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Gastrointestinal Parasitic Infections in Markhor Wild Goat (Capra Falconeri) at Chitral National Parks, Khyber Pakhtunkhwa, Pakistan

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Article Information

ABSTRACT

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Keywords

Chitral, Helminthes, Markhor (Capra Falconeri), National Parks

Markhor (Capra Falconeri), is notably endangered ungulate native to the northern mountains of Chitral, Pakistan, specifically within the Chitral Goal National Park and Tooshe Shaha Conservancy Area (TSC), is facing a serious threat from gastrointestinal (GI) parasites. These parasites adversely affect the health of Markhor, resulting in pathological and immunological degeneration. The conservation plan for Markhor is further complicated by climate change, introducing challenges, particularly in terms of hunting trophies. This study, conducted from October 2022 to May 2023. The primary goal was to evaluate the prevalence and distribution of gastrointestinal parasites in Markhor within the Chitral national parks. A total of n=200 fecal samples were processed and analyzed using flotation, sedimentation techniques, and the McMaster slide method to identify the prevailing parasites in these samples, 64% tested positive for various helminthes eggs and larvae. The prevalence rates for specific helminth species were identified as follows: strongyles 20%, trichostrongyles 14.5%, ostertagia 11%, haemonchus 8.5%, and trichuris 10%. Notably, 44% of positive animals exhibited coinfections. The collected fecal samples detected no eggs or larvae of trematodes (flukes) or cestodes (tapeworms). An overall protozoan infection proportion of 47.5% indicated that nearly half of the fecal samples were positive for protozoan parasites. Eimeria was present in 31.5% of the samples, while Entamoebiosis caused by Entamoeba was found in 15.5%. Gastrointestinal parasites were observed to be widely distributed among Markhor, posing a significant burden of various parasites and increasing the risks of morbidity and mortality. This research highlights the health challenges resulting from parasitic infections in the Markhor (Capra Falconeri) population as substantial contributors to poor health outcomes. It calls for collaborative efforts among stakeholders to address these challenges and ensure the long-term survival of Markhor conservation. Furthermore, it highlights the importance of preserving the natural habitat of Markhor populations in the northern Chitral region to prevent infections and habitat degradation.

INTRODUCTION

Chitral, located in the North-Western, region of the Khyber Pakhtunkhwa, Province in Pakistan, within Hindukush, and Himalayan mountains, and is a diverse population of wild goat species, including the Markhor (C. f. Cashmiriensis) a wild goat belonging to the subfamily Caprine and the family Bovidae (Schaller, G. B. (1977). First documented by Wagner (1839) and later discussed by Huffman (2004). Markhor is classified into two groups, namely the flare-horned Markhor and straighthorned Markhor, based on body characteristics and horn structure. Notably, females in both sub-species exhibit significantly smaller horns than males (Roberts, T. J. (1977). In Pakistan, seven species of Caprine inhabit the region (Roberts, T. J. (1977), including Asiatic Astor Markhor (C. falconeri falconeri), Chitral Markhor (C. f. cashmiriensis), straight-horned, Suleiman Markhor (C. f.jerdoni), Chiltan wild goat (C. a. chialtanensis), ibex (Capra sibirica), and Marco Polo sheep (O. ammonpolii). The distinctions among the three subspecies of urial (Ladakh urial, Afghan, and Punjab) are unclear, creating a similarity between Chitral and Gilgit Markhor. The International Union for Conservation of Nature (IUCN) Caprine specialists

have further classified the Markhor into two subspecies (Shackleton, D. M. (2001). The habitat of the Markhor is characterized by diverse elevations and vegetation types. Markhor typically resides in regions at lower elevations, ranging from approximately 700 to 1000 meters on the lower slopes of the Hindukush Range. During winter, they may ascend to around 2700 meters, and in summer, they can be found as high as 4000 meters in the Chitral valley (Schaller, G. B. (1977). Markhor predominantly occupies dry grassland forests (Roberts, T. J. (1969) ; Huffman, B. (2004). Markhors exhibit a preference for areas characterized by vertical slopes and cliffs with minimal precipitation (Roberts, T. J. (1969). The plant species within the Markhor's habitat vary with altitude. Below 2600 meters, dominant species include Oak (Quercus ilex). Additionally, at higher elevations, scattered wild Almond (Amygdalus sp) can be found. Ground cover is provided by grasses such as Cymbopogon and Stipa (Malik, M. M. (1981). The conservation strategy for Markhor is an initiative implemented by the government of Khyber Pakhtunkhwa, supported and executed by the local community. This sustainable management system aims to protect endangered wild animals and preserve

