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GASTROINTESTINAL HELMINTHOSES: PREVALENCE AND ASSOCIATED RISK FACTORS IN SMALL RUMINANTS OF DISTRICT KOHAT, PAKISTAN

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ABSTRACT

Gut contents of 500 freshly slaughtered small ruminants (300 goats and 200 sheep) were studied using floatation and sedimentation methods followed by direct microscopic examination aims to investigate the prevalence of gastrointestinal tract helminthes in small ruminants of district Kohat, Khyber Pakhtunkhwa Pakistan during March-November 2013. Overall, 45.6% samples were found positive for helminthes parasites comprising nematodes (23.0%), cestodes (22.4%) and trematodes (0.2%). The prevalence of GIT helminthes was higher in goats compared to sheep (49.0% vs. 40.5%), but the same was statistically not significant ($p>0.05$). Gender-wise prevalence of GIT helminthes in goats and sheep was also not significant ($P>0.05$) whereas prevalence of GIT helminthes was significantly higher ($p<0.05$) in young compared to adult sheep, however, the phenomenon was not observed in goats. Prevalence of GIT helminthes was significantly different ($P<0.0001$) across different seasons and GIT parts of host animals.

Key words: Gastrointestinal helminthoses, small ruminants, goats, sheep, Pakistan.

INTRODUCTION

A wide variety of gastro-intestinal (GI) helminthes have been implicated in huge morbidity and mortality in small ruminants like goats and sheep. Heavy parasitic burden in these animals is characterized by symptoms like diarrhea, gastritis, stunted growth, poor weight gain, reduced milk production, lack of appetite, and loss of wool and body hairs (Rehman and Ali, 2001). Cestodes infestation has been the leading cause of severe sheep loss in terms of poor performance (Hussain *et al.*, 2004). In addition to cestodes, *Trichostrongylidaes* (nematode family) are perhaps the most important parasites of small ruminants throughout the world, causing devastating effects on the health and production of these animals.

Livestock rearing in Pakistan is primarily a subsistence activity and is characterized by small herds/flocks with wide spread ownership, one million of which are landless. Goats and sheep are primarily reared for meat production with hair/wool and milk as by-products. Over the last few decades, population of goats has been growing exponentially among all species of livestock in Pakistan (Hasnain and Usmani, 2006). Currently, Pakistan is home to 53.7 million heads of goats and 26.4 million heads of sheep with an annual growth rate of 4.48% and 2.63%, respectively. In Pakistan, Khyber Pakhtunkhwa (KP) is rich in livestock where 9.5 millions heads of goats and 3.3 million heads of sheep are reported. District Kohat shares 0.29 million heads of goats and 0.10 million heads of sheep (PBS, 2006).

District Kohat is located between coordinates 32° 47' to 33° 53' north latitude and 70° 34' to 72° 17' east longitude with an average elevation of 1764 feet above sea level. Total area of the district is 2545 sq. Kms. Kohat has a human population of 0.56 million individuals with a population density of 221 persons/Km^2. A major proportion of the total population (73%) lives in the rural areas (PBS, 1998). The mean maximum and minimum temperature recorded in the area is 40°C and 6°C in summer and winter, respectively. Rainfall in Kohat varies from 24 to 321 mm per month with the highest precipitation received in the months of July and August. June is the hottest whereas January is the coldest month of the year in district Kohat. Maximum humidity is usually found in the months of July and August during summer whereas December is the most humid month of winter.

Several studies regarding GIT helminthoses in sheep and goats have been conducted in Punjab and Sindh provinces. However, little is known about GI helminthoses in these animals in Khyber Pakhtunkhwa province of Pakistan (Ijaz *et al.*, 2009). Therefore, the present study was designed with the aim to document the prevalence of GIT helminthoses and its associated risk factors in small ruminants of district Kohat in north-west Pakistan.

MATERIALS AND METHODS

Study area

Sample collection: This study was approved from the departmental ethical review committee of Hazara University Mansehra before starting the experimental work.

Samples were collected from the freshly slaughtered host animals in the Kohat Slaughter House by March - November, 2013.

