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PREVALENCE OF GASTROINTESTINAL NEMATODES IN EQUINES OF BAJAUR AND MOHMAND AGENCIES, NORTH-WEST PAKISTAN

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ABSTRACT

An epidemiological study was performed with the aim to elucidate the prevalence of gastrointestinal nematodes in equines of Bajaur and Mohmand Agencies in North-Western Pakistan from September 2013 to May 2014. The fecal samples from one thousand equines, comprising horses (n=83), donkeys (n=715), and mules (n=202) were collected and processed for identification of nematode parasites using floatation and sedimentation methods. Results revealed that 72% samples were positive for certain nematode parasites. Overall, 43.74% of the positive samples had mixed infection. The predominant nematode species identified included *Trichostrongylous axei* (19.81%), *Dictyocaulus arnfieldi* (13.62%), *Strongylous spp.*(11.14%), *Parascaris equorum* (7.57%), *Cyathostoma spp.* (3.85%), *Delofondia vulgaris* (0.14%) and *Strongyloides westeri* (0.14%). Significantly higher prevalence of nematodes was observed in equines of Bajaur than in the Mohmand Agency (81.2% vs. 64.2%, $p<0.0001$). Similarly, the highest prevalence of nematodes was recorded from donkeys followed by mules and horses (77.8%>66.3%>44.6%). In summary, prevalence of equines nematodes showed significant variation ($p<0.05$) with respect to parameters like geography, host's species, gender, and health condition; however, the difference in prevalence of nematodes between young and adult equines was not significant ($p>0.05$). In addition to its significance in the control and management of the pathological conditions of equines in the area, this study would provide a baseline for future research in the field.

Key words: Equines**,** Helminthes, Nematodes, Bajaur Agency, Mohmand Agency, Pakistan.

INTRODUCTION

The world is home to around 122.4 million equines that comprise 35% horses, 33% donkeys, 20% zebras and camels and 12% mule (Abayneh *et al*. 2002). Equines are used as a major substitute for transportation of goods in the areas with underdeveloped infrastructure like lack of roads, narrow streets and rough topography (Feseha *et al*. 1991). In addition, due to their most readily recognizable function against traction and drought, equines have made a significant contribution to the human welfare and advancement, especially in the agriculture and industrial sectors (Perveen *et al*. 2011). The domestication of donkeys (*Equus asinus)* dates back to nearly 4000 BC. At present, around 96% donkeys are confined to the developing world; however, their population is much lower in the developed countries (Starkey, 1997; Krecek and Waller, 2006). The use of donkeys has not only reduced the domestic burden in the rural areas but has also increased employment opportunities for people living in such communities (Pearson, 2000).

Gastrointestinal helminthiases, particularly the nematode and trematode infection, has considerably decreased the livestock production by increasing mortality and morbidity rates as well as costs related to control measures and treatment (Lashari and Tasawar, 2011; Rashid *et al*. 2016). Moreover, the infection is accompanied by loss of digestive function, decreased fertility and worse physical condition of the animals (Brady and Nichols, 2009). Thus, parasitic infections have proven to be one of the major hurdles against the successful raise of equines in the world. Though a number of parasitic species are involved in infecting the equines, gastrointestinal helminthiases have been found as serious threats to the grazing animals all around the world with far reaching consequences in the unindustrialized countries. Gastrointestinal helminthiasis in equines is characterized by symptoms like a rough and dull coat, weight loss, stunted growth, colic, weakness, diarrhea, dysentery, and tail-rubbing disease (Hendrix, 1998; Goraya *et al*., 2013a, 2013b; Mezgebu, 2013; Tahir *et al*. 2016).

In Pakistan, a number of studies are available from different parts of the country regarding prevalence and control of gastrointestinal parasitism in equines (Goraya *et al*. 2013a, Goraya *et al*. 2013b, Tahir *et al*. 2016). While a majority of these studies were reported from urban and semi-urban Pakistan, very little is known about gastrointestinal parasitism in equines of rural communities such as Federally Administered Tribal Areas, which constitute the most underprivileged, socioeconomically backward and geographically

inaccessible part of the country. Therefore, the current study was designed with the aim to understand the prevalence of gastrointestinal nematodes in equines of two rural communities in Pakistan.

