Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.016

RESEARCH ARTICLE

Prevalence, Hematology and Chemotherapy of Gastrointestinal Helminths in Camels

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ARTICLE HISTORY (17-292)

Received: August 14, 2017
Revised: September 05, 2017
Accepted: September 07, 2017
Published online: January 23, 2018

Key words:
Albendazole
Cholistan
Gastrointestinal helminths
Hematological parameters
Neem leaves

ABSTRACT

Gastrointestinal helminths (GI helminths) are of utmost importance in camels affecting their working potential and productivity. This study was aimed to monitor the status of GI helminths in camels of Cholistan region. This accompanied evaluation of effects exerted by helminthosis on various hematological parameters and comparative therapeutic trails of albendazole and Neem (*Azadirachta indica*) leaves against GI helminths in camels. A total of 384 camels were randomly selected in this study. The presence of helminths ova was observed using direct smear method, sedimentation and flotation techniques. An overall prevalence of GI helminths was recorded 66.67% in the study area. Trematodes were found the most prevalent parasite's type followed by nematodes and cestodes. Animals in age range of >10 years were significantly ($P<0.05$) affected more with nematodes than younger animals (5-10years age). A non-significant ($P>0.05$) difference in the prevalence between Berella and Marrecha breeds of camel was observed except for the trematodes where Marrecha breed was affected more (34.67%) than the Berella breed (25.16%), ($P<0.05$). *Hemonchus spp.* were the most prevalent compared to other nematodes ($P<0.05$), a non-significant difference was seen in the prevalence among *Fasciola gigantica* (*F. gigantica*) and *Fasciola hepatica* (*F. hepatica*), ($P>0.05$). The only cestode found in this study was *Monieza expansa* (*M. expansa*). The hematological study found a significant decrease in values of packed cell volume (PCV %), total erythrocyte count (TEC), hemoglobin (Hb) and increased in values of total leukocyte count (TLC), ($P<0.05$). The therapeutic trial conducted revealed albendazole as a superior drug over Neem leaves in control of GI helminths in camels; however, Neem leaves proved to be a successful candidate as an alternative to albendazole.

To Cite This Article: Ijaz M, Zaman MA, Mariam F, Farooqi SH, Aqib AI, Saleem S, Ghaffar A, Ali A and Akhtar R, 2018. Prevalence, hematology and chemotherapy of gastrointestinal helminths in camels. Pak Vet J, 38(1): 81-85. http://dx.doi.org/10.29261/pakvetj/2018.016

INTRODUCTION

Camel is a versatile animal of arid and semi-arid zones of the world. It not only serves the people living in desert areas with transportation but also with milk and meat (Hussain *et al.,* 2016). In Cholistan (Pakistan) Mareecha or Mahra is used for dancing and racing while Berella is a milch breed as it has capacity to give 10-15 liters of milk per day (Ali *et al.,* 2016). The low level of cholesterol and sugar and higher level of vitamin C as compared to cattle milk make camel milk a healthy food for people living in arid and semi-arid zones (Mullaicharam, 2014). Parasitic diseases are the key constraints in livestock industry as they affect the quality of meat and milk in camels (Anvari-Tafti *et al.,* 2013).

The enteric infection in camels is caused by a number of helminths. Camels are less prone to helminthic diseases because of the typical browsing habit. However, there are several parasites which affect camels. Gastrointestinal nematodes which commonly affect the camels are *Strogyloides*, *Trichostrogylus*, *Haemonchus*, *Nematodirus*, *Nematodirella*, *Marshallagia*, *Trichuris*, *Camelostrongylus*, *Coopera* and *Ostertagia* while trematodes of camels are *Fasciola hepatica*, *Fasciola*

gigantica, *Paramphistomum species*, *Dicrocelium dendriticum*, *Schistosoma species* and *Eurytrema pancreaticum* and major cestodes reported from camels are *Stilesia vittata*, *Moniezia expansa*, *Cysticercus tenuicollis*, Hydatid cyst and *Cysticerus dromedarii* (Parsani *et al*., 2008). Helminthosis may be subclinical or asymptomatic, lowers the performance potential of camels (Borji *et al.*, 2010).