A total of 500 host animals, comprising 300 goats and 200 sheep, were studied for presence of GIT helminthes. GIT of each host animal was divided into three parts i.e., stomach, small intestine and large intestine by ligation method.

About 150-200 grams gut content was collected in clean polythene bags from each GIT part of the freshly slaughtered animals. Before slaughtering the animal, age, sex and health of the host animal were properly checked by relevant veterinary doctor of the slaughterhouse.

Conventionally, animals aged ≤ 18 months were classified as young and those aged > 18 months as adults.

Each sample, labeled with corresponding date, age, sex and species, was transported to Parasitology Laboratory in Department of Zoology, Kohat University of Science and Technology where it was refrigerated till further use.

Sampling period was classified into three seasons' i.e., spring (March, April, May), summer (June, July, August) and autumn (September, October, November). Due to some logistic and financial constraints the researchers in the present study were unable to collect samples in winter season.

Sample processing and helminthes identification: All samples were examined using standard floatation and sedimentation methods (Khan, 1993) which was followed by direct microscopic examination. The helminthes were identified using different resolution powers of phase contrast microscope (Nikon Instruments Inc. USA) and according to the keys and morphological characteristics according to Soulsby (1986).

Statistical Analysis: Data obtained were entered into Microsoft Excel Sheet (2007) and tabulated using different options of the MS Excel. The prevalence of helminthes parasites was expressed in percentages. For association of GIT helminthes with different risk factors, Fisher's exact test and Pearson's Chi-square test were applied using GraphPad Prism software (Ver. 5). Statistical analyses were conducted using 95% confidence interval and p-value < 0.05 was considered as statistically significant.

RESULTS

The overall prevalence of GIT helminthes in small ruminants was found to be 45.6% (Table 1). The GIT helminthes were further categorized into nematodes, cestodes and trematodes and their respective prevalence in goats and sheep was determined. Collectively, nematodes, cestodes and trematodes were observed to be 23.0%, 22.4% and 0.2%, respectively (Table 1). Individually, goats were predominantly infested by nematodes (25.0%) followed by cestodes (23.7%) and trematodes (0.7%). Sheep on the other hand, were almost equally infested by both nematodes and cestodes (20.0% vs. 20.5%); however, no trematodes infestation (0.0%) was reported in sheep. Furthermore, the collected helminth parasites were identified up to species level and their respective prevalence in both hosts was calculated accordingly. In this respect, helminthes species identified in the present study comprised *Trichrius globulosa*, *Stronglyoidus papillosus*, *Haemonochus contortus*, *Trichrius trichrua*, *Moneiza expensa*, *Avitellina centripunctata*, *Stilesia globipunctata*, *Thysaniezia girdi* and *Parapostomum curvi* with the respective prevalence shown in Table 1. The prevalence of helminthes species between goats and sheep was statistically not significant ($p>0.05$) (Table 1).

Moreover, to check any association between helminthes infestation rate and some potential risk factors, data were analyzed with respect to different variables like seasons, host's species, gender, age and GIT parts (Table 2). In this regard, prevalence of GIT helminthes was higher in goats (49.0%) compared to sheep (40.5%), however the difference was statistically not significant ($p>0.05$). Likewise, prevalence of GIT helminthes infestation was compared across gender i.e., between male and female hosts. In this context, no statistical difference ($p>0.05$) was observed between male and female hosts in both categories (Table 2). In addition, data were analyzed age wise. Interestingly, prevalence of GIT helminthes was significantly higher ($p<0.05$) in young sheep (<3 years) compared to adult sheep (≥3 years). Among goats however, no significant difference ($p>0.05$) was observed between young and adult hosts (Table 2). The data were further analyzed with respect to different GIT parts of the host animals. Overall, GIT helminthes showed highest prevalence in large intestine (72.5%) followed by small intestine (59.2%) and stomach (15.6%). This trend of prevalence was also observed in both hosts individually, thus confirming highest prevalence of GIT helminthes in large intestine followed by small intestine and stomach i.e. (76.0% > 61.3% > 17.0% in goats; 66.7% > 55.6% > 13.8% in sheep, respectively). Thus, the results showed significantly higher difference ($p<0.0001$) in prevalence of helminthes across different GIT parts of the host animals.

