected with a motorable road, whereas Laya is about 5 – 6 hours' walking distance from the nearest road. For the sake of convenience, the names of the regions will be used instead of the names of the blocks.

Data collection

A cross-sectional study was carried out, focusing one yak herds meeting specific criteria: each herd owning more than 20 animals and located within herds located within a 4-hour walking distance from the livestock extension centre. A total of 28 herds were selected for the study, evenly distributed between the western ($n = 14$) and central ($n = 14$) regions. Faecal samples were collected from these herds, followed by face-to-face interviews with yak

herders using a semi-structured questionnaire. The interviews were conducted in the Bhutanese national language, which had been translated from English by a qualified local translator. The semi-structured questionnaire gathered information on various aspects of animal healthcare management practices, including, yak mortality, accessibility to animal health care services, deworming practices, awareness of GI parasites.

The herds were visited from December 1, 2022 to January 15, 2023, during the winter season then they were easily accessible. A total to 166 faecal samples were collected, with 80 from the western region and 86 from the central region. Samples were collected from different age groups: calves (0 – 2 years old), heifers (3 – 4 years old) and adult yaks (> 4 years old). During the visits, faecal samples were collected from animals that were available at the time. However, bulls (both breeding and castrated) could not be sampled because they freely graze on rangelands 24 hours a day, 7 days a week and were not gathered by herders. Faecal samples were collected either by directly swabbing from the animal's rectum using disposal sterile plastic gloves (approximately 30 g per sample) or by collecting freshly defecated faeces from the ground immediately after observation.

To preserve the samples, each faecal sample was individually sealed in sterilised vials containing about 2 ml of 10% formalin. These vials were labelled with unique IDs indicating the date of collection, location age of the yak, herd name, and serial numbers. The samples were then placed in ice boxes and stored in a refrigerator at 4°C. They were transported to the National Centre for Animal Health (NCAH) laboratory as soon as possible for examination of GI parasites.

Coprological examination

The faecal samples collected were examined for GI parasites using both sedimentation and Stoll's dilution techniques, following the protocol of NCHA laboratory. GI parasitic eggs/

larvae were identified microscopically based on their morphology (Figure 2).

The sedimentation technique was used specifically to detect trematode eggs, which are heavier than the nematodes in faecal samples. In this process, approximately 3 g of each sample were mixed with 45 ml of water and was stirred thoroughly using a fork. The mixture was then filtered through cheesecloth and the filtered materials were transferred into a test tube. After allowing the sediments to settle for 5 minutes, the supernatant was removed using a pipette. To the sediments, 5 ml of water was added and was allowed to settle for another 5 minutes. The supernatant was again removed using a pipette, and a drop of methyl blue was added to the sediment. The sediment was then transferred to a microscope slide, covered with a cover slip, and examined under a microscope.

In Stoll's technique, 3 grams of each sample were added in a sterilised beaker containing 45 ml of water. The mixture was stirred until the faecal matter turned into a slurry, which was then poured through a tea strainer to remove debris particles. The filtrate of faecal samples was further stirred using a fork. Using a graduated pipette, 1.5 ml of the filtrate was drawn and was placed on a microscope slide, covered with a coverslip, and examined under 40 x magnification.

Based on specific characteristics of GI parasite, the total number of eggs/larva present in the 0.15 ml of diluted faecal sample was multiplied by 100 to determine the number of eggs per gram (epg) in the original sample. The prevalence of GI parasites was calculated as the ratio of the number of yaks found positive for at least one parasite to the total number of yaks sampled. Likewise, the prevalence of GI parasites was also estimated separately by species.

Data analysis

The data were initially entered into Excel, which were then exported to R-Studio (version 3.5.0) for further analysis. Numerical data such as age of respondents, herd size, epg were

checked for normality using the Shapiro-Wilk test. The respondents' awareness of GI parasites in yaks, sources of information on GI parasites, effects of GI parasites on yaks, and deworming practices were displayed in percentages. Median values were used to summarize GI parasite burdens within herds, given that the data deviated from normality. The burdens of GI parasites were categorised as mild (< 500

epg), moderate (500-2000 epg) or heavy (> 2000 epg) as per the study of Obonyo et al. (2012).

A Mann-Whitney U test was performed to compare the burdens for GI parasites between the regions at $p < 0.05$. Furthermore, a Pearson correlation was used to determine the relation between GI parasites burden and the frequency of deworming practices in animals.