MATERIALS AND METHODS

Study area: The current study was carried out in two north-western tribal areas of Pakistan (Bajaur and Mohmand Agencies) along the Pakistan-Afghanistan border. Bajaur Agency (34.8569° N, 71.4299° E) covers an area of 1290 sq. Km and has a human population of 595,227 individuals whereas Mohmand Agency (34.5655° N, 71.4774° E), has a total area of 2296 sq. Km and house to a population of 334, 453 people (PBS, 1998). Due to the availability of very few livelihood opportunities within the area, people of Bajaur and Mohmand Agencies are mainly pastoral. Most of the families in the region are dependent upon primary-level activities for their livelihoods, for instance, agricultural activities, livestock raising or minor business (fata.gov.pk, accessed April 7, 2017).

Study Design and Samples Collection: A total of 1000 fecal samples were collected from the study area through a door to door survey from September 2013 to May 2014 as per method suggested by Soulsby (1982). About 200 grams of fecal material was collected from each host animal during the field survey. Only one host animal per household was selected in this study. To ensure uniform sampling in order to represent the whole study area, Bajaur Agency was further divided into 14 union councils or village clusters while Mohmand Agency was sub-divided into 12 union councils or village clusters. Variable numbers of samples were obtained from each union councils/village clusters of both agencies. For example, in Bajaur Agency, the highest number of samples were obtained from union council Nawagai (n=49) while least number of samples were obtained from union council Rashakai (n=15). In Mohmand Agency, on the other hand, the highest number of samples were obtained from union council Chamar Kand (n=68) while least number of samples were ascertained from union council Gandhab (n=21). Of the total samples collected (n=1000), donkeys comprised highest number (n=715) followed by mules (n=202) and horses (n=83). Clean plastic bottles were used for the collection of fecal material (~200 grams) from the host animals. In order to avoid embryogenesis, samples were stored in an ice-added container. Each sample was labeled with its corresponding date, location, species, age, gender, and health status/body condition of the host animal. All samples were soon transported to the Veterinary Research Institute Peshawar where they were stored at 4°C until further processing and identification of parasites.

Processing and Examination of Fecal Samples: Samples were microscopically examined using floatation and sedimentation methods as described by Hendrix (1998). For this purpose, around 15-20 ml of distilled water was added into about one gram of fecal sample and mixed thoroughly. Muslin cloth or fine sieve was used to filter the mixture. The filtrate was mixed with flotation solution (4-5 ml) such as NaCl, $ZnSO_4$, or saturated sugar solution. As a next step, the mixture was transferred into a glass tube and more flotation solution was added until the tube was filled. The tube was then covered with a glass cover slip and left for 20-30 minutes in a rack in standing position. The glass cover slip was then removed gently and placed on a glass slide for microscopy. The glass was examined under the phase contrast microscope at 10-40x magnification as described by Thienpont and colleagues, (1979). The eggs and larvae of nematodes species found in the positive fecal samples were identified on the basis of their morphological characteristics using the standard key as described by Soulsby (1982).

Statistical Analysis: Data obtained were entered into MS Excel Sheet and arranged with respect to different variables like geography, age, gender, health status/body condition and species of the host animals. Host animals were categorized as young or adult on the basis of their ages. Conventionally, host animals aged ≤3 years were considered as young whereas host animals aged >3 years were categorized as adults. Similarly, host animals were divided into three categories, that is, healthy, weak and emaciated on the basis of their physical appearance/body condition. Prevalence of nematodes infection was expressed in percentages. Chi-square test was applied to check any statistical difference in prevalence of nematodes within each variable like host's species, age, gender, and health status. $p<0.05$ was considered statistically significant.