Resistance to drugs used against nematode infections has become a serious problem in livestock. Furthermore, there is evidence of resistance to ivermectin, and the results of efficacy evaluations coupled with detection of resistance mutations in soil transmitted helminths raise the possibility that it may be developing in gastrointestinal nematodes of livestock and humans. The main anthelmintic classes, based on drug chemistry and drug receptors/modes of action, are the benzimidazoles (BZs), the macrocyclic lactones (MLs), and a variety of drugs that act on acetylcholine receptors (AChRs), such as levamisole and monepantel. Resistance has arisen in several nematode species to all of these drug classes (Prichard, 2017).

The antihelminthic property of certain plants such as; Neem has been evaluated and has revealed that, the probable mode of action can be due to the presence of an active alkaloid compound, azadirachtin, which interferes with the central nervous system of parasite via inhibition of excitatory cholinergic transmission and partly blocks the calcium channel resulting in expulsion of parasites from host body (Veerakumari and Priya 2006; Qiao *et al.*, 2013; Hamad *et al.*, 2014).

This study describes the prevalence, hematological analysis and chemotherapy of GI helminths in camels of Cholistan region, Pakistan and will provide baseline data for further work on GI helminths in camels.

MATERIALS AND METHODS

Study site: This study focuses Cholistan desert lying in the south-west of Punjab province, Pakistan. Cholistan desert is covering an area of 26,000 km^2 with patches of highly saline soil. It is situated between longitudes of 57° to 60°E and latitudes of 27° to 42° and 29°N (Akhter and Arshad, 2006). This desert is located at 112m above the sea level with 28.33ºC of mean annual temperature, occasionally exceeding 46ºC.

Study design and sampling: This study was designed on random based selection of subjects from the population. Keeping in view the unknown status of helminth's prevalence in the study area, a total of 384 animals were selected based on assuming 50% prevalence (Thrushfield, 1986). A data capture form was accompanying each sampling unit where information related to age, sex, and breeds of study animals were entered.

Collection and examination of fecal samples: About 5 grams of fresh fecal samples were collected from the rectums of camels and were packed in polythene bags. The samples were transported to the Medicine laboratory, Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences Lahore maintaining the cold chain. According to their consistencies samples were categorized into different fecal scores, score-1 (normal feces), score-2 (semisolid feces) and score-3 (diarrheic feces). The presence of helminths ova were observed using direct smear method, sedimentation and flotation techniques following the manual devised by Kaufmann (2013). The parasites genera were identified based on morphological evidences and micrometry as described by Bhoyar *et al*. (2012). The ova count was performed by McMaster technique described by Vadlejch *et al*. (2011).

Hematological examination: The hematological study was carried out in a total of 10 camels suffering from GI helminthiasis. In addition 10 healthy animals free from GI helminths were included as control group. Effect of helminths on various hematological parameters like; Hb, PCV%, TEC and TLC were monitored. The values were compared between the healthy and affected animals.

Therapeutic trial: A total of 20 camels of mixed breed, sex and age positive for helminths and having EPG above 200 were selected randomly and separated into two groups (A and B) comprising 10 camels each. The camels of group A were treated with dry Neem (*Azadirachta indica*) leaf powder 100 gm/camel for five days, group B was treated with albendazole granules® (Albendazole) at a dose rate of 15mg/kg body weight. Fecal samples were collected at day 0 (pre-medication) and on days 3, 7, and 14 (post-medication), respectively. Drug efficacy was calculated as described by Varady *et al*. (2004):

$$\left[\frac{(\text{Pre treatment EPG} - \text{Post treatment EPG})}{\text{Pre treatment EPG}}\right] \times 100$$

Statistical analyses: Data regarding prevalence of GI helminths were evaluated using the X^2 test (Aqib *et al.*, 2017). Probability levels; $P<0.05$ was considered significant whereas data regarding hematology and periodic EPG counts was analyzed through independent *t*-test. Data regarding the therapeutic trials was analyzed by formula as described by Varady *et al.* (2004). All the analyses were performed using SPSS (Statistical Package for the Social Sciences) version 20.