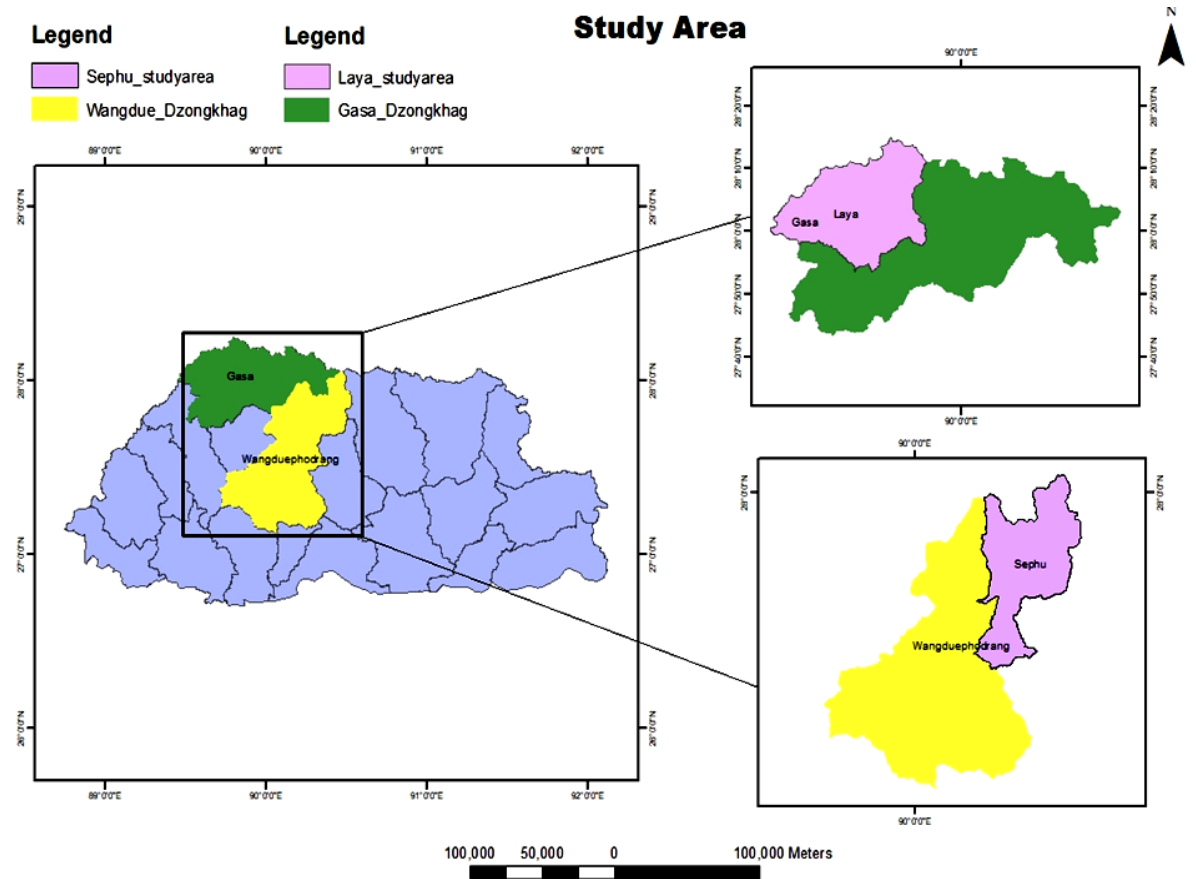


Figure 1. Location of two study areas in Bhutan

Results

Respondent awareness of the GI parasites

All respondents reported that they had experienced yak mortality in the preceding year (December 2021-November 2022). Several factors such as wildlife predators, cold and bad weather conditions, accidents, parasites and diseases were said to have caused 179 yaks deaths (60 in the western region and 119 in the central region).

Over half of the respondents indicated awareness of GI parasite infections in yaks,

with 78.57% in the western region and 35.71% in the central region acknowledging this issue, while the remainder were not aware of it. Based on these findings, there is a recommendation for continued governmental support to raise awareness among yak herders and provide support in combating these parasites. Some of the sources of information regarding the parasites in yaks expressed by the respondents included extensionists, social media, and friends and relatives.

All respondents of the western region reported that they dewormed their yaks at least

once (14.29%), twice (35.71%), thrice (14.29%) or four times (35.71%) per year. On the contrary, in the central region, a minority (28.60%) dewormed their animals once a year, while the majority did not engage in deworming practices. Respondents in both regions administered anthelmintic drugs to yaks either by using an oral drenching (85.71% in the west, 100% in the central) or by mixing drugs with animal feed (14.29% in the west). Also, all respondents of the two regions opined that the yaks infected with GI parasites negatively affected the health of animals (animals become weak, suffer diarrhoea and lose appetite) and productivity.