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their pastures, particularly focusing on the endangered Markhor species. The comprehensive conservation program, spearheaded by the government of Pakistan, has resulted in tangible benefits for the local community, livestock farmers, and wildlife, including free vaccination and treatment, awareness programs, training and skill development, direct financial incentives, and infrastructure development. Chitral Goal National Park and Tooshe Shasha Conservancy (TSC) areas, both are under the jurisdiction of the wildlife department, constitute the primary segments of the national parks in the region. These parks, situated at a one-hour drive from Chitral town, are collectively known as Chitral National Parks, divided into three valleys and featuring glaciers and springs with a cold water stream flow of 18-25 kilometers toward the east. The park's lush environment, characterized by thick forests and diverse flora including herbs, shrubs, cedar, and alpine trees, provides essential biodiversity and serves as a habitat for various wild animals, especially the endangered Markhor species. The national parks inhabit a variety of wildlife, including Kashmir Markhor, Siberian ibex, snow leopard, Ladakhi urial, wolf, and black bear. However, the Markhor population faces a threat from parasites and pathogens, with gastrointestinal parasites being particularly prevalent. Parasites are transmitted among genetically related hosts sharing grazing pastures and drinking water channels (Loukopoulos, P. et al., 2007). Gastrointestinal parasites, predominantly present in the duodenum, ileum & cecum, and large intestines, pose a significant management challenge and are a growing concern for conserving the threatened Markhor species. Initially listed as an endangered species (IUCN (1996); IUCN (2007), due to overhunting for meat and horns, the Markhor population has shown improvement according to recent government data, estimating between 6,000 and 7,000 individuals. Trophy hunting, considered a significant wildlife management strategy in many countries, has positively influenced local attitudes toward wildlife, encouraged community involvement in natural resource projects, and contributed to conservation goals. However, the Markhor's habitat is under threat due to a substantial increase in human population and changes in land use, leading to habitat shrinkage. In Chitral, Markhor inhabits coniferous forests and adjacent alpine meadows, representing the largest concentration of this species globally. While literature on the parasitic load of wildlife in Pakistan is limited, with case reports addressing a small number of animals, it is crucial to conduct regular habitat analysis and monitoring for sustainable wildlife habitat management. Despite annual monitoring of the Markhor population, habitat monitoring and assessment are currently lacking. Environmental education is recognized as a vital conservation tool, and there is a need for more scientific research focusing on parasitic diseases to protect endangered species. Parasitic infections can adversely affect health, leading to breeding abnormalities, untimely reduction in population size, and contribute to the extermination of species. Hence, it is

crucial to investigate and document the prevalence of gastrointestinal parasites among the endangered Markhor wild goat species. Simultaneously, parasitological studies are essential to comprehend the potential transmission and interaction of parasites between various species, both wild and domestic. Various gastrointestinal helminthes parasites have been documented in different ibex species, highlighting the need for such investigations (Barji, H.et al.,(2011). Studies and case reports on zoo animals have indicated a prevalence of gastrointestinal nematodes ranging from 65% to 100%, with mortality rates due to parasitic gastro-enteritis ranging from 5% to 17% (Gorman, T.R. et al., 1986). Considering the significant impact of gastrointestinal parasites as a leading cause of economic losses, the present study was undertaken to determine the prevalence of gastrointestinal parasites in Markhor (Capra falconeri) wild Goat residing in high pasture national parks in Chitral. The primary objective of this research was to evaluate the occurrence of gastrointestinal parasitic infections in Markhor (Capra falconeri) fecal samples within the Chitral Goal National Park and Toshe Shasha Conservancy area (TSC). The investigation was carried out on utilizing diagnostic techniques like Flotation, Sedimentation techniques and McMaster slide method for the detection and characterization of these parasites eggs or oocysts from the fecal material. The predictable results of the study are expected to provide valuable knowledge regarding the health status and parasite biology of the Markhor (Capra falconeri) population in these designated conservation zones.