RESULTS

This study showed an overall prevalence 66.67% (256/384) of GI helminths in Cholistan region. Trematodes were the most prevalent parasites' type followed by nematodes and cestodes presenting 30.73%, 26.30% and 09.64% prevalence, respectively. Prevalence of nematodes was significantly ($P<0.05$) higher in male than in female animals presenting 35.71% and 22.43%, respectively. Trematodes were more prevalent in female 31.62% than male 28.57% animals. Similarly, cestodes were also found more in female 10.29% than male 08.04% animals. The variation in prevalence among the two sexes was significant ($P<0.05$) in case of nematodes but was found non-significant ($P>0.05$) in case of trematodes and cestodes. Age was found as a major factor for the occurrence of various parasite types. Prevalence of nematodes was more in >10years of animals (38.69%) as

compared to the animals of 5-10years age group (19.43%). The presence of trematodes was more in >10years age group (40.14%) as compared to the age group 5-10years (25.51%); while, cestodes were found to be slightly more prevalent in 5-10years (09.72%) as compared to >10years (09.49%). The prevalence difference for the two age groups was significant (P<0.05) for nematodes and trematodes while, a non-significant (P>0.05) difference was seen in case of cestodes. Breed wise prevalence was monitored for GI helminths in Marrecha and Berella breeds. Prevalence of nematode, cestode and trematode in Marrecha breed was 26.67, 34.67 and 10.22%, respectively. While, in case of Berella breed, prevalence of nematodes, trematodes and cestodes was 25.79, 25.16 and 08.80%, respectively. A significant (P<0.05) difference in prevalence of trematodes was seen between the two breeds, however, difference was non-significant (P>0.05) in case of nematodes and cestodes (Table 1).

Genus wise prevalence of GI helminths in camel is shown in table 2. Amongst nematodes *Haemonchus* was the most prevalent followed by; *Trichiuris*, *Camelostrongylus*, *Nematodirus*, *Strongyloides* and *Trichostrongylus* presenting 7.81, 7.29, 4.17, 2.86, 2.34 and 1.82% prevalence, respectively. There was a significant (P<0.05) difference in the prevalence among various nematodes in camels. In case of trematodes, *F. hepatica* and *F. gigantica* were 16.93 and 13.80%, respectively. There was no significant (P>0.05) difference among prevalence of trematodes. The only cestode found in this study was *M. expansa* showing 9.64% prevalence.

Effect of GI helminths on various hematological parameters of camel is shown in (Fig. 1). Blood samples of healthy and affected animals were analyzed. A decreasing fashion in values of Hb (g/dl), PCV (%) and TEC (10^6/µl) was observed i.e. 8.48±0.85, 27.69±0.88, 3.98±0.70, while a raise in TLC (19.24±1.14 10^3/µl) was noticed in affected animals. These values were compared with those of healthy animals revealing; 11.54±0.45, 34.76±1.67, 6.99±0.61 for Hb, PCV % and TEC, while 12.29±0.46 for TLC.

EPG count of GI helminths in groups A and B is shown in (Fig. 2). The EPG counts of group A and B pre-treatment (Day 0) were 410±86.763 and 385± 105.541, respectively. Group A was treated with Neem leaves and the effect on EPG counts at day 3, 7 and 14 were 342.5±66.719, 290±69.920 and 185±57.975, respectively. Albendazole was given to group B animals and the EPG counts recorded post-treatment on day 3, 7 and 14 were; 320±97.752, 250±91.287 and 165±81.819, respectively. Decrease in EPG was seen in both the treatment groups. However, in terms of comparative efficacy group B (albendazole) showed significantly (P<0.05) higher efficacy as compared to group A (Neem leaves). Comparative details of efficacy have been shown in Table 3. On post-treatment day 3 Neem leaves cured 20%, while albendazole cured 30%. On 7th and 14th day post-treatment the Neem leaves cured 40% and 60% animals while albendazole cured, 60% and 80% animals, respectively.