Prevalence of the gastrointestinal parasites

The majority of the faecal samples (west, 96.25%; central, 97.67%) showed presence of the GI parasites in yaks, highlighting welfare concern across all herds assessed. The high prevalence of GI parasites prevalence in animals allowed to graze freely is probably due to

species of the GI parasites (*Strongyloides*, coccidia, *Trichuris*, *Strongyles* and *Balantidium coli*) were identified in the faecal samples of yaks from Bhutan (Figure 2). Three GI parasites *Strongyloides* sp. (west, 91.66%; central, 95.34%), coccidia (west, 85.0%; central, 66.28%) and *Trichuris* sp. (west, 8.33%; central, 4.65%) were detected in yaks from both regions. In addition, *Strongyles* sp. (6.97%) and *Balantidium coli* (24.42%) were identified exclusively in yaks from the central region, and not in the west. In addition, more than half of the samples (west, 82.89%; central, 75.0%) had multiple GI parasites infection (presence of two or more parasites) and the rest with a single infection (Table 1). In this study, no tapeworm eggs were found in the faecal samples collected, despite the fact that some herders kept dogs.

The overall burden of GI parasites in yaks of Bhutan ranged from mild to heavy infections (Figure 3). Moreover, the median GI parasite burden in faecal samples from yaks in the

Table 1. Types of gastrointestinal parasites infection in yaks (%)

Parasites species	West (n = 80)	Central (n = 86)
<i>Strongyloides</i> sp.	11.84	23.81
Coccidia	5.26	1.19
<i>Strongyles</i> sp.	0	1.19
<i>Strongyloides</i> sp.+ <i>B. coli</i>	0	5.95
Coccidia+ <i>B. coli</i>	0	1.19
<i>Strongyloides</i> sp. + Coccidia	75	46.43
<i>Strongyloides</i> sp. + <i>Strongyles</i> sp.	0	1.19
<i>Strongyloides</i> sp. + Coccidia + <i>Trichuris</i> sp.	7.89	0
<i>Strongyloides</i> sp. + <i>Strongyles</i> sp. + <i>B. coli</i>	0	1.19
<i>Strongyloides</i> sp. + Coccidia + <i>B. coli</i>	0	13.1
<i>Strongyloides</i> sp. + <i>Strongyles</i> sp. + <i>Trichuris</i> sp.	0	1.19
<i>Strongyloides</i> sp. + Coccidia + <i>Trichuris</i> sp. + <i>B. coli</i>	0	2.38
<i>Strongyloides</i> sp. + <i>Strongyles</i> sp. + Coccidia + <i>B. coli</i>	0	2.38

their increased exposure to the parasite eggs and larvae present in contaminated forage, often shared with other infected animals. Five

central region (900 epg) was significantly higher than that in the western region (500 epg) at a significance level of $p < 0.05$. In the

western region, the majority of faecal samples showed mild infections with *Strongyloides* sp., whereas more than half of the samples from the central region showed moderate infections (Figure 3). In addition, more than half of the samples showed mild infections with coccidia (85.29% in the west, 68.42% in the central), with the remainder showing moderate infections. Also, three GI parasites (*Trichuris* sp., *Strongyles* sp. and *B. coli*) infections in the central region were all mildly infected. Overall, the GI parasite burden in Bhutanese yaks was moderate, with

a median of 700 epg, ranging from a minimum of 100 epg to a maximum of 2800 epg. In the central region, the burden of *Strongyloides* sp. (median 700 epg, range 100-2,000 epg) and *Coccidia* sp. (median 300 epg, range 100-1,200 epg) was significantly higher compared to yaks in the west (*Strongyloides* sp. and coccidia both at 300 epg) at $p < 0.05$, though this difference was not observed for *Trichuris* sp.. A negative correlation was observed between the frequency of deworming practiced by herders and the burden of GI parasites in yaks ($r_{(26)} = -0.61$, $p < 0.01$), indicating that more frequent anthelmintic treatment could benefit the health of animals.

