

## Original papers

# Parasites of markhor, urial and Chiltan wild goat in Pakistan

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**ABSTRACT.** Parasites are transferred between domestic and wild animals, when host animals come in contact with each other, particularly while grazing the same pastures, or when using same water bodies for drinking. Chances of parasite transmission and adaptation are high when hosts are genetically related. Afghan urial (*Ovis vignei blanfordi*), Suleiman markhor (*Capra falconeri jerdoni*) and Chiltan wild goat (*C. aegagrus chialtanensis*) are wild kin of domestic sheep and goats, sharing numerous parasitic diseases with each other. The present study was conducted in 2014–2015, to determine parasitic infections of Suleiman markhor and Afghan urial of Torghar Game Reserve, and the endemic wild goat of Chiltan National Park. For comparison, parasites of domestic small ruminants of these areas were also studied. A total of 11 species of helminth and 20 species of protozoa were recorded. Highly prevalent helminth among wild ruminants were *Trichuris* spp., *Nematodirus* spp., *Protostrongylus rufescens* and *Moniezia benedeni*, while highly prevalent *Eimeria* were *E. arloingi* and *E. ninakohlyakimovae* in caprines and *E. ovinoidalis* in urial. Chiltan wild goats were also found infected with *Entamoeba* spp. A short tabulated review of the helminth and protozoan parasites of wild sheep and goats of Pakistan, India, Iran and Turkey has been presented.

**Keywords:** markhor, urial, wild goat, Chiltan, Torghar, parasites

## Introduction

Pakistan is home to greatest diversity of wild sheep and goats found anywhere on Earth. Controversies in systematics and nomenclature of animals belonging to the genera *Capra* and *Ovis* are greatly endeavored by scientists [1–3]. In Pakistan these nine species and subspecies of wild sheep and goats are recognized [4]: Asiatic ibex (*Capra [ibex] sibirica*), Astor/Gilgit markhor (*C. falconeri falconeri*), the Pir Panjal/Chitral markhor (*C. f. cashmirensis*), straight-horned/Suleiman markhor (*C. f. jerdoni*), Persian pasang/bezoar/Sindh wild goat (*C. aegagrus blythi*), Chiltan wild goat (*C. a. chialtanensis*), Afghan urial (*Ovis vignei blanfordi*), the Punjab urial (*O. v. punjabiensis*) and argali/Marco Polo sheep (*O. ammon polii*). Difference among the three subspecies of urial, i.e., Afghan, Punjab and Ladakh urial is not very clear, and so is between Chitral and Gilgit markhor.

Balochistan is the largest province of Pakistan by land, having many Government owned and private wildlife refuges, Chiltan and Torghar being two of the famous among them. Chiltan National Park is

famous for its endemic population of Chiltan wild goat (*C. a. chialtanensis*), while Torghar Game Reserve conserves numerous species of mammals, important being Suleiman markhor (*C. f. jerdoni*) and Afghan urial (*O. v. blanfordi*). In Chiltan National Park, poaching led into extinction of urial in 1980s [4], yet Chiltan wild goat survived, with unsteady population trends seen in coming years [1]. In Torghar in late 1980s, due to extensive hunting, only 100 markhor and urial each were remaining [5]. The local tribal leaders established a licensed trophy hunting program in participation with inhabitants of the area for protection of markhor and urial. Two decades later the number of markhor reached 1,684, while that of urial reached 1,742 [6], making the program one of the brightest examples of sustainable use of natural resources [7].

Grazing of domestic animals inside the boundary of Chiltan National Park is illegal, yet breaches being frequently observed. In Torghar, urial and markhor habitat is community owned land, and the domestic sheep and goats forage the same pastures, without any grazing strategy. Hence, based upon information gathered from local tribesmen, it was



Table 2. Percentage prevalence of protozoan parasites in wild and domestic ovines of Chiltan and Torghar

Protozoan Parasites	Chiltan National Park		Torghar Game Reserve	
	Domestic		Afghan	Domestic
	Sheep		Urial	Sheep
	(n=111)		(n=115)	(n=37)
Overall infection	76.58		81.74	56.76
<i>Eimeria ovinoïdalis</i>	33.33		53.04	37.84
<i>Eimeria bakuensis</i>	42.34		18.26	16.22
<i>Eimeria parva</i>	14.41		28.70	29.73
<i>Eimeria crandallis</i>	18.92		–	13.51
<i>Eimeria intricata</i>	8.11		0.87	32.43
<i>Eimeria weybridgetensis</i>	15.32		0.87	–
<i>Eimeria ahsata</i>	14.41		–	–
<i>Eimeria faurei</i>	4.50		6.09	5.41
<i>Eimeria pallida</i>	3.60		0.87	–
<i>Eimeria marsica</i>	3.60		–	–