DISCUSSION

This study showed higher prevalence of nematodes in males (35.71%) as compared to females (22.43%) which is in agreement with the results of Birhanu *et al.* (2014) who also observed higher prevalence of GI nematodes in males 64.7% than in females 55.04%. However, the results does not correlate with Swai *et al.* (2011) who reported increased prevalence of GI nematode in female 68.1% than in male 46.9%. The higher prevalence in males can be attributed to more drastic work load and exposure to parasites than female. The contrasting results shown by Swai *et al.* (2011) can be attributed to various stress factors associated with different physiological statuses in female animals. In this study, overall presence of nematodes in >10 years age group was more 38.69% as compared to 5-10 years age group 19.43% which is not in line with the studies of Birhanu *et al.* (2014) and Swai *et al.* (2011) who reported increased prevalence of nematodes in 5-10 years i-e 69.05% and 70% than >10 years i-e 53.8% and 60.5% respectively. The possibility of this difference might be the unawareness of our farmers in taking good care of their animals. Secondly suppression in immunity build up with aging is also a major factor.

The genus wise prevalence of GI nematodes in this study is in line with the study of Bekele, 2002, who dictated that *Hemonchus* as the most prevalent among all other nematodes. In this study, the overall prevalence of fasciolosis (trematode) in camel was recorded 30.73% which is in agreement to the study of Khosravi and Babaahmady, (2012) who found the 34.6% prevalence of Fasciolosis. The overall prevalence of *M. expansa* was 9.64%. These results are nearly closer to those of Duguma *et al.* (2014), where the prevalence of *M. expansa* was 6.91%.

This study found significant effects of GI helminths over various hematological parameters like PCV (%), TEC, Hb and TLC. Decreasing pattern was seen in Hb, PCV (%) and TEC; while, the TLC showed increasing pattern in affected animals. These findings are in line with those of Rabana *et al.* (2011) who also reported that PCV, mean hemoglobin concentration MCH, mean corpuscular hemoglobin concentration MCHC and TEC were significantly (P<0.01) affected in the infected camels compared to the non-infected ones. Osman *et al.* (2014) also conforms to this study reporting decreasing

Table 1: Sex, age and breed wise prevalence of gastrointestinal helminths in camel

Parameter	Variable	Total observed (n)	Nematodes			Trematodes			Cestodes		
			Positive (n)	Prevalence (%)	P value	Positive (n)	Prevalence (%)	P value	Positive (n)	Prevalence (%)	P value
Sex	Male	112	40	35.71	0.007	32	28.57	0.556	09	08.04	0.495
	Female	272	61	22.43		86	31.62		28	10.29	
Age	5-10years	247	48	19.43	0.001	63	25.51	0.003	24	09.72	0.942
	>10 years	137	53	38.69		55	40.15		13	09.49	
Breed	Marrecha	225	60	26.67	0.847	78	34.67	0.047	23	10.22	0.643
	Berella	159	41	25.79		40	25.16		14	08.80	

P<0.05 indicate significant results.

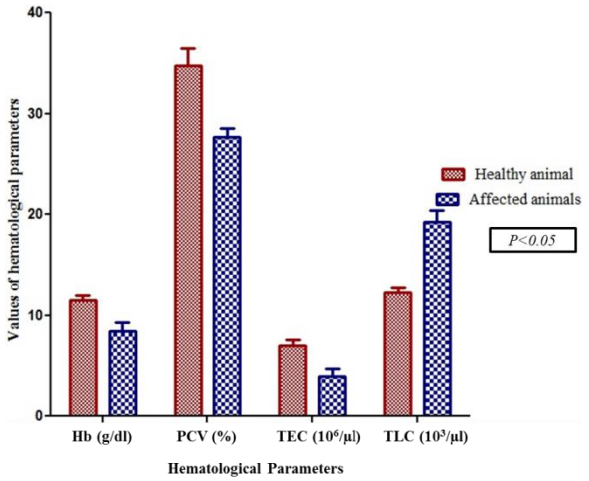

Fig. 1: Comparison of hematological parameters between healthy and parasites affected animals.

