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PREVALENCE OF GASTROINTESTINAL HELMINTHS IN PASTORAL SHEEP AND GOAT FLOCKS IN THE CHOLISTAN DESERT OF PAKISTAN

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ABSTRACT

Small ruminants are a major source of cash for many rural populations, especially in semi-arid and arid regions of developing countries. Extensively managed animals often host gastrointestinal parasites, and even chronic infestations lead to economic losses. We evaluated the prevalence of gastrointestinal helminths in sheep and goats of the Cholistan desert, Pakistan, where livestock is the backbone of the regional economy. Fresh faeces (10 - 15 g) were collected from 500 sheep and 500 goats across five different localities. Standard parasitological techniques served to identify parasite eggs, and copro-culture enabled larval determination of specific nematodes. Overall helminth prevalence was 78.1% across the 1000 animals; pure nematode infestations were most prevalent (37.5%), followed by pure trematode (7.9%), pure cestode (2.6%) and pure protozoa infestations (0.8%). Mixed infestations with nematodes and trematodes occurred in 6.4% of all animals, mixed nematode-cestode infestations in 3.8%, and all three groups were found in 19.1% of the sheep and goats. In goats more males (81.1%) than females (77.0%) were infested, the opposite was found in sheep (73.6% males, 79.5% females). Parasites were especially prevalent in suckling goats (85.2%) and sheep (88.5%) and to a lesser extent in young (goats 80.6%, sheep 79.3%) and adult animals (goats 72.8%, sheep 73.8%). Given the high infestation rates, particular attention should be paid to management of suckling animals. A general means of reducing infestation rates might be the systematic testing of traditional plant-based remedies against helminths for cheap and regular deworming of the herds.

Keywords: Cestodes; Extensive grazing system; Gastrointestinal parasites; Small ruminants; Nematodes; Trematodes.

INTRODUCTION

Livestock production contributes substantially to the livelihoods of resource-poor rural farmers in Pakistan and plays an imperative role in poverty alleviation by strengthening the socio-economic conditions of pastoralists (Gadahi *et al*., 2009). Pakistan's livestock sector provides approximately 55.1% of the agricultural value added and 11.6% of the national GDP. The gross value added of this segment at constant factor cost has increased by 4% from 5.29 billion Euro (2010-2011) to 5.51 billion Euro (2011-2012; Economic Survey of Pakistan, 2012). Historically the livestock sector was subsistence-oriented and dominated by smallholders, and even today livestock are considered a more secure source of income for small farmers and landless poor, and as a source of employment generation at the rural level (Gadahi *et al*., 2009; Khajuria *et al*., 2012). This is particularly true for sheep and goats (Khajuria *et al*., 2012), of which numbers have doubled in Pakistan during the past 15 years (Iqbal and Jabbar, 2005). This can be ascribed to the relatively low inputs needed such as startup capital, feedstuffs and maintenance expenditures as compared to large ruminants (Terefe *et al*., 2012).

Small ruminants are under sober coercion of clinical and sub-clinical gastrointestinal helminth infestation in developing countries, which reduces their productive and reproductive potential (Zeryehun, 2012; Ayaz *et al*., 2013) due to decreasing voluntary feed intake and/or feed conversion efficiency (Kanyari *et al.*, 2009). Especially the ineffective use of absorbed nutrients leads to retarded growth (Sykes, 1994; Terefe *et al*., 2012) and provokes anemia and even mortality at heavy infestation (Hassan *et al.*, 2011). In addition to these threats, a helminth infestation lowers the animal's immunity and renders it susceptible to other pathogenic infections; finally this may result in heavy economic losses (Garedaghi *et al.*, 2011). The problem is however much more severe in tropical countries due to very favorable environmental conditions for helminth transmission (Mohanta *et al*., 2007; Zeryehun, 2012), poor nutrition of the host animal (Mbuh *et al*., 2008) and poor sanitation in rural areas (Badran *et al*., 2012). As a result diseases caused by helminths remain a major impediment to small ruminant production in the tropics (Kumsa *et al*., 2011), and up to 95% of small ruminants are reported to show helminth infestation in these latitudes (Opara *et al*., 2005; Mbuh *et al*., 2008; Terefe *et al*., 2012). However, the majority of animals infested with helminths do not show clinical signs owing to the chronic nature of the disease.