**Samples from study animals.** Fecal material of five types of animals were collected between September 2014 and July 2015, i.e., domestic sheep, domestic goat, Suleiman markhor, Afghan urial and Chiltan wild goat. Fecal pellets of domestic sheep and goats were collected directly from animals. To collect fecal samples of markhor, urial and Chiltan wild goats, services of local game guards were hired. Trapping of these wild animals is not just very difficult for researchers, but also very stressful for host animals. Hence, fecal samples were collected from their grazing grounds, recording the collecting sites with GPS. Wild animals were sighted for some time, to allow grazing and defecation in particular area. Preference was paid to collection of fresh fecal samples and to collection of samples located distant from human settlement. Dubious and old samples were not collected.

**Lab-work.** Fecal samples were collected in small polythene bags, avoiding contamination of soil or free living nematodes. These bags were left for few days at room temperature for larval development and for sporulation in case of *Eimeria*. Sporulation was let done without adding 2% K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> solution to samples. For preservation of protozoa and helminth, 10–20% formaline was added to samples that were to be studied months later. Fecal pallets were crushed, homogenized and the wet mounts were studied directly under microscope as illustrated by Pritchard and Kruse [22], without going through sedimentation or floatation techniques. Nematode eggs and larvae were identified with the help of different literature [23–26], while species of *Eimeria* were identified

consulting work of Eckert et al. [27].

## Results

### Helminths

A total of 11 helminth eggs and larvae were identified in 1,015 fecal samples studied. Widely prevalent helminth were *Trichuris* spp., *Nematodirus* spp., *Protostrongylus rufescens* and *Moniezia benedeni*, while less prevalent parasites were *Strongyloides papillosus*, *Paramphistomum/Fasciola* spp., *Moniezia expansa* and *Dictyocaulus filaria* (Table 1). Eggs of *Skrjabinema ovis* were seen in feces of three markhor only, one of which was found dead and decomposed in Torghar game reserve. *Trichostrongylus* spp. and *Oesophagostomum* spp. were found in one or two domestic goats of Torghar only. Among wild animals, Chiltan wild goat had highest prevalence of helminth (84.69%), followed by Afghan urial (83.45%) and Suleiman markhor (68.22%). Due to random use of anthelmintics, low prevalence of helminth was observed in domestic animals, except in domestic goats of Torghar.

### Protozoa

Ten species of *Eimeria* were found in 263 fecal samples of wild and domestic sheep (Table 2). Urial were found infected with 7 species of *Eimeria*. The most prevalent species among sheep and urial were *E. ovinoïdalis*, followed by *E. bakuensis* and *E. parva*, while the least prevalent species was *E. marsica*, being found in 4 urial only. *Eimeria intricata* was quite prevalent in domestic sheep of

Table 3. Percentage prevalence of protozoan parasites in wild and domestic caprines of Chiltan and Torghar

Protozoan Parasites	Chiltan National Park		Torghar Game Reserve	
	Chiltan Wild Goat (n=139)	Domestic Goat (n=27)	Suleiman Markhor (n=65)	Domestic Goat (n=106)
Overall infection	81.29	100.00	81.54	82.08
<i>Eimeria arloingi</i>	35.97	59.26	36.92	39.62
<i>Eimeria ninakohlyakimovae</i>	27.34	55.56	46.15	46.23
<i>Eimeria alijevi</i>	14.39	37.04	29.23	27.36
<i>Eimeria hirci</i>	15.11	48.15	26.15	16.04
<i>Eimeria caprina</i>	13.67	25.93	10.77	23.58
<i>Eimeria jolchijevi</i>	25.90	14.81	6.15	3.77
<i>Eimeria caprovina</i>	11.51	11.11	6.15	18.87
<i>Eimeria apsheronica</i>	–	11.11	23.08	5.66
<i>Eimeria christenseni</i>	–	11.11	1.54	2.83
<i>Entamoeba</i> spp.	3.60	–	–	2.83

Torghar (32.43%), yet it was found in a single urial only, and so were *Eimeria weybridgensis* and *E. pallida*. The only ovine *Eimeria* that was neither found in domestic nor in wild hosts was *E. granulosa*.

