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Diversity, Prevalence, and Risk Factors of Gastrointestinal Parasites in Sheep Herds Grazing in Upper Dir district, Northern Pakistan

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Abstract

IN DEVELOPING countries parasitic diseases caused by intestinal parasites pose a significant challenge to livestock production. Therefore, this study focuses on evaluating the incidence rate of gastrointestinal parasites (GIP) in grazing herds of sheep in Northern Pakistan. Fresh fecal samples were collected from herds of Hastnaghari breed (n = 91), Rambouillet cross breed (n = 88) and non-descript sheep (n = 121) from November 2020 to May 2021. Samples were examined for isolation and identification of GIP by direct smear method, fecal floatation and sedimentation techniques. The overall prevalence of GIP observed was 64% out of which 53.6% sheep were harbouring single parasite species, whereas, 46.4% had multiple parasite species. Effect of sex (P = 0.00), age (P = 0.024), housing system (P = 0. 035) and the use of anthelmintic (P = 0.002) were observed as significant sources of variation in the incidence of GIP. Likewise, GIP were significantly prevalent more in ewes (65.8%) than rams (61.1%), young sheep (< 1 year; 82.80%) than adult sheep and in sheep reared in "Kaccha" housing system than reared in Pakka houses. Likewise, in sheep that did not receive anthelmintics, the prevalence was significantly higher (92.7%) than those received (7.3%). Specie-wise analysis of the GIP revealed higher (P = 0.003) incidence rate for *Haemonchus* (28.2%), *Eimeria crandallis* (23.3%) and *Moniezia* species (13.6%). The *Haemonchus* + *Trichuris* category was more prevalent (47.0%) in double parasitic infection, while *Haemonchus* + *Strongyloides* + *Moniezia* category infection was more (52.2%) prevalent in triple infection. The high prevalence of GIP in sheep poses a serious threat to the small ruminant industry in Northern Pakistan. Proper control measures keeping in view of the contributing factors and awareness of the farmers are therefore recommended to optimize farmer's income from sheep rearing.

Keywords: Gastrointestinal parasite, *Haemonchus, Coccidia,* Sheep, Upper Dir, Northern Pakistan.

Introduction

Sheep product consumption, including meat, milk, and milk products, has seen a global increase over the past few decades. In 2018, global sheep milk production was 10.4 million metric tons, with an expected rise to approximately 2.7 million metric tons and a 26% increase by 2030 [1]. Pakistan, with a substantial sheep population of 31.6 million, holds the 11th position in global sheep production [2]. Small ruminant farming plays a crucial role in providing food security and livelihood, particularly in arid and semi-arid regions of the country [2,3]. Small ruminant farming, including sheep, is extensively practiced in grazing-based production systems in the tropical arid regions of Pakistan [4]. The use of good quality forages and concentrates is common to supplement grazed-based diets [5]. Gastrointestinal (GI) parasitic infestation, especially in subsistence production systems in the tropics, poses a significant threat to the health of small ruminants. Parasites can have detrimental effects on the productivity and well-being of sheep [6].

Parasitic infections especially GI nematodes, cestodes, and trematodes can cause subclinical and clinical parasitism in livestock species. These parasites frequently pose a serious health threat and reduce livestock productivity [7, 8]. Notably, the presence of GI parasites in animals can lead to heavy economic losses in terms of production (like poor weight gain, reduced milk production, and wool quality), blood loss, impaired reproductive performance, and depression of appetite [9, 10].

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Therefore, the accurate management and judicious use of anthelmintics as key pillars of parasite control in small ruminants. However, it also highlights a significant challenge in the form of anthelmintic resistance (AR) due to extensive and indiscriminate use [6].

The development of AR is a serious animal health problem and has a major economic impact worldwide, leading to production and financial losses [11, 12]. The persistent use of anthelmintics remains a primary choice for prophylaxis in farm animals, particularly in the context of controlling parasitism among small ruminants. Additionally, it addresses the significant economic impact associated with parasitism control in developed countries, with specific cost estimates for helminth infections in various livestock categories. For example, annual estimated costs of helminth infections in dairy cattle were € 941 million, in beef cattle were € 423 million, in dairy sheep were € 151million, and in dairy goats were € 86 million [13].