Moreover, the data were analyzed across three seasons i.e. spring, summer and autumn (Table 2). In this respect, highest prevalence of GIT helminthes in small ruminants was witnessed in summer (61.3%) followed by autumn (40.6%) and spring (34.7%), thus showing significant seasonal variation ($p<0.0001$). This phenomenon was also observed in each host individually. For example, among goats highest proportion of GIT helminthes was observed in summer (64.0%) followed by autumn (45.0%) and spring (38.0%). Likewise, among sheep GIT helminthes showed highest prevalence in summer (57.4%) followed by autumn (33.8%) and spring (29.9%). Thus, it is pertinent to say that seasonal variations in the present study have significantly influenced the prevalence of GIT helminthes in small ruminants ($p<0.0001$) (Table 2).

Prevalence of GIT helminthes infestation in small ruminants was further analyzed month wise (Table 3). In this regard, a steep increase in prevalence of GIT helminthes was witnessed in small ruminants from March until July which was subsequently followed by an abrupt decline till November. For instance, the prevalence of GIT helminthes infestation was increased from 26.9% in March to 68.4% in July which subsequently declined up to 33.3% in November. Thus, June, July and August were found to be the most dangerous months for small ruminants in terms of GIT helminthes infestation. By analyzing the data statistically using chi-square test, the prevalence of GIT helminthes revealed significant difference ($p<0.0001$) across month-wise data. More interestingly, this pattern of prevalence was also noticed in individual categories of goats and sheep when the data were analyzed month-wise (Table 3).

Table 1. Genera-wise prevalence of GIT helminthes in small ruminants of district Kohat.

	Goats (n=300)		Sheep (n=200)		Total (n=500)	
Helminths*	**No. Obs.**	**Prevalence (%)**	**No. Obs.**	**Prevalence (%)**	**No. Obs.**	**Prevalence (%)**
Nematodes						
Trichrius globulosa	32	21.8	13	16.0	45	9.0
Stronglyoidus papillosus	23	15.6	14	17.3	37	7.4
Haemonochus contortus	16	10.9	11	13.6	27	5.4
Trichrius trichrua	4	2.7	2	2.5	6	1.2
Total	75	25.0	40	20.0	115	23.0
Cestodes						
Moneiza expensa	21	14.3	12	14.8	33	6.6
Avitellina centripunctata	16	10.9	11	13.6	27	5.4
Stilesia globipunctata	19	12.9	5	6.2	24	4.8
Thysaniezia girdi	15	10.2	13	16.0	28	5.6
Total	71	23.7	41	20.5	112	22.4
Trematodes						
Parapostomum curvi	1	0.7	--	--	1	0.2
Grand Total	147	49.0	81	40.5	228	45.6

*: $\chi^2 = 0.6359$; df = 2; p = 0.7276 (non-significant)

Table 2. Prevalence of GIT helminthes in small ruminants with respect to different risk factors

Risk Factor		**Negative**	**Positive**	**Total**	**Prevalence (%)**	¡ ^{0}	**P-value**
1.	***Species***						
	Goats	153	147	300	49.0	3.495	0.0616 ns
	Sheep	119	81	200	40.5		
2.	***Gender (Goats)***						
	Male	90	80	170	47.1	0.592	0.4418 ns
	Female	63	67	130	51.5		
3.	***Gender (Sheep)***						
	Male	85	51	136	37.5	1.587	0.2077 ns
	Female	34	30	64	46.9		
4.	***Gender (Overall)***						
	Male	175	131	306	42.8	2.474	0.1157 ns
	Female	97	97	194	50.0		