Three classes of helminths are distinguished, namely nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes). Several authors (Raza *et al.*, 2007, 2009; 2012; Ijaz *et al*., 2009; Khan *et al.*, 2010; Farooq *et al.*, 2012) have explored various aspects of helminth infestation in different localities of Pakistan and reported prevalence ranges of 25 - 92%. There are still numerous geographical regions in Pakistan where the livestock population needs to be screened for the presence of gastrointestinal helminths in view of their high economic significance. No report is available on prevalence of gastrointestinal helminths in the Cholistan desert of Pakistan, where livestock husbandry is the primordial occupation of the pastoralist communities and where traditionally wealth has been assessed based on the number of animals, especially goats and sheep, owned by an individual. Since the resource-poor pastoralists of this area have very limited access to veterinary services, an analysis of the *status quo* of helminth infestation of their animals should precede the design of appropriate and accessible means to effectively counteract eventual problems. This was the objective of the present study.

MATERIALS AND METHODS

Study area: The Cholistan desert covers area of 2.6 Mio. hectares and is located in the southern Punjab between latitudes 27°42′ and 29°45′ N and longitudes 69°52′ and 75°24′ E (FAO, 1993). Average annual rainfall is only 128 - 175 mm, therefore crop cultivation is only possible under irrigation near streams or wells. The region’s total livestock population has been estimated at 1.29 Mio. heads (Livestock Census of Pakistan, 2006), which is almost twice that of the human population in this area.

The people of Cholistan lead a semi-nomadic life, moving from one place to another in search of water and fodder for their animals. For their livestock and for themselves, the local tribes store rainwater in man-made ponds in the ground or between sand-hills – these ponds are called ‘*tobas*’. Habitations are small and scattered around the *tobas*. For this study five localities that were at least 30 km distant from each other were selected, each locality comprising several villages and *tobas*, respectively (Figure 1).

Sample collection: Five hundred sheep and 500 goats, from a total of 100 pastoral flocks (comprising each between 5 – 250 animals) across the 5 localities were randomly selected. A ratio of 20: 40: 40 of suckling, young, and adult animals was sampled in each species, and the male to female ratio was 30 : 70. From January to May 2011, one sample of fresh faeces (approx. 10 - 15 g) was collected from the rectum of each animal by using plastic gloves. The sample was put in a sterile zipper polythene bag coded with the locality, household ID, species, age and sex of the animal. Samples were placed into an air tight cool box until arrival at the laboratory and were refrigerated at 4°C until analysis (Hayat and Akhtar, 2000). After faecal examination, samples were preserved in 10% formalin for backup purpose.

Faecal examination: Examination of faecal samples was performed by using standard direct and indirect parasitological techniques (flotation and sedimentation) as suggested by Hayat and Akhtar (2000) and Soulsby (1987). Eggs of the different helminths were identified on the basis of morphological appearance and size with the help of keys (MAFF, 1979; Soulsby, 1987; Urquhart *et al*., 1996). For the identification of certain nematodes, copro-culture was performed to obtain larval stages as described by MAFF (1979). Faecal samples containing parasitic eggs that could not exactly be identified were finely crushed with a pestle and mortar or a spatula, and were placed in a glass jar or petri-dish which was closed and incubated at 26°C for 7 days. After incubation, samples were examined for the presence of larvae, which were identified with the help of keys (MAFF, 1979).