Importantly, the incidence of GI parasites in ruminants varies according to local climatic conditions like humidity, temperature, precipitation, vegetation, and management approaches [14]. These factors have a great impact on the prevalence and severity of numerous endo-parasitic infections. Therefore, this study was conducted to instigate the endo-parasitic profile of sheep in Northern Pakistan and assess the potential risk factors under the traditional grazing-based production systems.

Material and Methods

Study area

This study was conducted in the Upper Dir district, an administrative unit in Northern Pakistan. Upper Dir covers an area of 3,699 km² and is situated at 35° 9′ latitude and 71° 52′ N longitude. The district is a mountainous area characterized by a prolonged winter season. During winter, the mean minimum temperature is -2.3 °C, while the mean maximum temperature is 11.2 °C. The average annual rainfall in the area ranges from 1,000 to 1,600 mm. Three different sites, namely Kair-Dara, Barawal Banday, and Patrak, were selected for this study (Figure 1).

Study population

This study involved 300 sheep including 91 Hastnaghari breed, 88 Rambouillet cross and 121 local non-descript sheep. All the sampled animals were apparently healthy and in good body condition at the time of sampling.

Collection of fecal samples

From November 2020 to May 2021, each fecal sample (~ 10 g) per sheep was directly collected from the rectum [15]. The samples were immediately transferred to pre-labeled, clean plastic bottles. The sampling bottles were stored in screw caped container with ice packs and transported to the laboratory. All samples were analyzed for detection of endoparasites within 24 h of collection [16]. Data on breed, sex, age, parity status, herd size, deworming status and housing condition, date and place of sampling were recorded for each sampled sheep at the time of sampling.

Parasitological examination

Fecal samples were processed and examined by direct and indirect parasitological technique (centrifugation floatation and sedimentation) according to [16]. The GI parasites were identified using identification keys described by [17] and [18]. Briefly, fecal materials (1 g) were mixed with 0.9% normal saline wet mount solution in a mortar and relatively homogenous preparation was obtained. The suspension was then filtered through a tea strainer. Finally, a drop of suspension was added on a glass slide and was examined under microscope. From each fecal sample, three direct smears were examined.

To examine sample in floatation process, 2 grams of feces was put in sterile screw caped bottle, containing 5 ml of the saline solution, was mix, and strained through a sieve. The mixed matters were riddled into a centrifuge tube or a walled test tube. Flotation solution was added to the test tube until a convex meniscus was formed. A cover slip was positioned on the top of test tube carefully and left for 5 minutes. The cover slip was removed from the glass tube and placed on the slide and was tested for helminths eggs and oocysts under microscope at 10 X.

In sedimentation method, 2 g of feces was mixed in 50 ml water and sieved. The suspension was centrifuged at 1000 rpm for 2-3 minutes with a solution of sodium chloride (NaCl). The supernatant was decanted and with Pasteur's pipette, a drop from sediment was obtained and then placed on clean slide. Finally, the slide was examined for the presence/absence of endoparasite under microscope at 10 X.

Statistical analysis

The data was presented in percentile. Statistical Package for Social Sciences (SPSS 16.0 software, Inc., Chicago, USA) was used for data analysis, using the Chi-square test.

Results

Data showing effects of sheep breed, sex, age, parity, herd size, housing and deworming status on the prevalence of GI parasites is presented in Table 1. The overall prevalence of GI observed in this study was 64%. Out of the total positive cases, the incidence of single parasitic infection was higher (53.6%) than multiple parasitic infections (46.4%) but the difference was non-significant ($P > 0.05$). Similarly, the result of

relationships among breeds and endoparasitic infection was non-significant (P = 0.827). The ratio of parasitic infection in Rambouillet, Hashtnagri and non-descript breeds were 65.9, 64.8 and 62%, respectively. Higher (68.1%; P = 0.00) infection rate was reported in ewes than rams (61.5%). Likewise, significant relationships were found between age and endoparasitic infection (P = 0.024). Sheep 1-2 years old had higher (65.2%) rate of endoparasitic infection than 3-4 years (56.8%).