A total of 9 and 7 species of *Eimeria* were found in 65 fecal samples of markhor and 139 fecal samples of Chiltan wild goat, respectively. Among wild and domestic goats of Chiltan and Torghar, the most prevalent *Eimeria* were *E. arloingi*, *E. ninakohlyakimovae*, and *E. alijevi*, while the least prevalent species was *E. christenseni* (Table 3). *Eimeria apsheronica* and *E. christenseni* were not found in Chiltan wild goats, yet they were present in markhor. A total of 5 (3.60%) wild goats of Chiltan and 3 (2.83%) domestic goats of Torghar were seen

infected with uninucleated *Entamoeba* species (Fig. 2). Cysts were not observed by the author, yet size of round stationary trophozoites (n=22), from fecal sample of single Chiltan wild goat measured on average 39µm (30–47µm) in length and 36µm (27–47µm) in width.

## Discussion

Reports on parasitic infections in domestic animals are common in Pakistan. Yet, reports on parasitic infection of wild animals are lacking. Almost all the helminth reported in present research have previously been reported in domestic animals of Quetta and nearby regions [28–33]. Research on enteric protozoan species of both wild or domestic

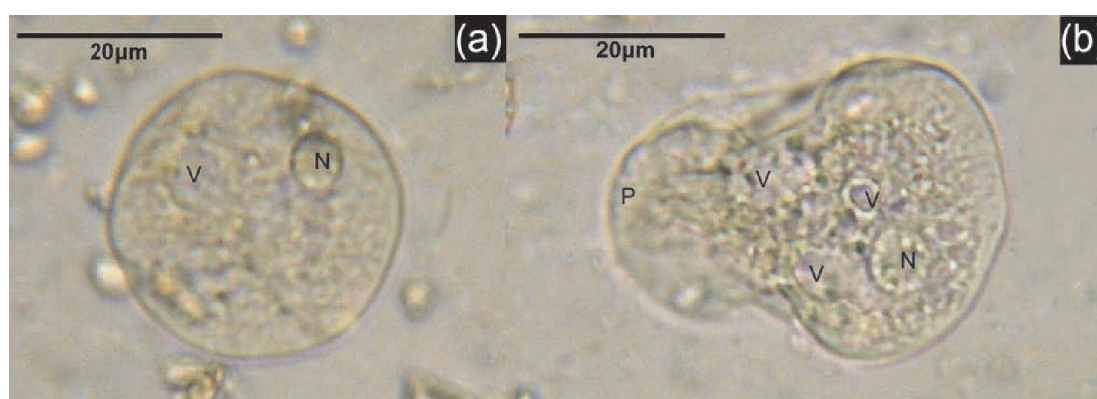


Figure 2. Unstained uninucleated *Entamoeba* spp. in Chiltan wild goat (a) trophozoite in stationary form with peripheral karyosome in nucleus (=N), and (b) trophozoite form with pseudopodia (=P) and vacuoles (=V) visible.

Table 4. Helminth and enteric protozoan parasites previously reported from wild sheep and goats of India, Iran, Turkey and Pakistan