5.	***Age (Goats)***						
	Young	82	68	150	45.3	1.614	0.2039 ns
	Adult	71	79	150	52.6		
6.	***Age (Sheep)***						
	Young	43	42	85	49.4	4.872	0.0273*
	Adult	76	39	115	33.9		
7.	***Age (Overall)***						
	Young	125	110	235	46.8	0.261	0.6094 ns
	Adult	147	118	265	44.5		
8.	**Season (*Sheep)***						
	Spring	47	20	67	29.9	12.36	0.0021*
	Summer	29	39	68	57.4		
	Autumn	43	22	65	33.8		
9.	**Season (Goats)**						
	Spring	62	38	100	38.0	14.49	0.0007*
	Summer	36	64	100	64.0		
	Autumn	55	45	100	45.0		
10.	**Season (Overall)**						
	Spring	109	58	167	34.7	26.33	< 0.0001*
	Summer	65	103	168	61.3		
	Autumn	98	67	165	40.6		
11.	**GIT parts (Sheep)**						
	Stomach	69	11	80	13.8	40.75	< 0.0001*
	Small Intestine	40	50	90	55.6		
	Large Intestine	10	20	30	66.7		
12.	**GIT parts (Goats)**						
	Stomach	83	17	100	17.0	64.69	< 0.0001*
	Small Intestine	58	92	150	61.3		
	Large Intestine	12	38	50	76.0		
13.	**GIT parts (Overall)**						
	Stomach	152	28	180	15.6	106.6	< 0.0001*
	Small Intestine	98	142	240	59.2		
	Large Intestine	22	58	80	72.5		

*ns: p-value is not significant; *: p-value is significant.*

Table 3. Month-wise prevalence of gastrointestinal helminths in small ruminants of district Kohat.

	Sheep (n=200)*		**Goats (n=300)****		**Total (n=500)****	
Months	**Obs. Sample**	**Obs. Prevalence**	**Obs. Sample**	**Obs. Prevalence**	**Obs. Sample**	**Obs. Prevalence**
March	22	5 (22.7)	30	9 (30.0)	52	14 (26.9)
April	23	6 (26.1)	35	13 (37.1)	58	19 (32.8)
May	22	9 (40.9)	35	16 (48.6)	57	25 (43.9)
June	24	13(54.2)	30	19 (63.3)	54	32 (59.3)
July	22	15 (59.1)	35	24 (71.4)	57	39 (68.4)
August	22	11 (50.0)	35	21 (60.0)	57	32 (56.1)
September	23	9 (39.1)	35	18 (51.4)	58	27 (46.6)
October	20	7 (35.0)	30	14 (46.6)	50	21 (42.0)
November	22	6 (27.3)	35	13 (37.1)	57	19 (33.3)

*χ^2=16.41, p=0.0368; **χ^2=18.10, p=0.0205; ***χ^2=33.56, p<0.0001; Figure in parentheses shows percentage.

DISCUSSION

Goats and sheep, among livestock, are the fastest growing ruminants in Pakistan which are primarily reared for meat production with hair, wool and milk as by-products (Hasnain and Usmani, 2006). Parasitic infestation of these animals with gastrointestinal tract helminthes, especially nematode and trematode, results in low productivity due to stunted growth, poor weight gain and poor feed utilization which ultimately leads into huge morbidity and mortality (Lashari and Tasawar, 2011). According to several earlier studies conducted throughout Pakistan, prevalence of helminthes infestation in small ruminants ranged from 25% to 92% (Farooq *et al.,* 2012; Ijaz *et al.,* 2009; Khan *et al.,* 2010; Raza *et al.,* 2007; Raza *et al.,* 2012). Keeping in view the high economic significance of livestock, it is imperative to document prevalence of GIT helminthes in unexplored areas of Pakistan (Raza *et al.,* 2014). Hence, the present study was conducted in district Kohat to check the prevalence of GIT helminthes infestation in goats and sheep.

The overall prevalence of GIT helminthes infestation in the small ruminant was 45.6% which is sufficiently lower than a previous finding that suggested an overall prevalence of 78.1% in Cholistan desert of Pakistan (Raza *et al.,* 2014). However, nearly similar results were noticed in the present study only during summer season. Therefore, highest prevalence of GIT helminthes infestation in Cholistan desert and district Kohat during summer may partly be attributed to the elevated temperature in both regions. Prevalence of GIT helminthes infestation was comparable in goats and sheep. The current study is in concordance with the previous literature regarding comparative prevalence in goats and sheep which show mixed results (Asif *et al.,* 2008; Emiru *et al.,* 2013; Farooq *et al.,* 2012; Mehmood *et al.,* 2013; Zeryehun, 2012).