Data analyses: Differences between independent variables (location, host species, sex and age group) with respect to prevalence of individual parasite species, or helminth groups, respectively, were explored using Chi-square test (categorical variables) or Kruskal-Wallis test (continuous variables), whereby continuous variables had first been tested for normality (Kolmogorov-Smirnov test). Data was analyzed using SPSS 17.0 software (SPSS Inc., Chicago, USA). Relative prevalence of different helminth species or groups, respectively, was calculated as follows:

Prevalence (%) = [Number of positive samples / Total number of samples examined] x 100

RESULTS AND DISCUSSION

The overall prevalence of gastrointestinal (GI) parasites in the 1000 sheep and goats was 78.1%, with 78.2% (n=391) in goats and 78.0% (n=390) in sheep across the five different locations (Table 1). In goats, 81.1% (116 of 143) male and 77.3% (275 of 357) female animals harbored GI parasites, whereas 73.6% (95 of 129) male and 79.5% (295 of 371) female animals were infected in sheep (Table 2). It is interesting to note that in goats the prevalence of helminths was higher in males compared to females, while in sheep the opposite was found. Normally females are assumed to be more heavily infested due to hormonal differences and stress during pregnancy. In goats the present results may be due to the stall feeding of female animals during pregnancy, which reduces exposure to pasture contamination (Pal and Qayyum, 1992; Maqsood *et al.*, 1996; Ayaz *et al*., 2013).

As far as different age groups were concerned, 85.2% (75 of 88) suckling, 80.6% (166 of 206) young and 72.8% (150 of 206) adult goats were parasite-infested, whereas in sheep infection rates amounted to

88.5% (69 of 78) in suckling, 79.3% (138 of 174) in young and 73.8% (183 of 248) in adult animals (Table 2). The age of the host animal was an important factor influencing the prevalence of GI parasites. The higher infection of suckling than that of young and adult animals may be attributed to a weaker immunological response of young animals. Several authors (Urquhart *et al*., 1996; Tariq *et al.*, 2010; Zeryehun, 2012) reported that older animals recover from parasitic infection more quickly as the immunity of the host increases with age; animals may hence become immune, especially as they undergo repeated exposure (Dagnachew *et al*., 2011).