Country (Host)	Parasite (Prevalence) <sup>a</sup>	References
<b>India</b>		
( <i>Capra [ibex] sibirica</i> )	<i>Eimeria arloingi</i> (?) <i>Paramphistomum</i> spp. (66.6%) <i>Echinococcus granulosus</i> (cyst) (33.3%), <i>Moniezia</i> spp. (33.3%) <i>Gongylonema verrucosum</i> (?), <i>Haemonchus contortus</i> (33.3%), <i>Marshallagia marshalli</i> (= <i>Ostertagia orientalis</i> , <i>O. occidentalis</i> , <i>M. orientalis</i> ) (50%), <i>Oesophagostomum asperum</i> (?), <i>O. columbianum</i> (?), <i>O. venulosum</i> (100%), <i>Protostrongylus indicus</i> (?), <i>Setaria cervi</i> (= <i>S. buxi</i> ) (?), <i>Teladorsagia circumcincta</i> (= <i>Ostertagia circumcincta</i> ) (?), <i>Trichuris</i> spp. (100%), <i>Varestrongylus capricola</i> (?), <i>V. pneumonicus</i> (?)	[14, 34–42]
( <i>Capra falconeri</i> )	<i>Haemonchus contortus</i> (?)	[43]
( <i>Ovis ammon hodgsoni</i> ) <sup>b</sup>	<i>Dictyocaulus filaria</i> (= <i>D. unequalis</i> ) (?), <i>Varestrongylus pneumonicus</i> (?)	[34]
( <i>Ovis vignei</i> )	<i>Gongylonema pulchrum</i> (= <i>G. scutatatum</i> ) (?)	[44]
<b>Iran</b>		
( <i>Capra aegagrus aegagrus</i> )	<i>Eimeria</i> spp. (80%) [ <i>E. arloingi/jolchijevi</i> , <i>E. caprina</i> , <i>E. caprovina</i> , <i>E. ninakohlyakimovae</i> ] <sup>c</sup>	[45]
( <i>Ovis orientalis</i> ) <sup>b</sup>	<i>Eimeria</i> spp. (100%), <i>E. ahsata</i> (6.5%<), <i>E. faurei</i> (6.5%<), <i>E. ovinoidalis</i> (9.7%<), <i>E. parva</i> (32.3%<) <i>Dicrocoelium dendriticum</i> (0.4%), <i>Fasciola gigantica</i> (0.4%), <i>F. hepatica</i> (2%), <i>Helicometra giardi</i> (10%) <i>Avitellina centripunctata</i> (5.2%), <i>Echinococcus granulosus</i> (cyst) (4–4.8%), <i>Moniezia benedeni</i> (3.6–4%), <i>M. expansa</i> (6%), <i>Stilesia globipunctata</i> (4.4%), <i>Taenia hydatigena</i> (cysticerci) (5.6–8%), <i>T. ovis</i> (cysticerci) (0.4%) <i>Chabertia ovina</i> (56%), <i>Dictyocaulus eckerti</i> (1.6%), <i>D. filaria</i> (21.4–78%), <i>Gongylonema pulchrum</i> (5.6%), <i>Haemonchus contortus</i> (0.8%), <i>Marshallagia marshalli</i> (= <i>Ostertagia occidentalis</i> ) (29.5–100%), <i>Muellerius capillaris</i> (1.1–70%), <i>Nematodirella longissimespiculata</i> (26%), <i>Nematodirus abnormalis</i> (2%), <i>N. archari</i> (14.8%), <i>N. davtiani</i> (36.6%), <i>N. filicollis</i> (21.6%), <i>N. gazellae</i> (3.6%), <i>N. oiratianus</i> (76%), <i>N. spathiger</i> (36%), <i>Ostertagia ostertagi</i> (= <i>O. lyrata</i> ) (14%), <i>Parabronema skrjabini</i> (0.8%), <i>Protostrongylus raillietii</i> (17.6%), <i>P. rufescens</i> (4.8–38%), <i>Setaria</i> spp. (0.4%), <i>Skrjabinema ovis</i> (10–72%), <i>Teladorsagia circumcincta</i> (= <i>Ostertagia circumcincta</i> , <i>O. trifurcata</i> ) (2–10.6%), <i>Trichostrongylus vitrinus</i> (0.8%), <i>Trichuris</i> spp. (41.5%), <i>T. discolor</i> (18–100%), <i>T. gazellae</i> (6.4%), <i>T. georgicus</i> (36%), <i>T. infundibulum</i> (86%), <i>T. ovis</i> (4–10%), <i>T. parvispiculum</i> (15.6%), <i>T. skrjabini</i> (2%), <i>T. vondwei</i> (24%)	[45–51]
<b>Turkey</b>		
( <i>Capra aegagrus aegagrus</i> )	<i>Marshallagia marshalli</i> (= <i>Teladorsagia occidentalis</i> ) (100%), <i>Nematodirus abnormalis</i> (100%), <i>N. spathiger</i> (100%), <i>Teladorsagia circumcincta</i> (= <i>T. trifurcata</i> ) (50–100%), <i>Trichostrongylus colubriformis</i> (50%)	[52]
( <i>Ovis orientalis</i> ) <sup>b</sup>	<i>Eimeria</i> spp. (100%) <i>Echinococcus granulosus</i> (cyst) (100%), <i>Taenia hydatigena</i> (cysticerci) (100%), <i>T. multiceps</i> (coenuri) (100%), <i>Thysaniezia ovilla</i> (100%) <i>Chabertia ovina</i> (100%), <i>Cystocaulus ocreatus</i> (100%), <i>Dictyocaulus filaria</i> (100%), <i>Marshallagia marshalli</i> (= <i>Ostertagia occidentalis</i> ) (100%), <i>Nematodirus</i> spp. (100%), <i>Teladorsagia circumcincta</i> (= <i>Ostertagia circumcincta</i> ) (100%), <i>Trichuris</i> spp. (100%)	[53–55]
<b>Pakistan</b>		
( <i>Capra [ibex] sibirica</i> )	<i>Ostertagia ostertagi</i> (?), <i>Protostrongylus indicus</i> (?)	[14, 56]
( <i>Capra falconeri falconeri</i> )	<i>Haemonchus contortus</i> (40%)	[19]
( <i>Ovis vignei punjabiensis</i> )	<i>Haemonchus contortus</i> (74%), <i>Trichostrongylus axei</i> (3%), <i>Trichuris ovis</i> (16%)	[20]

