Pakistan J. Zool., pp 1-10, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20231015102923

Prevalence, Associated Risk Factors of Gastrointestinal Parasite and Molecular Characterization of *Haemonchus contortus* in Small Ruminant

Muhammad Azhar Memon[1], Javaid Ali Gadahi[1]*, Sana Noor Panhwer[1], Sambreena Tunio[2] Mansoor Tariq[3], Zubair Ahmed Laghari[1], Shaharbano Memon[4], Housh Mohammad Solangi[1], Feroza Soomro[1] and Abdullah Arijo[1]

[1]*Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam, 70060, Pakistan*
[2]*Department of Animal Product Technology, Sindh Agriculture University, Tandojam, 70060, Pakistan*
[3]*Department of Veterinary Pathology, Sindh Agriculture University, Tandojam, 70060, Pakistan*
[4]*Department of Anima Breeding and Genetics, Sindh Agriculture University, Tandojam, 70060, Pakistan*

Article Information
Received 15 October 2023
Revised 15 July 2024
Accepted 23 July 2024
Available online 08 November 2024 (early access)

Authors' Contribution
MAM: Conceptualization, data curation, investigations, visualization, writing and editing, original draft. SN and ST: Conceptualization, visualization, writing and editing original draft. MTS and AA: Formal analysis, writing. SBM, FS and HM: Formal analysis, review and editing. JAG and ZAL: Conceptualization; Formal analysis; Supervision.

Key words
Prevalence, Risk factors, Gastrointestinal parasites, Molecular characterization, *H. contortus*, Small ruminants

ABSTRACT

Gastrointestinal parasites significantly reduce the growth and productivity of livestock. The morbidity and mortality of kids and lambs due to GIT parasites may lead to profound economic losses in rural communities. This study was conducted on GIT parasites of small ruminants in Tandojam and its adjoining areas in January-April 2020. A total of 800 fresh fecal samples were collected and analyzed through the flotation technique. The partial sequence of the Nuclear Ribosomal Internal Transcribed Spacer 2 (*ITS-2*) gene of *H. contortus* was used for the identification and molecular analysis of *H. contortus* isolates. The prevalence of different GIT parasites in small ruminants was recorded as *Eimeria* spp. (75.5%), *Haemonchus* spp. (11.6%), *Strongyloides* spp. (11.1) %, *Moneizia* spp. (4.6%), *Oestertagia* spp. (1.9%), *Trichostrongylus* spp. (1.9%), *Toxocara* spp. (1.4), *Trichuris* (0.9%) and *Nematoduris* spp. (0.5%). The sheep were found more infected as compared to the goats and females were more prone to infection and among them, pregnant were found more infected. Eimerian infection was higher in lambs and kids whereas gastrointestinal nematodes are higher in young followed by adults. Breed-wise prevalence of the GIT parasite was higher in Kacchi followed by Kooka breed of sheep and Pateri, Jattan, Tapri, and lowest in the Kamori breed of goat. It was interestingly reported that the infection rate was very low in animals that use babul pods in feed as compared to other types of feed. The amplicon of the *ITS-2* gene of *H. contortus* was 248bp. The obtained sequence was confirmed by aligning with homolog sequences available on GenBank using the BLAST algorithm. Multiple alignments and phylogenetic tree analysis indicated that *H. contortus* isolated from Tandojam and its vicinity was closely related to isolates of India, Myanmar, and Ghana. It is concluded that GIT parasitic infection in small ruminants is endemic in Tandojam and its adjoining villages. Based on our findings it is suggested that the anthelmintic potential of babul pods needs to be further explored.

INTRODUCTION

Parasitic infestations are one of the major constraints to livestock productivity across all agroecological zones and production systems in the world, and gastrointestinal nematodes remain of major economic importance in domesticated livestock throughout the world (Adedapo *et al*., 2007; Prichard, 1994). Production potential of livestock development programs is plagued in tropical and subtropical areas due to the prevalence of helminths which causes high mortality and great economic losses

* Corresponding author: jagadahi@sau.edu.pk
0030-9923/2024/0001-0001 $ 9.00/0

(Al-Quaisy *et al*., 1987; Ghosh *et al*., 2016).

Small ruminants are exposed to a variety of internal and external parasites due to close contact with the infected animals, due to improper care, an unhygienic environment, and extreme climate. The species of the internal parasite that affect mostly sheep and goats, belong to the Superfamily *Trichostrongyloidea* and includes *Haemonchus, Trichostrongylus, Cooperia, Ostertagia Oesophagostomum* (Bowman, 2014), *Chabertia, Nematodirus, Trichuris, Moniezia* and *Fasciola***,** *Haemonchus contortus*, *Ostertagia ostertagi* and *Trichostrongylus colubriformis* are notorious owing to impaired productivity of small ruminants. These parasites negatively affect the livestock industry. Gastrointestinal nematodes are the most prevalent and major parasite of domestic and wild ruminants in tropical, sub-tropical and temperate regions worldwide (Brasil *et al*., 2012; von Samson-Himmelstjerna *et al*., 2002). Several studies were conducted on the prevalence of gastrointestinal parasites in sheep and goats in different parts of Pakistan (Al-Shaibani *et al*., 2008; Gadahi *et al*., 2009; Khalil-ur-Rehman *et al*., 2009; Lashari and Tasawar, 2011; Qamar *et al*., 2009; Qayyum *et al*., 2010; Tasawar *et al*., 2010).