MATERIALS AND METHODS Study Animal and Area

The study focused on the Markhor (Capra falconeri) population in Chitral Goal National Park and Toshe Shasha Conservancy Area. Notably, the Markhor groups in each location were characterized by variations in sex, age, and potentially distinct social groups. Chitral Goal National Park was established in 1984, this park serves as a protected area for the conservation of endangered Kashmir Markhor (Capra falconeri cashmiriensis). The park is situated between 350 51 to 350 57N latitude and 71 to 43 to 71-47 longitude, covering an area of 78.6 square kilometers. It is located 3-5 kilometers west of Chitral town. The Tooshe Shasha Conservancy (TSC) is located to the north of Chitral town adjacent to the main Lotkoh River, covering an area of approximately 200 square kilometers as per the Wildlife Department 1998. It was officially designed as a conservancy in 1998 and is managed by the Khyber Pakhtunkhwa, Wildlife Department. Understanding the health and parasite dynamics of the Markhor population can have broader implications for the conservation of bio-diversity in this region.

METHODOLOGY

The study is divided into two seasons, winter and summer, spanning one Calendar year from October 2022

to May 2023. The study was designed as a cross-sectional study, which typically involves collecting data from a specific population at a single point in time to assess the prevalence and characteristics of a particular condition. A simple random sampling technique was used to collect fresh fecal samples. This approach ensures that each individual or group within the population had an equal chance of being included in the sample, helping to reduce bias and increase the representativeness of the collected samples.

Sample Collection, Preservation, and Transportation

After observing a group of Markhor and allowing them to vacate the area, fresh fecal samples were systematically collected. These samples were carefully placed in sterile plastic bags, appropriately labeled to uphold their integrity and identification. For transportation to the Veterinary Research & Disease Investigation Center Chitral Parasitology laboratory (VR&DIC), cold ice boxes were utilized, and the fecal samples were stored at 4°C until further processing. A total of n=200 samples were randomly gathered from various locations within the parks, ensuring representativeness. To prevent duplication or contamination, a distance of 200-300 meters was maintained between sample collection points. Subsequently, the fecal samples processed a series of parasitological tests, which included floatation and sedimentation techniques. Additionally, the McMaster test, a quantitative method for counting parasite eggs, was employed to evaluate the prevalence of eggs and larvae of helminthes in the samples (Soulsby, E.J.L.,(1982); Urquhart, G.M.et al., (2003).

Laboratory Processing and Examination

The laboratory methods of processing and examination of parasites explained in the literature Basnett *et al.*, (2018). Thus, the collected samples were stored in sterile vials with 2.5% potassium dichromate solution and were brought to the laboratory, where they were put at 4°C in the refrigerator. About 5gm of the sample was taken in the mortar, and 10mL saline (0.9% NaCl) was added to it. The mixture was filtered with the help of tea strainer. The filtrate was examined by the following three techniques separately.

Direct Wet Mount Technique

Used to detect the, eggs, larval and trophozoites, oocysts stages of the endo-parasites, the filtrate of fecal sample was directly observed at 2.5% potassium dichromate, 0.9% saline solution, and Lugol's Iodine.

Saturated Salt Floatation Technique

About 3mL of filtrate and 12mL of NaCl (45%) were kept in the centrifuge tubes. The mixture was centrifuged at 900-1200 rpm for 5-8 min, and the centrifuge tubes with mixtures were kept in the test tube stands. The concentrated solution of NaCl was added entirely to the brim forming convex surface at the top of the tube. The tube was covered by the cover slip so that the solution touched the cover slip. After 10 min, cover slips were removed and kept on glass slides. The slides were examined under the microscope.

Parasite Identification

Lightmicroscopy, specifically employing stereomicroscopes, was utilized in this study at magnifications of 40X and 100X. Images of the specimens, sized by Google pixels-5 mobile Camera, were captured using PX View (March7, 2023), and subsequent measurements were conducted. The analysis focused on parasitic stages, including eggs, larvae and oocysts, trophozoites (Khan, *et al.*, (2010).

Data Analysis

Descriptive statistical methods utilized for the analysis of the data related to the prevalence and mean Eggs per Gram (EPG) of the helminthes egg count in Markhor (*Capra falconeri*) fecal samples. The Chi-square test was used to assess the association between categorical variables. It was applied to measure relationships between the prevalence of different genera of helminthes parasites in the fecal pellet groups and used of significance level of P>0.05.