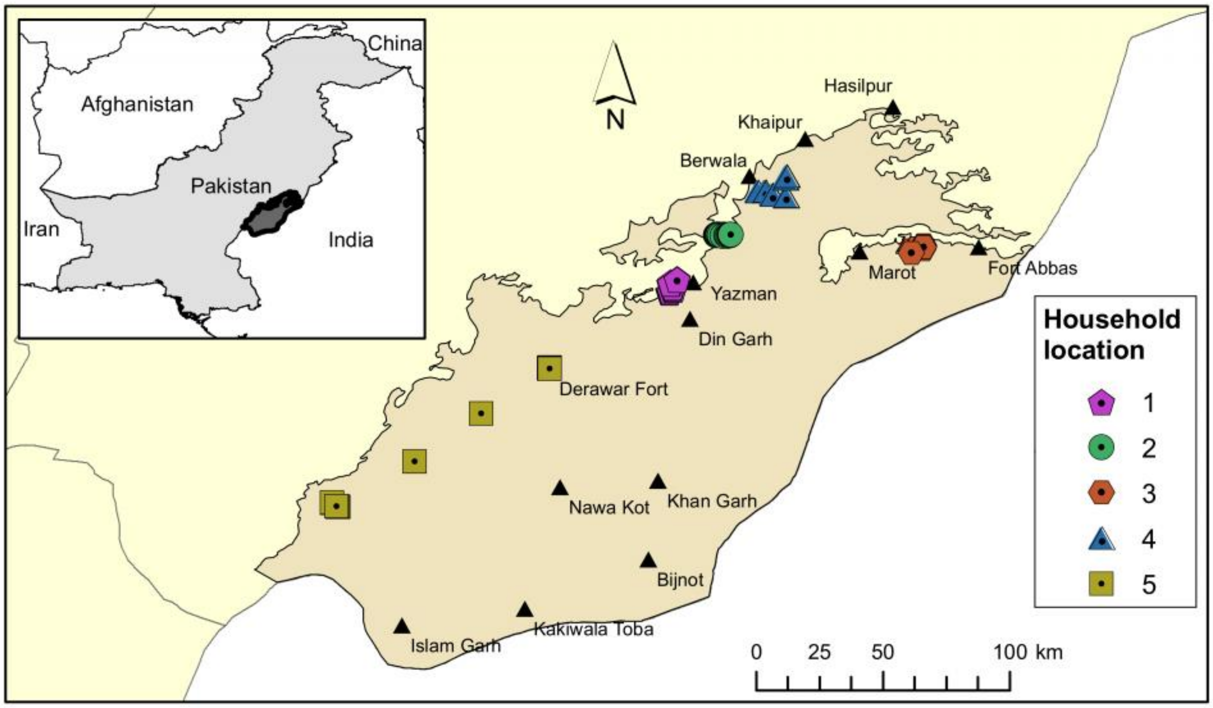

Figure 1. Map of the study area in the Cholistan desert, Pakistan, with the homesteads of the sampled sheep and goat herds and the overall distribution of the five locations.

As far as the identified 24 parasite species are concerned, 15 were nematodes, 4 were trematodes, 4 were cestodes and one was a protozoa. The protozoa species was mostly found in combination with helminths, its exclusive prevalence was 0.8%. While suckling animals hosted on average 1.5 to 7.6 different parasite species, this range reduced to 1.8 – 6.0 in young and 1.4 – 5.6 in adult animals, with significant differences between locations, sex and age groups but not between species (Table 3). The prevalence of 18, 16, 13 and 11 individual parasites varied significantly between locations, animal species, sex and age groups, respectively (Table 4). *Haemonchus contortus* was the most dominant parasite (13.3%), followed by *Trichuris ovis* (5.6%) and *Trichostrongylus axei* (4.7%).

Location significantly affected ($P \leq 0.01$) the overall prevalence (n=1000) of only nematodes (37.5%) and only trematodes (7.9%) in individual animals, with locations 2 and 5 having the highest nematode (9.6%) and trematode infestation (2.8%), respectively, and at the same time the lowest prevalence of trematodes (0.5%) and nematodes (5.6%). Sex had a significant effect ($P \leq 0.05$) on the prevalence of only cestodes (0.2% in males versus 2.4% in females). Mixed infections (Figure 2) were subdivided into (i) simple poly-parasitism, that is infection with species belonging to two different parasite groups (protozoa or helminth species) and (ii) multiple poly-parasitism, namely infection with parasites belonging to more than two different groups. Location and animal species had a significant effect ($P \leq 0.01$) on the prevalence of simple poly-parasitism, whereas occurrence of multiple poly-parasitism was significantly affected by location ($P \leq 0.01$), sex ($P \leq 0.05$) and age group ($P \leq 0.05$). Location and species together had a significant effect on the prevalence of only nematodes and only trematodes, and on the occurrence of simple and multiple poly-parasitism ($P \leq 0.01$ in all cases). In female animals, interactions between animal species and age group significantly affected poly-parasitism of nematodes plus trematodes ($P \leq 0.01$) and nematodes plus cestodes ($P \leq 0.05$). In young animals, interactions between animal species and sex significantly ($P \leq 0.01$) affected the infestation with only nematodes (female sheep 15.6%, male sheep 3.8%, female goats 13.0%, male goats 5.1%) as well as poly-parasitism of nematodes plus trematodes ($P \leq 0.05$), while in adult animals the interactions between animal species and sex affected simple poly-parasitism (nematodes plus trematodes as well as nematodes plus cestodes; $P \leq 0.01$) and multiple poly-parasitism ($P \leq 0.05$). Only in sheep, but not in goats, the interaction between sex and age group significantly affected the prevalence of cestodes ($P \leq 0.01$) and multiple poly-parasitism ($P \leq 0.05$). No case of simple poly-parasitism with trematodes and cestodes was found (Figure 5).