RESULTS

Our results showed 727/1000 samples positive for nematode species, thus, indicating 72.7% prevalence in the region. While 43.74% of the positive samples had mixed infection, the major nematode species that were found individually include *Trichostrongylous axei* (19.81%), *Dictyocaulus arnfieldi* (13.62%), *Strongylous spp.* (11.14%), *Parascaris equorum* (7.57%), *Cyathostoma* (3.85%), *Delofondia vulgaris* (0.14%) and *Strongyloides westeri* (0.14%) (Fig. 1). Individually, donkeys revealed highest frequency for mixed infection (33.8%) followed by *Trichostrongylous axei* (14.2%), *Dictyocaulus arnfieldi* (11.3%), *Strongylous spp.* (8.4%), *Parascaris equorum* (6.3%), *Cyathostoma* (2.2%), and *Delofondia vulgaris* as well as *Strongyloides westeri* (0.1% each). Mule indicated 6.9% mixed infection which

was followed by *Trichostrongylous axei* (4.7%), *Dictyocaulus arnfieldi* (2.3%), *Strongylous spp*. (2.3%), *Cyathostoma* (1.2%) and *Parascaris equorum* (1.0%). While the prevalence of mixed infection in horses remained 3.2%, the nematodes like *Trichostrongylous axei*, *Strongylous spp., Parascaris equorum,* and *Cyathostoma* had a prevalence of ≤1% (Table 2).

The prevalence of nematodes' infection was significantly higher in Bajaur Agency compared to Mohmand Agency (81.2% vs. 64.2%, $p<0.0001$) (Table 1). The data were further analyzed with respect to different variables such as age, gender, health status and species of the host animals. In addition, the results obtained were compared between two selected populations using chi-square test with $p<0.05$ considered as statistically significant. With respect to host species, the highest prevalence of nematodes was found among Donkeys (77.8%) followed by Mules (66.3%) and Horses (44.6%) and the difference was statistically highly significant ($p<0.0001$) (Table 1). Individually, this trend (Donkey>Mule>Horse) was also followed in both Agencies; however, the difference was statistically significant in Bajaur Agency ($p<0.0001$) but not in Mohmand Agency ($p>0.05$). Moreover, the overall prevalence of nematodes was statistically not significant when compared between young and adult equines (73.6% vs. 72.2%, respectively, $p>0.05$). Nevertheless, the prevalence of nematode infection showed a significant variation ($p<0.05$) between young and adult hosts in both Agencies. Interestingly, in Bajaur, more young than adult hosts were infected with nematodes (86.3% vs. 78.1%, $p<0.05$) whereas in Mohmand Agency, more adults than young hosts were infected with nematode parasites (67.0% vs. 57.6%, $p<0.05$) (Table 1).

With respect to gender of the host animals, the prevalence of nematode infection was significantly higher in male as compared to female hosts (81.6% vs. 70.1%, $p=0.0007$) (Table 1). While similar trend was documented in Mohmand Agency where more male than female hosts were infected with nematodes (84.4% and 58.6%, respectively, $p<0.0001$), the prevalence of nematode infection showed non-significant difference between male and female hosts in Bajaur Agency (78.9% and 81.9%, respectively, $p>0.05$).

The data were further analyzed with respect to the health status of the host animals, that is, healthy, weak and emaciated. In this respect, the prevalence of nematodes was always highest in emaciated followed by weak and healthy animals and the difference was always statistically highly significant ($p<0.0001$). For instance, the overall prevalence of nematodes in emaciated, weak and healthy animals was found to be 100%, 85.9%, and 63.9%, respectively ($p<0.0001$). Independently, this trend (Emaciated>Weak>Healthy) was also followed in both populations (Table 1).

Table 1. Prevalence of Gastrointestinal Nematodes in Equines of Bajaur and Mohmand Agencies.