Discussion

Understanding the prevalence of GI parasites in yaks is crucial for preventing transmission and benefiting farmers. This study represents the first-ever assessment of GI parasites in yaks of Bhutan. In this study, we found that more than half of the respondents had not heard about the GI parasites in yaks. Providing continued awareness on GI parasitism to yak herd-

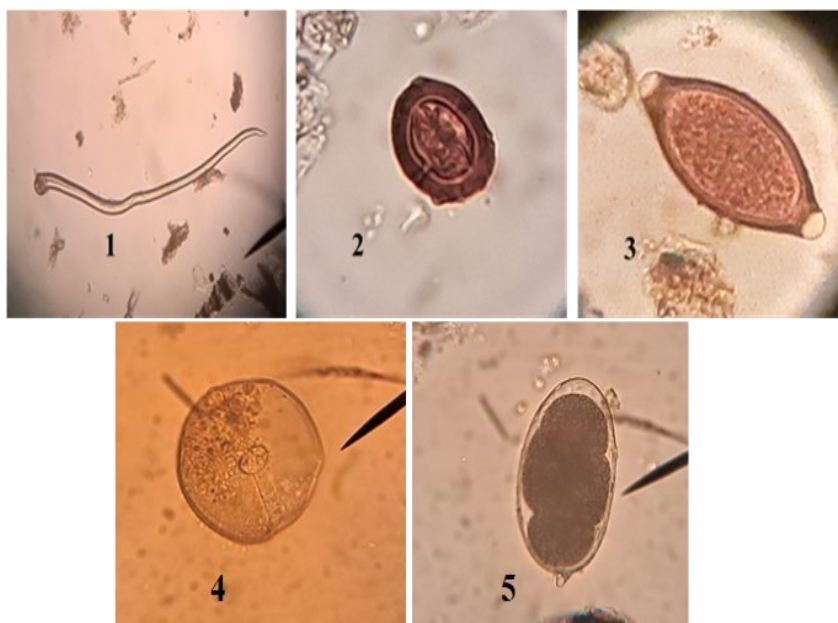


Figure 2. The gastrointestinal parasites detected in the yak faecal samples. 1, *Strongyloides* sp. (larvae); 2, coccidia (ova); 3, *Trichuris* sp. (egg); 4, *Balantidium coli* (egg); 5, *Strongyles* sp. (egg).

ers and other livestock farmers (who do not own yaks), for example, observing the animals, treating the livestock animals and dogs, probably is one of the most successful control strategies to prevent outbreaks of diseases in the community.

The majority of the faecal samples showed presence of GI parasites in yaks, which was close to GI parasites prevalence in the yaks of Nepal (Shrestha & Bindari, 2011; Acharya et al., 2016). However, the high prevalence of GI parasites in this study was higher than those GI parasites found in yaks of Indian Himalayas (Bam et al., 2012; Goswami et al., 2013; Pal et al., 2015) and China (Qin et al., 2019). The prevalence of GI parasites in yaks differed among several studies as a result of combination of several favourable factors for parasites to thrive and proliferate, such as herd management practices (e.g., feeding/grazing conditions, deworming practices, and persistent nutritional stress), climatic conditions (humidity, temperature), sampling seasons and size, and animal health conditions. For example, the poor body condition of animals was detected with higher GI parasites

prevalence than the animals with ideal body condition (Tiele et al., 2023; Terfa et al., 2023). Previous investigators also recorded the highest positive for protozoa and helminth infections in yaks during spring and summer and the least during winter (Bam et al., 2012), which indicates that the season for collection of samples for GI parasites examination would determine the presence of it. Such observation of high prevalence of parasites in yaks during spring to summer could be explained by high humidity which is conducive for egg/larvae of parasites to survive on the pasture (Colwell et al., 2014). Moreover, yaks allowed to graze within the farm-managed rangelands (i.e., limit mixing of herds or ruminants of neighbours) have less probability to get infected with parasites than those animals allowed to graze freely on communal range-

probably had ingested parasitic eggs along with forages, which might have contaminated by infected ruminants (e.g., cattle) sharing common rangelands in winter (Pal et al., 2015; Angell et al., 2018). In fact, Khan et al. (2009) showed that the mixing of different ruminants increases chances to be infected with parasites than solitary animals. Although faecal samples of cattle from the central region were not collected to confirm the presence of *Strongyles* sp. and *B. coli*, however, *B. coli* was reported in the ruminants of Pakistan (Bilal et al., 2009) and *Strongyles* sp. in the cattle of Ethiopia (Tiele et al., 2023). We also found that more than half of the samples had multiple GI parasites infections. The findings of this study were comparable to the findings of Byanju et al. (2011), who observed that yaks of Nepal had multiple para-