<sup>a</sup> Conflicts in synonyms and taxonomic position of species and their morphs in genera *Ostertagia*/*Teladorsagia*/*Marshallagia* are resolved here in accordance with findings [36, 57, 58], while other species are dealt in accordance with Yamaguti [59]. Junior synonyms are shown in parenthesis, when relevant country authors used them in their publications. Parasites with unknown prevalence are marked with question mark in parentheses.

<sup>b</sup> Host names as *Ovis ammon orientalis* and *O. gmelinii anatolica* of Iran and Turkey are treated as *O. orientalis*, and *Ovis hodgsoni* of India is treated as *O. ammon hodgsoni* here, as is now widely considered [3].

<sup>c</sup> Species of the unsporulated *Eimeria* not declared in relevant article [45], but are guessed by present author from micrographs provided therein.

ungulates of Balochistan has never been conducted before. It is assumed that wild caprines of present article are infected with more helminth than actually reported here, because many parasites were identified down to genus level only, as *Trichuris* and *Nematodirus*. Wild sheep of Iran have been found infected with eight species of *Trichuris* and seven species of *Nematodirus* (Table 4) [14,19,20,34–59]. Methodology of the present article also falls short of detecting certain other parasites, for example tissue inhabiting cysts/cysticerci of different *Taenia* and *Echinococcus* species.

### Highly and lowly prevalent parasites

Findings of the present research resemble scientific studies conducted in Iran, rather other provinces of Pakistan with humid environment or canal irrigation systems, mainly because Chiltan and Torghar have arid steppe environment resembling many of the eastern areas of Iran. Hence, the most prevalent parasites in wild sheep of Iran [46] and present study were *Protostrongylus rufescens* and species of *Nematodirus*, *Trichuris* and *Moniezia*. In contrast, *Haemonchus contortus*, which is a parasite of humid/canal irrigated regions of Pakistan [60–62], as was also seen in Chitral markhor [19] and the Punjab urial [20], was not seen in present research either in domestic or wild animals. Same way, *Trichostrongylus* spp. was found in two goats of Torghar only, while this parasite commonly infects small ruminants and urial of the Punjab [20,61]. The high prevalence of *Nematodirus* in Chiltan and Torghar is chiefly due to the remarkable capacity of eggs and larvae of this genus to withstand extreme dry and cold environments for so long [63–65]. Eggs of *Marshallagia marshalli* cannot be distinguished from those of *Nematodirus*, hence in areas where both parasites coexist, they are reported together (e.g., [66]). With the exception of one skeptical report on presence of *M. marshalli* in small ruminants of Quetta and Kalat regions [29] there is no other recent or significant report on presence of this parasite in small ruminants of the region. The lack of additional evidence on presence of minor morphs and synonyms of this parasite in this region rules out its presence in wild animals of the present study.

Aridity of an ecosystem disfavors survival of certain water dependent parasites as *Fasciola* spp. and lungworm as *Dictyocaulus filaria*. The intermediate hosts of *Fasciola* in Pakistan are

different species of *Lymnaea/Galba* snails, which require water for their survival. Yet, review of literature regarding trematodes of Balochistan [28,33], as well as the author's personal experience suggest that rumen flukes are more common than liver flukes, hence the *Fasciola*-like eggs seen in present research might belong to *Paramphistomum cervi*, if not the host animal is brought in from distant *Fasciola*-abundant region. Pakistan appears sharply divided over geographic distribution of parasites, *Fasciola* spp., *D. filaria* and trichostrongylids being more prevalent in the eastern canal irrigated region [60,61,67], while *Pa. cervi*, *P. rufescens* and *Nematodirus* spp. being very common in the western colder arid steppe region.