RESULTS

Prevalence Status of Parasites

In the present investigation, 128 out of 200 fecal

 Table 1: Prevalence of Nematodes, Trematodes and Cestodes in Markhor (Capra Falconeri) in Two Conservancy

 National Parks at Chitral

Locations	Name species	No of examination (n)	No of positive samples	Nematodes Prevalence (%)	Trematodes Prevalence (%)	Cestodes Prevalence (%)	Protozoa prevalence (%)
Chitral Goal National park	Markhor wild goat	100	65	65	0	0	48(48%)
Tooshe Shasha Conservancy area	Markhor wild goat	100	63	63	0	0	50 (50%)
Overall		200	128	128 (64%)	0	0	98 (49.5)
Parameter tested		Chi-square test result					
Locations vs Nematodes Prevalence		χ -squared = 0.021701, df = 1, p-value = 0.8829					
Locations vs Protozoa prevalence	χ -squared = 0.020008, df = 1,p-value = 0.8875						

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samples, constituting 64%, were identified as infected with gastrointestinal parasites in the Markhor wild goat population. The prevalence of parasitic burden in both geographic areas reflects similar outcomes. The pastures within the national parks' conservancy are interconnected, and seasonal animal migrations occur for both feeding and breeding purposes in both seasons, as elaborated in (Table 1).

The prevalence of parasites (Nematodes and Protozoa) across geographical location has no significant affects p-value = 0.89). The prevalence of each parasite was as follows: Strongyles were the most prevalent at 20%,

followed by trichostrongyles at 14.5%, ostertagia at 11%, haemonchus at 8.5%, and trichuris at 10% (Table 2).

Chi-square test were applied to statistically evaluate the prevalence of Overall helminthosisfor each Conservancy area where by the P-value for each are less than 0.05% level of significance suggests that Season play a significant part in prevalence of the Parasite.

Chi-square test was applied to statistically evaluate the prevalence of Overall helminthosis for both Conservancies across seasons whereby P-value of 0.001977 is less than 0.05% level of significance suggests that Season also play a significant role in prevalence of

 Table 2: Helminthes Parasites Prevalence Percentage in Wild Markhor (*Capra Falconeri*) in National Goal Parks and Tooshee Conservancy (TSC) Chitral

Helminthes	Chitral National Park Markhor (n=100)			Tooshee (TSC) M	Over All Prevalence (%)			
	Winter (n=50)	Summer (n=50)	Percentage (%)	Winter (n=50)	Summer (n=50)	Percentage (%)		
Over all Helminthosis	27	38	65	26	37	63	64	
Strongolides spp	9	13	44	7	11	36	20	
Tricho Strongyloides spp	7	8	30	5	9	28	14.5	
Osteratagia spp	3	6	18	5	8	26	11	
Heamoncus contortus spp	5	5	20	4	3	14	8.5	
Trichirus spp	3	6	18	5	6	22	10	
Season-wise Overall helm	inthosis]	prevalence						
Parameter tested		Comparison		Chi-square test result				
Chitral National Park		Winter & Summer		χ -squared = 4.3956, df = 1, p-value = 0.03				
Tooshee Shasha National Park		winter and summer		χ -square	= 0.03834			
Geographical-wise Overal	ll helmin	thosis prev	alence					
Parameter tested		Comparison		Chi-square test result				
National Goal Parks and Tooshe Shasha Conservancy Area Chitral		winter and summer		χ -squared = 9.5703, df = 1, p-value = 0.00197				

 Table 3: Helminthes Parasites Prevalence Percentage in Wild Markhor (*Capra Falconeri*) in National Goal Parks and Tooshee Conservancy (TSC) Chitral

Protozoan Parasites	National Park Markhor			e Shasha co Markhor (r	Over All Prevalence (%)			
	Winter (n=50)	Summer (n=50)	Percentage (%)	Winter (n=50)	Summer (n=50)	Percentage (%)		
Over All Protozoan	19	29	48	17	33	50	49	
Eimeria Spp	14	17	31	11	21	32	31.5	
Entamoeba Spp	5	12	17	6	8	14	15.5	
Season-wise Overall I	Protozoai	n prevalenc	ce					
Parameter tested Comparison			Chi-square test result					
Chitral National Park	hitral National Park Positive & Negative cases			χ -squared = 3.2452, df = 1, p-value = 0.07163				
Tooshee National Park	oshee National Park Positive & Negative cases			χ -squared = 9, df = 1, p-value = 0.0027				
Geographical-wise O	verall Pro	otozoan pro	evalence					
Parameter tested Comparison			Chi-square test result					
National Goal Parks and TooshePositive & Negative casesShasha Conservancy Area Chitralfor winter and summer			χ -squared = 12.505, df = 1, p-value = 0.0004059					

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the Overall helminthosis across both Areas.