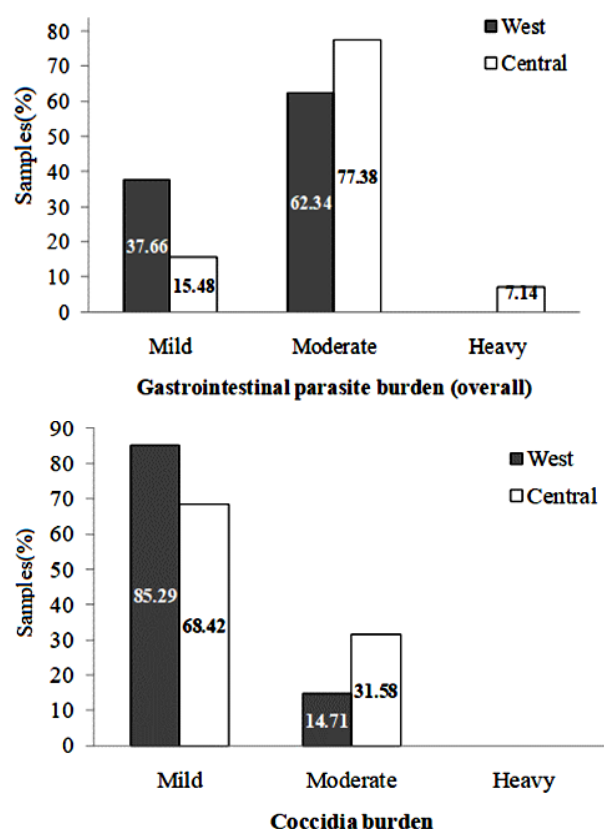


Figure 3. The gastrointestinal parasites burden detected in yaks

lands (Bandyopadhyay et al., 2010; Pal et al., 2015).

The prevalence of *B. coli* and *Strongyles* sp. in the central region could be because yaks

site infections. This result probably suggests that animals with one parasite species infection may also facilitate multiple infections (Win et al., 2020). Furthermore, animals with multiple parasite infections have suppressed immune systems making them more vulnerable to diseases (Wang et al., 2006) and are associated with high morbidity and low productivity (Bersissa et al., 2010).

Overall, GI parasites burden of Bhutanese yaks was moderately infected, which was higher than the yaks of Nepal with 310 epg (Acharya et al., 2016) and India with 43.86 epg (Pal et al., 2015). Region-wise comparison showed that yaks of the central region had comparatively higher *Strongyloides* sp. and

coccidia burden than the yaks of the western region. The yaks of the central regions shared common rangelands with cattle in winter (cattle might be infected with GI parasites), which partly explains the high burden of GI parasites in this region. Another plausible explanation could be that a few herders dewormed their animals once a year because herders are discouraged to travel long distance to receive the anthelmintic drugs (Dorji et al., 2020) and/or herders might be ignorant of the GI parasites (central, 64.29%). This finding was confirmed by the negative relation between deworming frequency practiced by herders and GI parasite burdens. A previous researcher found that dairy cattle given anthelmintic drugs four to six times a year had lesser GI parasites burden compared to those animals provided drugs once a year (Kouam et al., 2021). In essence, animals will benefit from anthelmintic treatment because the presence of parasites means the host shifts its energy to develop immune system instead of using it for growth and milk production. Besides, when these infected yaks are allowed to freely graze on summer rangelands often sharing with the wild herbivores such as blue sheep (*Pseudois nayaur*), and these would infect and threatened predators such as snow leopard (Hewavithana et al., 2022).

Conclusion

The majority of the yaks in western and central Bhutan were infected with the gastrointestinal (GI) parasites, and most of the yaks had multiple GI parasites infections. However, yaks of the central region had higher GI parasites burden than the yaks of the west. The study showed a clear that a negative relationship between frequency of deworming of yaks and the GI parasite burdens recommend for better and more accessible animal health care

services. Based on these findings, deworming of yaks at regular intervals (e.g., four times a year) is recommended to reduce the GI parasites infection in yaks that might improve the health of yaks. However, the frequent use of anthelmintics could also lead to development of GI resistance. The study also suggests the government (e.g. animal health workers) to provide continued awareness on the GI parasitism to yak herders and other livestock farmers (who do not own yaks), and also on the benefits of applying strategies to prevent outbreaks of parasites and to treat the animals.

Limitations

The limitations of the study are as follow:

1. Family owning less than 20 yaks were selected and those herds of more than 4 hr walking distances from the livestock extension center were excluded.
2. A little selection bias might be introduced because faecal samples of animals available during the time of the visit were sampled. Also, the sample needs to be collected from different regions across different points in time / seasons, which probably would provide overall status of parasites in yaks of Bhutan.
3. We used specific diagnostic techniques such as, sedimentation and Stoll's techniques, which probably could not detect entire parasitic species in yaks. In future study, there is a need for integration of comprehensive methods enhance accuracy to detect parasites.

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