Variable	*Mohmand Agency*				*Bajaur Agency*				*Total*			
	N	**Negative**	**Positive**	**% Prev.**	**N**	**Negative**	**Positive**	**% Prev.**	**N**	**Negative**	**Positive**	**% Prev.**
Host [#]												
Donkey	373	130	243	65.1	342	29	313	91.5	715	159	556	77.8
Horse	31	17	14	45.2	52	29	23	44.2	83	46	37	44.6
Mule	96	32	64	66.7	106	36	70	66.0	202	68	134	66.3
		χ2=5.289, df=2, p=0.071				***χ2=86.4, df=2, p<0.0001*** [*]				***χ2=46.43, df=2, p<0.0001*** [*]		
Age												
Young	151	64	87	57.6	190	26	164	86.3	341	90	251	73.6
Adult	349	115	234	67.0	310	68	242	78.1	659	183	476	72.2
		χ2=4.08, df=1, p=0.0434 [*]				***χ2=5.25, df=1, p=0.0219*** [*]				***χ2=0.2145, df=1, p=0.6433***		
Gender												
Male	109	17	92	84.4	114	24	90	78.9	223	41	182	81.6
Female	391	162	229	58.6	386	70	316	81.9	777	232	545	70.1
		χ2=24.75, df=1, p<0.0001 [*]				***χ2=0.491, df=1, p=0.4835***				***χ2=11.49, df=1, p=0.0007*** [*]		
Health [#]												
Healthy	322	148	174	54.0	316	82	234	74.1	638	230	408	63.9
Weak	152	31	121	79.6	152	12	140	92.1	304	43	261	85.9
Emaciated	26	0	26	100.0	32	0	32	100.0	58	0	58	100.0
		χ2=44.66, df=2, p<0.0001 [*]				***χ2=29.8, df=2, p<0.0001*** [*]				***χ2=72.90, df=2, p<0.0001*** [*]		
Total	**500**	**179**	**321**	**64.2**	**500**	**94**	**406**	**81.2**	**1000**	**273**	**727**	**72.7**
						χ2=36.40, df=1, p< 0.0001 [*]						

[*]*Statistically significant p-value, df: Degree of freedom;* [#]*Significant predictors of nematodes' infection*

Table 2. Host-wise Prevalence of Gastrointestinal Nematodes in Bajaur and Mohmand Agencies

Host Animal	Nematode Species	No.	%
Donkey (N=715)	*Mixed*	246	33.8
	Trichostrongylous axei	103	14.2
	Dictyocaulus arnfieldi	82	11.3
	Strongylous spp.	61	8.4
	Parascaris equorum	46	6.3
	Cyathostoma	16	2.2
	Delofondia vulgaris	1	0.1
	Strongyloides westeri	1	0.1
Horse (N=83)	*Mixed*	23	3.2
	Trichostrongylous axei	7	1.0
	Strongylous spp.	3	0.4
	Parascaris equorum	2	0.3
	Cyathostoma	2	0.3
Mule (N=202)	*Mixed*	50	6.9
	Trichostrongylous axei	34	4.7
	Dictyocaulus arnfieldi	17	2.3
	Strongylous spp.	17	2.3
	Cyathostoma	9	1.2
	Parascaris equorum	7	1.0
Total		**727**	**100.0**