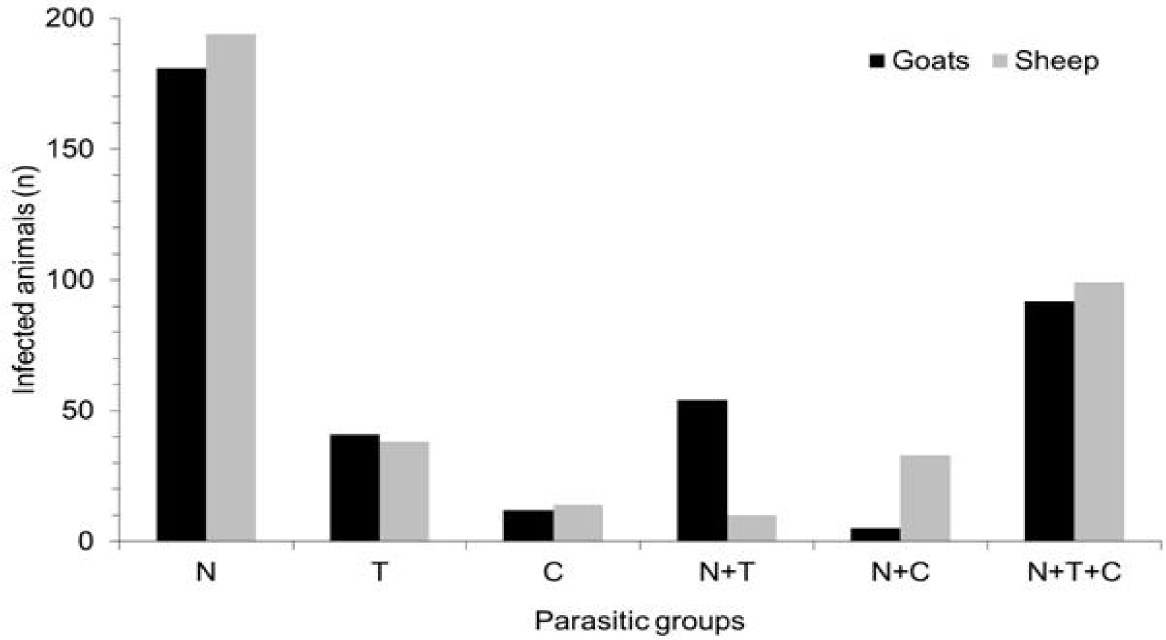

Figure 2. Prevalence of single or mixed infestations with gastrointestinal nematodes (N), trematodes (T) and cestodes (C) in 500 goats and 500 sheep of pastoralists in the Cholistan desert, Pakistan.

Epidemiology is the foundation on which control of parasitic diseases has to be based. There is no recent data available on the prevalence of GI parasites in small ruminants of the Cholistan desert, therefore the findings of this study are indicative for other small ruminant herds under similar management in this environment. The high prevalence of parasites in the screened pastoral sheep and goat flocks supports the notion that parasitic infestations are among the main health problems in small ruminants globally (Wang *et al*., 2006; Mbuh *et al*., 2008; Lone *et al*., 2012; Badran *et al*., 2012; Kantzoura, 2012).

The helminth species and groups recorded in the study area have also been reported previously by Raza *et al*., (2007), Gadahi *et al*., (2009), Khan *et al*., (2010), Akhtar *et al*., (2012) and Ayaz *et al*., (2013) from different areas of Pakistan and other parts of the world (Biu *et al*., 2009: Nigeria; Mohanta *et al*., 2007: Bangladesh; Kanyari *et al*., 2009: Kenya; Dagnachew *et al*., 2011: Ethiopia). Yet, the mentioned studies also reported some other helminth species in addition to those recorded in Cholistan, and variation also exists in the prevalence of different helminth species in different regions, which may be attributed to different host factors and climatic conditions required for the development of the free-living stages of different parasites (Tariq *et al.*, 2010).

The Cholistan sheep and goats were infected with the same principal parasitic species, and one reason for this could be that samples were collected from animals in mixed flocks. Grazing patterns and managerial practices of the pastoralists are almost the same for both species, and sheds, pastures and watering places are shared between sheep and goats. The year-round movements of sheep and goat flocks over a large area may greatly facilitate the spread of parasites Wild ruminants occurring in the region, such as chinkara (*Gazella bennettii)* and blackbuck (*Antilope cervicapra*) constitute a reservoir of helminths for their domestic relatives.