On the other hand, parity-wise observation revealed non-significant (P = 0.798) interaction between levels of parity and parasitic infection. Also, the results of endoparasitic infection did not differ (P = 0.871) due to herd size. In contrast, significant interaction (P = 0.035) was observed between endoparasitc infection and housing system. Higher (64.9%) parasitic infection was detected in sheep reared under "Kaccha" housing system. Likewise, sheep which did not receive any anthelmintic drug had higher (67.1%; P = 0.002) parasitic infection than those who had received anthelmintic drugs (20%).

Species-wise pattern of single and multiple parasitic infections was different (P = 0.003) in sheep (Table 2). In single parasitic infection, higher prevalence was reported for *Haemonchus spp* (28.2%), followed by *Eimeria crandallis* (23.3%) and *Moniezia spp* (13.6%). In double infection, *Haemonchus* + *Trichuris* infection was more prevalent (47.0%). While, in triple parasitic infection, *Haemonchus* + *Strongyloides* + *Moniezia* infection was more (52.2%) prevalent.

Discussion

Gastrointestinal parasitism is a serious and rapidly growing problem in domestic animals, particularly in sheep herds in the arid and semi-arid regions of the world [19], which holds most of the small ruminant population in Pakistan. This study was designed to explore the burden of GI parasites and to identify the potential risk factors in sheep that will help in designing control measures for GI parasites in this area. This study illustrates that 64% of samples were infected with gastrointestinal parasites (nematodes, trematodes, cestodes, and protozoans). In agreement with our findings a high prevalence rate of GI parasite in sheep has been reported by [20] (72%), [21] (53.33%), and [22] (79.24%). Discrepancy in these findings might be attributed to different ecological conditions and management practices. In addition, pattern-wise prevalence of GI parasite in the study area was higher for single parasitism. In agreement with our findings, [7] reported a high incidence rate of single parasitism in sheep. In contrast, other studies have reported a higher incidence of multiple parasitic infections in sheep [23, 14, 24]. Notably, the occurrence of multiple nematode species in a host has an additive pathogenic effect on the host, and usually, the pathogenicity is high [25]. Variations in the intensity of GI parasitic infections can be attributed to differences in grazing patterns, feeding modes, sampling seasons, and regions [26]. Moreover, the prevailing climates of the region and the overstocking of the grazing herds provide an ideal situation for the transmission of helminth parasites [21].

Generally, nematode infections were highly prevalent in the study area. Previous studies in other areas have also reported a high incidence of nematodes [27, 28, 29]. This might be because most species of nematode do not need an intermediate host during their life cycle [30]. Notably, *Haemonchus* was the most prevalent nematode reported in this study, highlighting its major role in small ruminant helminthiasis in the study area. Our findings agree with the earlier reports of [31] and [7]. The higher occurrence of *Haemonchus* in the host environment is noted for a comparatively short generation interval and their prolific egg-laying capacity (approximately 10,000 eggs per day/female) [32]. In addition, Literature reports indicate that nematodes including *Haemonchus* quickly develop resistance to anthelmintic drugs than other helminths [33]. Moreover, the climate of the study area is considered a factor that may favour the spread of *Haemonchus* larvae. The established high pathogenicity of *Haemonchus* suggests that sheep farmers in the study area are at a comparatively high risk of economic losses. These losses are attributed to the negative impact of *Haemonchus* infections on the productivity, overall performance and economics of the sheep farming [34, 35]. Infected sheep in the study area are found to harbour more than one species of nematode parasites. The coexistence of multiple nematode species adds complexity to the management of parasitic infections in sheep herds.

The present study demonstrated the effects of a variety of factors such as host age, sex, housing condition and use of anthelmintic drugs on the occurrence of GI parasite in sheep. In agreement with our findings, [36] and [37] reported that prevalence of gastrointestinal parasite infections are higher in rams than in ewes. The results of the present investigation have revealed the highest prevalence of parasitic infections in younger age (< 1 year) groups of sheep. Earlier studies also reported that susceptibility and pathogenicity of nematode infections were greater in young animals than in mature animals [36, 37, 38]. It might be attributed to the fact that adult animals may acquire immunity to the parasites through frequent challenges as compared to young animal [39, 40]. The present study also revealed that the application of anthelmintic drugs in small ruminants could significantly diminish the incidence of helminths parasite. Keeping in view the present findings, necessary steps for control, cost-effective strategies, and awareness about GI parasites among farmers should be taken promptly to improve the productivity of these animals.