It is noteworthy that single infections were absent in this study, and importantly, double species of parasites were observed, respectively. Moreover, protozoan parasites were identified in 49.5% of the samples, while Eimeria and Entamoebiosis parasites were detected in 31.5%

and 15.5% of the samples, respectively, as mentioned in (Table 3).

Chi-square test were applied to statistically evaluate the prevalence of Overall Protozoan for each Conservancy area whereby the P-value for each are less than 0.05% level of significance suggests that Season play a significant



(Microphotograph of larva of Haemancus species (Google pixel 5 Mobile Camera)

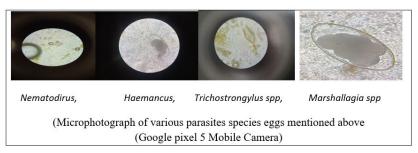


Figure 1: Markhor (Capra falconeri) is a wild Goat helminthes parasite SPP, Photographed from fecal samples

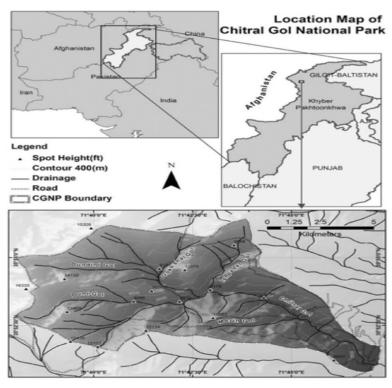


Figure 2: Location of study area at Chitral Goal national park and Tooshe Shasha Conservancy (TSC), in Khyber Pakhtunkhwa, Pakistan. *Source adopted Google Map*

Note. Markhor is deeply concerned in the core region. The fresh fecal samples were collected in the different spots. Location of study area

at Chitral Goal national park and Tooshe Shasha Conservancy (TSC), in Khyber Pakhtunkhwa, Pakistan



part in prevalence of the Protozoan for both seasons.

Chi-square test was applied to statistically evaluate the prevalence of Overall Protozoan for both Conservancies across seasons whereby P-value of 0.0004059 is less than 0.05% level of significance suggests that Season also play a significant role in prevalence of the Overall Protozoan across both areas. Among the identified helminthes parasites, notably, strongyles were the dominant helminthes parasites found in the samples, as shown in (Fig 1), while no eggs and larvae from trematodes and Cestodes were observed in (Table 1).

Multiple parasite infections were common, with some fecal pellets containing up to six different parasite species. The majority of specimens exhibited three or four varieties of parasites, with a notable prevalence of multiple parasitism, particularly among nematode parasites. Analysis of seasonal patterns revealed a higher parasitic burden during the summer compared to the winter, as elaborated in (Tables 1, 2). The widespread practice of goats grazing in pastures is a common occurrence during the summer, contributing to elevated parasitic loads in these areas, as mentioned in the national parks map (Fig 2).

DISCUSSIONS

The study of Gastro Intestinal (GI) parasites of the Markhor (Capra falconeri) in the national parks Chitral is believed to be exciting research mainly because the national parks obtains various types of wild goats are mixed grazing area reared in both seasonal environments, and therefore the Markhor (Capra falconeri) may accompany with different parasites in the Map mentioned (Fig 2). The current study has attempted to explore the prevalence of GI parasites in the Markhor wild Goat. In this study, the recorded prevalence of the study revealed that Markhor (Capra falconeri) acts as a host for a variety of helminthes parasites, specifically nematodes, while no trematodes or cestodes were detected. The overall prevalence of gastrointestinal parasitic infections and the highest seasonal infections were documented in Markhor elaborated in the (Table 1). Helminthes and protozoan infections were found in 64.0% and 49.5% of the Markhor (Capra falconeri) population, respectively. As a Previous studies have reported heavy parasitism by helminthes parasites in captive cervid and bovid (Hayat, C. S., et al., (1998) and supported our study. Among the helminthes, Strongyle spp. had the highest prevalence at 20.0%, followed by Trichostrongyloides spp. at 14.5%, Ostertagia spp. at 11.0%, Haemonchus contortus spp. at 8.5%, and Trichuris spp. at 10.5%. Protozoa, specifically Eimeria spp. at 31.5% and Entamoeba spp. at 15.5%, were also detected and mentioned in the (Table 2). Additionally, a comparable inquiry into parasitic loads was conducted on the Punjab Urial in the Kalabagh Game Reserve area (Jamal, Q. et al., (2016), and (Bajwa A.A., (2019). The presence of these parasites in the wild host population could potentially contribute to increased illness rates and mortality. Moreover, these