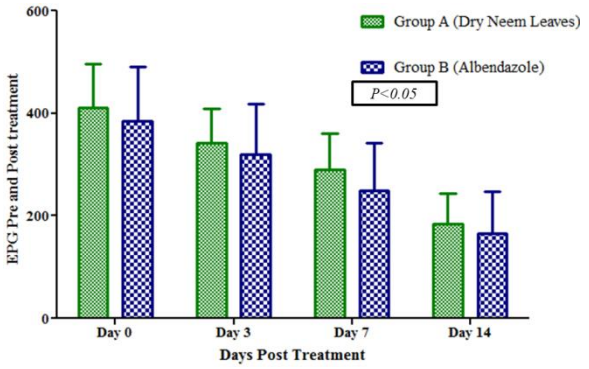

Fig. 2: Comparison of pre-treatment and post-treatment EPG levels between Group A and B.

pattern in Hb, PCV (%) and TEC while, increasing pattern in TLC. Sena *et al.* (2000) also found that GI helminths decrease hemoglobin content, total serum proteins, lymphocytes, albumin and albumin-globulin ratio, and increase total leukocyte count, eosinophils, neutrophils, globulin, serum glucose and monocytes.

This study showed that albendazole gave 80% cure in treated animals and proved superior to Neem leaves which cured 60% animals. These findings are in line with those of Delatour *et al.* (1989) who also reported albendazole as suitable anthelminthic in camels. Another study conducted by Yadav and Kumar, (1990) also conforms to our finding declaring albendazole as a superior anthelmintic over other preparations. Similarly, Mukhwana and Mitema, (1997); Mouldi *et al.* (2015) also reported albendazole as efficacious anthelmintic in different studies. The antihelminthic property of certain plants such as; Neem has been evaluated and has revealed that, the probable mode of action can be due to the presence of an active alkaloid compound, azadirachtin, which interferes with the central nervous system of parasite via inhibition of excitatory cholinergic transmission and partly blocks the calcium channel resulting in expulsion of parasites from host body (Veerakumari and Priya 2006; Qiao *et al*. 2013; Hamad *et al.,* 2014).

However, the results of Neem leaves in current study make it a successful replacement in remote areas where the farmers have less access to the market for purchase of allopathic preparations.

Table 2: Genus wise prevalence of gastrointestinal helminths in camel

Parasite specie	Positive (n=384)	Prevalence (%)	P value
Nematode			
Camelostrongylus	16	04.17	0.047
Trichostrongylus	07	01.82	
Strongyloides	09	02.34	
Haemonchus	30	07.81	
Nematodirus	11	02.86	
Trichuris	28	07.29	
Trematode			
Fasciola hepatica	65	16.93	0.310
Fasciola gigantica	53	13.80	
Cestodes			
Moniezia expansa	37	09.64	NA

*P<0.05 indicates significant value.

Table 3: Comparative efficacy of Neem leaves and Albendazole against GI Helminths

Drug	Efficacy (%) at day		
	3rd day	7th day	14th day
Neem leaves	20	40	60
Albendazole*	30	60	80

*Indicates significant difference (P<0.05).

This study concludes that GI helminths of camel are of considerable importance to camel industry. These parasites decrease the work potential of camels by affecting the vital physiological parameters. From therapeutic trial it is clear that though albendazole is superior drug in controlling the nematodes burden in camels however, Neem leaves have the potential to be used as alternative to albendazole in remote areas with lesser access of the farmers to medicines market. As anthelmintic activity was associated with the presence of alkaloid or other compounds in Neem so there is need for further studies in order to determine the active component, route of administration, their lethal dose and which parasite species or developmental stages are most susceptible to the effects exerted by the extract so as to further enhance their anthelmintic usefulness.

Acknowledgements: The author is thankful to higher education commission Pakistan, Medicine laboratory, University of Veterinary and Animal Sciences, Lahore for the provision of laboratory and technical assistance for the smooth run of this research study.

Authors contribution: MIZ and MI, designed and executed the study, FM, SHF, AIA did sampling from the study area, FM, SHF processed the samples, SS, AG, AA compiled the data into excel sheet and analyzed the data statistically. AG, SS, SHF wrote the manuscript, MI, AIA and MIZ reviewed the manuscript critically.

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