Conclusion

The results of this study showed that host sex, age, housing condition, and anthelminthic drugs are vital features, which affect the prevalence rate of gastrointestinal parasitic infection in sheep in Upper Dir. Moreover, the *Haemonchus* species was the major contributors to small ruminant helminthiasis in the study area. Keeping in view the present findings necessary steps for control, cost-effective strategies and awareness about GI parasite among the farmers should be taken in timely manner to improve the productivity from these animals.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical approval

All experimental protocols were maintained and performed according to standard ethics for care and use of experimental animals. This was based on the ethical standards protocol of the Department of Basic and Applied Zoology, Shaheed Benazir Bhutto University, Pakistan.

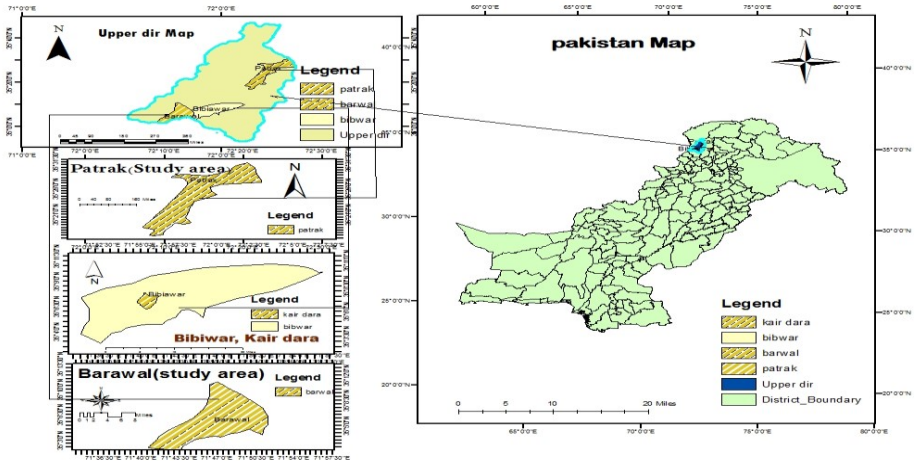

Fig. 1. Sampling sites of the study area indicating three important regions namely "Kair-Dara", "Barawal Banday" and "Patrak".

TABLE 1. The effect of breed, sex, age, parity, herd size, housing and deworming status on the prevalence of GI parasites in grazing herds of sheep in northern Pakistan.

Variables	Description	Sample size	Positive	Percentage	P-value
Breed	Hashtnagri	91	59	64.8	
	Rambouillet cross	88	58	65.9	0.827
	Non-descript	121	75	62.0	
Total		300	192	64	
Nature of infestation	Single	192	103	53.6	0.435
	Multiple	192	89	46.4	
Sex	Ewes	113	77	68.1	0.00
	Rams	187	115	61.5	
Age (Year)	1-2	244	159	65.2	
	3-4	44	25	56.8	0.024
	5-6	12	8	66.7	
Parity	Nulliparous	159	95	59.7	
	1	96	68	70.8	0.798
	2	41	26	63.4	
	3	4	3	75	
Herd size	1-5	120	83	69.2	
	6-10	127	82	64.6	0.149
	11-15	39	20	51.3	
	16-20	14	7	50	
Housing	"Kacha"	288	187	64.9	0.035
	"Pakka"	12	5	41.7	
Deworming status	Yes	20	4	20	0.002
	No	280	188	67.1	