wild animals may serve as a reservoir of parasites that could affect both domestic and other wild animal species. However, a comprehensive investigation is needed to understand the impact of these parasites on the host and their potential to infect other animals, Identifying these parasites at the molecular level, especially those affecting Markhor, could shed light on potential shared parasites among different ungulate species. The absence of trematodes in this study suggests that the necessary environmental and climatic conditions for snail habitat, which are essential for trematode infections, might not be present in the park. This could be due to the park's winter temperatures falling below freezing and its dry, humid temperate zone. A previous study supported our study climate and host species played similar role 18% by range described (Meradi,S., at el., (2011).While other studies have described helminthes infections in various wild animals, such as icebergs and antelopes, the specific parasites mentioned, including Haemancus spp., Ostertagia spp., Trichostrongylus spp., and Trichuris spp., are commonly associated with wild ungulates. This study is the first of its kind to investigate helminthes parasites affecting Markhor wild goats in Chitral Goal National Park, making it challenging to compare the results with previous surveys. Parasitological examinations, including qualitative and quantitative estimations of parasitic load and micrometry, confirmed the presence of Haemancus species eggs. These findings align with observations of parasite relationships between species, both wild and domestic animals. Heavy parasitism by helminthes parasites has previously been reported in captive cervids and bovids, including Markhor, in other studies. Previous studies have reported heavy parasitism by helminthes parasites in captive cervids and bovids (Rana M.A., (2015). The seasonal migration of Markhor wild goats occurs effortlessly, facilitated by interconnected pastures within both conservancy areas, primarily driven by the need for seasonal fodders, forages, and feeding. Our research aligns with similar studies, highlighting the significant impact of habitats and forages on the health status of Markhor, as elucidated (Ali S., 2008). Studied (Ashraf et al., (2014) emphasizes the potential severe consequences of a dried diet in winter for Markhor. Parasites play a crucial role in shaping competitive dynamics among various host species by influencing the availability of essential resources like food or habitat, as described (Price, P.W. et al., (1986). Our study reveals the prevalence of coccidiosis, a disease of high economic importance caused by Eimeria species, and embiosis among Markhor wild goats. The results indicate 31.5% for Eimeria spp. and 15.5% for Entamoeba spp. in both seasons, as presented in (Table 3). Our findings are supported (Walaa I. Mohamaden et al., 2018). Who reported a significant prevalence of subclinical coccidiosis and Eimeria infection in goats during summer (42%) and autumn (38%). The seasonal migration of Markhor wild goats occurs easily, facilitated by interconnected pastures within both conservancy areas, mostly for seasonal fodders& forages scarcity and feeding purposes,



and our research is supported a similar study. The health status of Markhor is influenced by habitats and forages described (Ali. S., (2008). A dried diet in winter may have severe consequences for Markhor studied (Ashraf et al., (2014).Parasites contribute to shaping the competitive dynamics among various host species by impacting the availability of essential resources, such as food or habitat describes (Price, P.W.et al., (1986).Coccidiosis is a disease of high economic importance caused by Eimeria species and embiosis that show distribution among Markhor wild goats and results showed Eimeria spp. 31.5% and Entamoeba spp. 15.5%, in both seasons respectively in the (Table 3) and Our research study is supported (Walaaet al.et al, (2018) revealed that goats suffering from subclinical coccidiosis and Eimeria infection were significantly prevalent in summer (42%) and autumn (38%). These data substantiate our research findings, indicating that parasitic infections can lead to compromised health.

CONCLUSION

The study results highlight a notable prevalence of helminthes parasites in Markhor (*Capra falconeri*) population, emphasizing the importance of further research aimed at devising effective strategies for parasites management, there is a possibility that Markhor, hosting gastrointestinal parasites serve as source of infection for others forest house species as well as domestic livestock and even humans. In general of conserving this endangered species, it becomes crucial to include the control of helminthes parasites as integral component of the comprehensive conservation plan.

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