As noted earlier by several researchers, parasitic infestation in small ruminants may be influenced by a variety of factors including grazing habits, age, gender, body weight and breed of the host animal. In addition, variation in agro-climatic conditions like rainfall, humidity and temperature have also shown profound effects on helminthes infestation in small ruminants (Bhat *et al.,* 2012; Demissie *et al.,* 2013; Komoin *et al.,* 1999; Ouattara and Dorchies, 2001; Sajid *et al.,* 1999). Thus, we have explored association of parasitic infestation with various risk factors encompassing gender, age, season, GIT parts and host species. In this context, gender-wise analysis of the data revealed no significant difference in prevalence of GIT helminthes infestation between male and female hosts in both species of small ruminants. Some of the previous studies gave notion that females are more prone to helminthes infestation compared to male small ruminant, whereas others have opposite results (Emiru *et al.,* 2013). Age-wise prevalence of helminthes infestation showed no significant difference between young (45.33%) and adult goats (52.67%). However, prevalence of helminthes infestation was significantly higher ($p<0.05$) in young (49.41%) than adult sheep (33.91%). Thus, our results are in close lines with a previous report which showed maximum parasitic infestation in lambs compared to adults which may be attributed to the development of significant immunity in adult hosts (Lashari and Tasawar, 2011). Furthermore, it is evident too from several studies that age of host animal greatly influences level of parasitic infestation in sheep with higher prevalence in young hosts than adult hosts. As mentioned earlier, the higher parasitic infestation of young than adult animals may be attributed to a weaker immunological response of young animals (Raza *et al.,* 2007; Raza *et al.,* 2014; Zeryehun, 2012). Similarly, Tasawar *et al.,* (2010) have recently reported that younger sheep are more prone to nematode infestation than their older counterparts.

Moreover, it is widely anticipated that nematodes are the most common helminthes reported in goats followed by cestodes and very few cases of trematodes. Thus, our findings are in close agreement with this statement as nematodes and cestodes were the predominant helminthes reported in the present investigation. However, the current results are not in conformity with a previous study which has reported an overall prevalence of 39.7%, 6.7% and 2.3% for nematodes, trematodes and cestodes in goats, respectively. Likewise, the prevalence of gastrointestinal nematodes, trematodes and cestodes in sheep was found to be 46.0 %, 6.0 %, and 3.3%, respectively (Raza *et al.,* 2011). Keeping all together, nematodes are the predominant helminthes inflicting GIT infestation in small ruminants.

The present investigation has revealed significant seasonal variation in the prevalence of helminthes infestation during the study period. In this regard, highest prevalence of GIT helminthes was reported in June, July and August (59.3%, 68.4% and 56.1%, respectively) compared to either March, April and May (26.9%, 32.8% and 43.9%, respectively) or September, October and November (46.6%, 42.0% and 33.3%, respectively). Thus, it depicts significantly higher ($p<0.0001$) prevalence of helminthes infestation in both hosts during summer compared to spring and autumn. The highest prevalence of helminthes infestation in goat and sheep during summer may be due to the underlying agro-climatic conditions like heavy rainfall in monsoon, thick vegetation, increased humidity and high temperature which highly favor growth of free larval stages of helminthes parasites. Our results confirm the previous findings which revealed higher infection rate of gastrointestinal helminthes in goats and sheep during monsoon compared to winter (Khajuria *et al.,* 2013). Among the various helminthes species observed in the

present study, *Trichrius globulosa* was the most predominant parasite whereas *Parapostomum curvi* as the least common parasite.

Conclusion: In conclusion, we report highest prevalence of GIT helminthes infestation in goats and sheep during summer season which may partially be attributed to the extreme agro-climatic conditions like heavy rainfall, increased humidity, thick vegetation and remarkably high temperature in the study area during summer. Thus, we recommend use of chemotherapy and extended health care campaign in the area during summer season in order to alleviate helminthes related morbidity and mortality in small ruminants.

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