In the present study nematode infections were highly prevalent, followed by trematodes and cestodes. Similar results have been reported for Owerri, southeastern Nigeria (Opara *et al*., 2005); Muzaffargarh, Pakistan (Raza *et al*., 2007), Central Oromia, Ethiopia (Kumsa *et al*., 2011) and Haramaya, southeastern

Table 1. Prevalence of gastrointestinal parasites (number of infected animals) in goats and sheep (100 each per location) of pastoralists distributed across five locations* in the Cholistan desert, Pakistan.

Locations	Goats (n)**	Sheep (n)**	Total infected (n)	Overall prevalence (%)
1	74	68	142	71.0
2	83	81	164	82.0
3	75	80	155	77.5
4	80	86	166	83.0
5	79	75	154	77.0

* For emplacement of locations see Fig. 1.
** Prevalence (%) and infected animals (n) are the same as the total number of animals is 100 per location.

Table 2. Prevalence of gastrointestinal parasites in goats (male=143, female=357) and sheep (male=129, female=371) of different age groups* (goat: suckling=88, young=206, adult=206; sheep: suckling=78, young=174, adult=248) of pastoralists in the Cholistan desert, Pakistan.

Species	Sex	Suckling (n)		Young (n)		Adult (n)	
		Infected	Non-infected	Infected	Non-infected	Infected	Non-infected
Goat	Male	27	30	47	60	42	53
	Female	48	58	119	146	108	153
Sheep	Male	28	30	35	52	32	47
	Female	41	48	103	122	151	201

*Age groups: suckling (≤3 months), young (4-18 months), adult (>18 months)

Table 3. Total mean number (±SD) of different species of gastrointestinal parasites found in 1000 goats and sheep* of pastoralists in the Cholistan desert, Pakistan, and the respective statistical effects of location, age group#, livestock species and sex.**

Location	Male			Female		
	Suckling	Young	Adult	Suckling	Young	Adult
1	7.6 ±6.32	3.0 ±5.45	2.1 ±3.81	1.5 ±2.04	2.7 ±3.10	1.4 ±2.70
2	7.0 ±5.48	3.1 ±4.80	2.2 ±3.49	5.0 ±4.63	3.6 ±4.34	2.6 ±3.94
3	3.6 ±3.86	3.9 ±4.22	2.5 ±3.02	3.1 ±4.21	1.8 ±2.85	1.7 ±2.79
4	4.0 ±4.17	6.0 ±5.25	5.6 ±4.84	4.9 ±5.14	3.9 ±4.37	3.6 ±4.05
5	5.1 ±5.29	4.3 ±5.26	3.8 ±4.80	2.7 ±3.53	3.5 ±4.58	3.4 ±3.99

Variable	df	Mean	SD	χ^2	*P*
Location	4	3.00	1.414	30.15	<0.001
Age group	2	2.29	0.733	19.41	<0.001
Species	1	1.50	0.500	0.59	0.444
Sex	1	1.73	0.445	4.46	0.035

* Kruskal Wallis test; as livestock species had no effect on number of species of gastrointestinal parasites per animal, average values are presented across goats and sheep.
** For emplacement of locations see Fig. 1.
# Age groups: suckling (≤3 months), young (4-18 months), adult (>18 months).

Ethiopia (Zeryehun, 2012). Similarly, the occurrence of simple and multiple poly-parasitism in small ruminants agrees with findings from several locations in Pakistan (Raza *et al*., 2007: Muzaffargarh; Gadahi *et al*., 2009: Rawalpindi and Islamabad) and Ethiopia (Kumsa *et al*., 2011; Tefere *et al*., 2012; Zeryehun, 2012). Poly-parasitism is an important cause of morbidity and loss of production in sheep and goats (Kumsa *et al*., 2011). The impairment of the host's immune system by poly-parasitism increases the animal's susceptibility to other diseases or parasites (Wang *et al*., 2006).