The very high prevalence of second lungworm species *Protostrongylus rufescens* in present research is due to situation slightly different from that discussed above. Like *Fasciola* spp. it requires intermediate host, in Pakistan this being snails of genus *Helicella* [68], which unlike *Lymnaea*, are geophilic preferring dry habitat. This parasite is commonly reported in sheep and goats of the Punjab [67]. Larvae is commonly observed by present author in feces of sheep and goats in northern areas of Balochistan, yet still it has been reported in single article only [31], mainly because local researchers fall short of correctly identifying the larvae. In Iran, three protostrongylid lungworm were found in Asiatic mouflon, i.e., *P. rufescens*, *P. railletii* and *Muellerius capillaris* [47].

*Strongyloides papillosus* was the most formidable parasite found in present survey. However, low prevalence of this parasite was found in Chiltan wild goat and domestic sheep in the surrounding areas. Free living stages of *S. papillosus* are short lived, even when the environment is favorable [69], hence the dry and cold environments of Chiltan and Torghar disfavor its proliferation. It appears that the parasite arrived Chiltan National Park in recent times from western side, as it was not found in samples collected from eastern side. Leaner wild goats were seen on western side of Chiltan, rather the eastern side. The low prevalence in domestic animals is because this parasite effects body weight so overtly [70], that farmers often get rid of emaciated animals by slaughtering for domestic consumption. This parasites has never been reported in wild sheep and goats of Pakistan, India, Iran and Turkey (Table 4).

Two species of *Moniezia* were seen in present research, *M. benedeni* being more prevalent here, in

contrast to higher prevalence of *M. expansa* as reported in domestic sheep and goats of Quetta [28], cervids of Pakistan [15–18], and wild sheep of Iran [48]. It is interesting to note that *M. benedeni* and *M. expansa* were present in domestic sheep and goats in the surrounding of Chiltan, but not in Chiltan wild goats. Overlooking the trespassing and grazing of domestic stock is always negated by the wildlife department. Hence, this parasite is considered as marker species, wherein future its emergence among Chiltan wild goats would positively mark grazing of the National Park area by domestic stock.

### *Eimeria* and *Entamoeba*

Important aspect of present study was presence of *Entamoeba* and *Eimeria* protozoan parasites. Whereas many *Eimeria* species commonly infect domestic animals of Pakistan [71–73], India [42,74,75] and Iran [76,77], presence of *Entamoeba* spp. in Chiltan wild goat is a surprise. Indeed, *Entamoeba* has remained unnoticed in parasitological studies of even the domestic small ruminants of Pakistan. *Entamoeba* has not been reported in wild sheep and goats of Iran and Turkey, yet it has been reported in argali and ibex of Mongolia [78]. Wild and domestic ruminants of India were found infected with *En. bovis*, *En. antilocapra*, *En. cervis*, *En. histolytica* and *En. ovis* [42]. At present, there are twelve species recognized as uninucleated *Entamoeba* organisms, with five species infecting ruminants [79]. Size of the round trophozoites measured in present findings were far greater than other species found in sheep, goat and cattle [78,80–82]. While, there is no doubt in identification of the genus, the present author is unable to decide species of this parasite, due to facts, as *En. histolytica* is capable of infecting canids and ruminants [42,80], and that recent molecular studies of *Entamoeba* of ruminants show a diversity not obvious from the apparent morphology [81,82]. Little is documented about *Eimeria* of wild sheep and goats of the region surrounding Pakistan [42,51,45].

In conclusion, Torghar and Chiltan areas of Pakistan have arid steppe environment. The parasitological data recorded during present research resembled steppe regions of Iran and Central Asia, rather humid and canal irrigated agricultural regions in the east of Pakistan. Methodology of the present research was noninvasive to the endangered markhor, urial and Chiltan wild goats, yet there is need to study adult

worms in necropsied trophy hunted animals of Torghar Game Reserve. In places as Chiltan National Park, with no trophy hunting program, PCR based genetic study would better determine parasites as *Nematodirus/Marshallagia* spp. or *Entamoeba* spp.

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