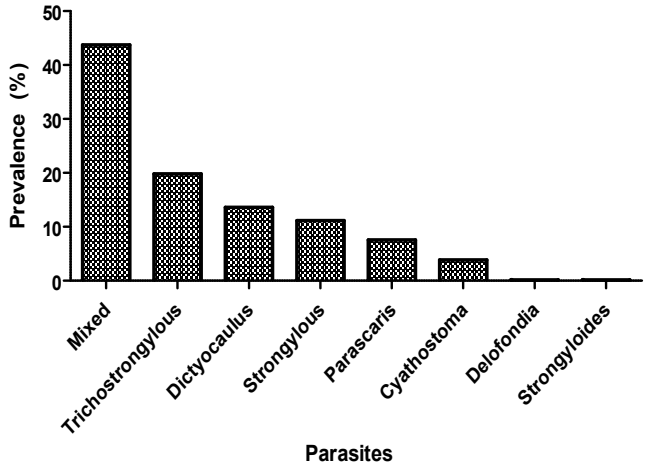

Figure 1. Prevalence of nematode parasites observed in equines of the study area

DISCUSSION

There are many species of the helminths that infect equines adversely, thus creating one of the major impediments in the successful rearing of these animals. In Pakistan, a number of studies are available from different parts of the country regarding prevalence and control of gastrointestinal parasitism in equines (Goraya *et al*. 2013a, Goraya *et al*. 2013b, Tahir *et al*. 2016). While a majority of the earlier studies hailed from urban and semi-urban Pakistan, information is lacking regarding gastrointestinal parasitism in equines of rural communities like Federally Administered Tribal Area. Equines play an important role in the subsistence of the rural communities of Pakistan, especially in the agriculture and transportation sectors. Survey of the literature has indicated that the prevalence of equines helminthiasis varies considerably from country to country, which may be attributed to the underlying differences in the agro-climatic conditions, and/or varied management and parasite control practices around the world (Chapman *et al*. 2002; Montenaro *et al*. 2002; Boxell *et al*. 2004; Capewell *et al*. 2005). For instance, the worldwide prevalence of gastrointestinal tract (GIT) helminthiases in horses ranged from 12.4% in France to 100% in Romania (Morariu *et al*. 2012).

There are many studies available, focusing on the prevalence of nematodes in horses and donkeys; information is scarce regarding the prevalence of nematode infection in horse, donkey, and mule in combination. Our results showed 72.7% prevalence of nematods in the studied equines which is in close agreement with an earlier study by Tesfu *et al*. (2014). Tesfu and colleagues, while conducting their research on the prevalence of gastrointestinal nematodes of horse and donkey in Ethiopia, have found an overall prevalence of 72.7%. While there is a close similarity between our findings and the earlier study regarding the prevalence of nematodes in Donkeys (77.85 and 78.5%, respectively), there is wide disagreement between the two studies with respect to the prevalence of nematodes in horses (44.6% vs. 63.7%).Similarly, our findings are not in agreement with yet another study which has suggested 66.5% prevalence of nematodes in horses (Saeed *et al*. 2010). Thus, the disagreement between our findings and previous studies could partially be attributed to the apparent difference in the number of samples obtained and/or the distinct geographic localities.

Researchers have associated helminths infection in equines with different parameters like age, gender, health status, geography, and species of the host animals. For example, several earlier epidemiological studies have indicated that young equines are more susceptible to acquire nematodes infection compared to the older equines (Saeed *et al*. 2010; Tesfu *et al*. 2014; Yadav *et al*. 2014). On contrary, our findings did not show any statistical difference in prevalence of nematodes infection between young and adult equines ($p>0.05$). Our results are supported by previous studies conducted in Turkey and Ethiopia (Aypak and Burgu, 2013; Mezgebu *et al*. 2013). With few exceptions, it is widely anticipated that female animals are more infected with nematode parasites compared to the male counterparts (Aypak and Burgu, 2013; Wosu and Udobi, 2014; Yadav *et al*. 2014). Most surprisingly, our results are in total disagreement with this notion by suggesting that male is more prone to

nematode infection compared to the female animals. This observed disharmony between our study and several previous reports could be attributed to the least number of male animals encountered in our study.

Finally, the prevalence of equines nematode in the present study showed a significantly higher difference ($p<0.0001$) with respect to body condition of the animals, that is, the prevalence of nematodes was always highest among the emaciated animals as compared to weak and healthy animals. This is strongly supported by earlier reports, which suggested higher prevalence of GIT helminths in equines with poor body conditions as compared to the healthy ones (Mezgebu *et al*. 2013; Tesfu *et al*. 2014).

Conclusions: The current study suggests 72% prevalence of nematodes in equines of the study area. The prevalence of equine's nematode showed significant variation with respect to parameters like geography, host's species, gender, and health condition; however, no difference in prevalence of nematodes was observed between young and adult equines.

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