Many nematode species do not require an intermediate host for the completion of their life cycle (Gulland and Fox, 1992), and for egg hatching and larval development they find suitable conditions around the natural water reservoirs of the *tobas*. The most prevalent nematode determined in this study was *Haemonchus contortus,* which agrees with findings from Himachal Pradesh, India (Katoch, 1999), Bokova, Cameroon (Mbuh *et al*., 2008) and Jatoi, Pakistan (Raza *et al.*, 2012). The higher prevalence could be related to the fact that this nematode has a relatively short generation interval and lays up to 10,000 eggs per day for several months. Additionally, this parasite is able to develop resistance against anthelmintics faster than other helminth species (Radostits *et al*., 1994; Katoch, 1999).

Table 4. Prevalence of different species of gastrointestinal parasites in goats and sheep of pastoralists in the Cholistan desert, Pakistan, and the respective statistical effects of livestock species, location, age and sex.

Parasite	**Prevalence (%)**			**Species**	**Location***	**Age****	**Sex**
	Goats (n=500)	**Sheep** (n=500)	**Overall** (n=1000)		*P*≤ [#]		
Bunostomum phlebotomum	1.4	1.4	1.4	n.s.	0.01	0.05	0.05
Chabertia ovina	0.6	0.4	0.5	0.05	0.05	n.s.	n.s.
Cooperia spp.	0.0	0.2	0.1	0.01	n.s.	0.05	0.05
Cotylophora cotylophorum	0.4	0.6	0.5	0.01	0.01	0.01	n.s.
Dicrocoelium dendriticum	1.4	0.4	0.9	0.05	0.01	n.s.	0.05
Echinococcus granulosus	0.8	0.6	0.7	0.05	n.s.	n.s.	0.01
Eimeria spp.	1.2	0.4	0.8	n.s.	0.01	0.05	0.01
Fasciola gigantica	0.6	0.8	0.7	0.01	0.01	0.05	n.s.
Fasciola hepatica	3.2	2.2	2.7	0.01	n.s.	n.s.	0.05
Haemonchus contortus	12.8	13.8	13.3	n.s.	n.s.	0.05	0.01
Haemonchus placei	0.0	0.2	0.1	n.s.	0.01	n.s.	0.01
Moniezia benedeni	0.2	0.2	0.2	0.01	0.05	0.01	0.01
Moniezia expansa	1.0	1.2	1.1	0.01	0.01	n.s.	n.s.
Nematodirus spp.	1.4	0.8	1.1	n.s.	n.s.	0.05	n.s.
Oesophagostomum columbianum	1.0	2.0	1.5	0.01	0.01	0.05	n.s.
Oesophagostomum radiatum	1.8	1.4	1.6	0.01	0.01	n.s.	n.s.
Ostertagia circumcincta	0.8	1.2	1.0	0.01	n.s.	n.s.	0.01
Ostertagia oestertagi	1.6	3.0	2.3	0.01	0.05	0.05	0.05
Paramphistomum cervi	3.0	4.2	3.6	0.01	0.01	n.s.	n.s.
Strongyloides papillosus	1.4	1.2	1.3	0.01	0.01	n.s.	n.s.
Trichostrongylus colubriformis	0.2	1.2	0.7	n.s.	0.01	0.01	0.01
Trichostrongylus axei	3.8	4.6	4.2	n.s.	0.05	n.s.	n.s.
Trichostrongylus ovis	2.2	3.4	2.8	0.01	0.01	n.s.	0.05
Trichuris ovis	7.2	4.0	5.6	n.s.	0.01	n.s.	n.s.

* For locations see Figure 1; ** Age groups: suckling (≤3 months), young (4-18 months), adult (>18 months);
[#] Chi square test; n.s. = non significant.

Conclusions: In Cholistan, pastoralist flocks of small ruminants are heavily infested with a variety of GI parasites including some that potentially entail substantial economic losses. Especially suckling animals carry a severe helminth burden, which indicates that particular attention should be paid to their management. In view of high prices, unavailability or inaccessibility of drugs and veterinary services, systematic deworming of animals with a broad-spectrum anthelminthic cannot be recommended to pastoralists unresentedly. Therefore inexpensive locally applied measures such as the use of plant-based remedies against GI parasites should be systematically evaluated for their effectiveness against the most prevalent helminth species, so as to devise cheap but effective remedies that pastoralists can synthesize themselves and use regularly.

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