% is calculated within each variable

TABLE 2. Overall prevalence of single, double and triple parasites in sheep*

Parasite species	No. of sheep infected
Haemonchus Spp	29 (28.2)
Coccidia Spp	24 (23.3)
Moniezia expansa	14 (13.6)
Dictyocaulus	10 (9.7)
Trichuris	9 (8.7)
Toxocara	7 (6.8)
Fasciola hepatica	3 (2.9)
Strongyloides	3 (2.9)
Ostertagia	2 (1.9)
Oesophagostomum radiatum	2 (1.9)
Haemonchus + Trichuris	31 (47.0)
Haemonchus + Dictyocaulus	13 (19.7)
Haemonchus + Fasciola hepatica	7 (10.6)
Trichuris + Dictyocaulus	10 (15.2)
Strongyloides + Coccidia	5 (7.6)
Haemonchus + Dictyocaulus + Trichuris	4 (17.4)
Haemonchus + Strongyloides + Moniezia	12 (52.2)
Dictyocaulus + Ostertagia + Coccidia	7 (30.4)

*Data showing in the parenthesis is in percentage. The percentage value has been calculated from the total number of positive fecal samples observed (n = 192)
The ratio of parasitic infection in various breeds are significant ($\chi 2 = 39.025$, df = 18, P = 0.003)

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تنوع وانتشار وعوامل الخطر للطفيليات المعوية في قطعان الأغنام التي ترعى في منطقة دير العليا، شمال باكستان

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الملخص

في البلدان النامية، تشكل الأمراض الطفيلية التي تسببها الطفيليات المعوية تحديًا كبيرًا لإنتاج الثروة الحيوانية. لذلك، تركز هذه الدراسة على تقييم معدل الإصابة بالطفيليات المعوية في قطعان الرعي من الأغنام في شمال باكستان. تم جمع عينات براز طازجة من قطعان سلالة هستنغاري(n = 91) ، وسلالة رامبوييه المهجنة (n = 88) والأغنام غير الموصوفة (n = 121) من نوفمبر 2020 إلى مايو 2021. تم فحص العينات لعزل وتحديد الطفيليات المعوية بطريقة اللطخة المباشرة وتقنيات تعويم البراز والترسيب. كان معدل انتشار الطفيليات المعوية العام الملحوظ 64٪ منها 53.6٪ من الأغنام كانت تؤوي نوعًا واحدًا من الطفيليات، في حين أن 46.4٪ كان لديها أنواع متعددة من الطفيليات. لوحظ تأثير الجنس (P = 0.00) والعمر (P = 0.024) ونظام السكن (P = 0.035) واستخدام طاردات الديدان (P = 0.002) كمصادر مهمة للتباين في حدوث التهاب الجهاز الهضمي العلوي. وبالمثل، كان التهاب الجهاز الهضمي العلوي منتشرًا بشكل ملحوظ في النعاج (65.8٪) مقارنة بالكباش (61.1٪) والأغنام الصغيرة (< سنة واحدة؛ 82.80٪) مقارنة بالأغنام البالغة وفي الأغنام التي تربى في نظام الإسكان "كاتشا" مقارنة بالأغنام التي تربى في بيوت باكا. وبالمثل، في الأغنام التي لم تتلق طاردات الديدان، كان معدل الانتشار أعلى بكثير (92.7٪) من تلك التي تلقت (7.3٪). كشف التحليل النوعي لالتهاب الجهاز الهضمي العلوي عن معدل حدوث أعلى (P = 0.003) لأنواع هيمونيكوس (28.2٪) و إيميريا كارانداليس (23.3٪) و مونييزيا (13.6٪). كانت فئة هيمونيكوس + ترايكيوراس أكثر انتشارًا (47.0٪) في الإصابة الطفيلية المزدوجة، بينما كانت فئة هيمونيكوس + سترونجبلويدز + مونييزيا أكثر انتشارًا (52.2٪) في الإصابة الثلاثية. يشكل الانتشار المرتفع للطفيليات المعوية في الأغنام تهديدًا خطيرًا لصناعة المجترات الصغيرة في شمال باكستان. لذلك يوصى باتخاذ تدابير مكافحة مناسبة مع مراعاة العوامل المساهمة ووعي المزارعين لتحسين دخل المزارعين من تربية الأغنام.

الكلمات الدالة: طفيليات الجهاز الهضمي، هيمونيكوس ، المكورات ، الأغنام ، دير العليا ، شمال باكستان..