Genetic characterization is extremely important to consider the presence of *H. contortus* from different species from different geographical areas (Achi *et al*., 2003; Cerutti *et al*., 2010; Jacquiet *et al*., 1998; Kumsa *et al*., 2008; Lavín *et al*., 1997; Ndao *et al*., 1995; Sharhuu and Sharkhuu, 2004; Taylor *et al*., 2005). Moreover, the pathology of *H. contortus* has encouraged the development of vaccines, anthelmintic resistance, and drug target identification (Molento *et al*., 2011; Redman *et al*., 2008). In recent years, DNA-based techniques have been implemented to specific identification and characterization of these parasites and to determine the level of infections. For this purpose, several PCR-based methods targeting mainly the nuclear and mitochondrial genes have been applied and used in the phylogenetic relationships among Trichostrongylid nematodes (Akkari *et al*., 2013; Brasil *et al*., 2012; Hussain *et al*., 2014; Molento *et al*., 2011).

Parasitic diseases of goats and sheep sharply reduce the growth, milk, and meat production, damage skin, hide, and wool and cause morbidity and mortality of kids and lambs, which may lead to profound economic loss in rural communities. However, there has not been performed a systemic work on the prevalence and associated risk factors of the gastrointestinal parasite in small ruminants in Tandojam, District Hyderabad, therefore the present study was conducted to identify the prevalence and associated risk factors of gastrointestinal parasites in small ruminants. This study will also provide a roadmap for future researchers and investigators.

MATERIAL AND METHODS

Collection of samples and coprological examination

The study was conducted in the period of four months from January to April 2020. A total of 800 fecal samples were collected by random sampling regardless of breed, age, and gender into the sterile plastic bags from Tandojam and its vicinity of District Hyderabad. The information regarding the sample and its associated risk factors was recorded at the time of sample collection on the proforma. Abomasum of the goat and sheep were collected as soon as possible, usually within 30 mins of evisceration from the butchers of Tandojam and brought to the Department of Veterinary Parasitology, Sindh Agriculture University, Tandojam. Abomasum were opened and examined for the worm infection. *Haemonchus contortus* were morphologically identified and collected according to the standard procedure designated by Zajac and Conboy (2012). Fecal samples were qualitatively analyzed for the identification of gastrointestinal parasite eggs/oocysts by using flotation technique (Urquhart *et al*., 1996).

DNA extraction

Genomic DNA of *H. contortus* was extracted from adult worms by using Genomic DNA purification Kit (Thermo Scientific, USA) according to the manufacturer's instructions. The DNA was quantified by NanoDrop Spectrophotometer (The Thermo Scientific NanoDropTM 1000) and kept at -20 °C for further use.

PCR amplification

The amplification of ITS-2 genes was done by using previously published conserved primers forward 5'GTTAACCATATACTACAATG-3' and reverse 5'GAGCTCAGGTTGCATTATAC-3' (Brasil *et al*., 2012; Kanzaki and Futai, 2002). The PCR reaction mixture consisted of 12.5ul master mix (green master mix GoTaq1 promega Madison, WI, USA) each primer 25 pmol, 50ng of DNA sample and water (nuclease free) was added up to 25ul PCR reaction mixture. PCR amplification was performed in the thermal cycler (Applied Biosystem 2720, USA) Following cyclic conditions were followed: initial denaturation for 2 min at 94°C followed by 35 cycles at 94°C for 30s, 55°C for 30 sec, 72°C for 60 sec and final extension was performed at 72°C for 10 min (Brasil *et al*., 2012). The PCR product was separated by 1% agarose gel electrophoresis stained with ethidium bromide and visualized by using the gel documentation system.

Sequence and phylogenetic analysis

The PCR product was purified using a PCR purification kit. Purified DNA was sent to the Medical

Research Center, Liaquat University of Medical and Health Sciences, Jamshoro for DNA Sequence. Obtained sequences were confirmed by aligned with homologues available on GenBank using the BLAST algorithm. Sequences was retrieved from the NCBI GenBank and aligned with *H. contortus* Tandojam strain by using ClustalX 1.83 program (http://www.clustal.org/). The phylogenetic tree was created using the neighbor-joining (NJ) method based on the Kimura 2 parameter model in Mega 5.2 with 1000 boot-strap replicates for each tree (Tamura *et al*., 2011).

Statistical analysis

Data was analyzed for descriptive type analysis through Microsoft Excel.

RESULTS

To record the prevalence of GIT parasite infection, 800 samples were collected and processed for fecal examination from randomly selected areas of Tandojam and its surrounding villages. Among 800 samples, 560 (70 %) were found positive. The results showed that the prevalence of GIT parasite was higher in sheep (82.14%) as compared to goat (75.63%). Species-wise prevalence of different GIT parasites in small ruminants was recorded as *Eimeria* (75.5%), *Haemonchus* (11 %), *Strongyloides* (11 %), *Moneizia* spp. (5%), *Oestertagia* (2 %), *Trichostrongylus* spp. (2 %), *Toxocara* spp. (5 %) *Trichuris* spp. (1 %), *Nematoduris* spp. (0.5%) and coinfection (29.5%) (Table I). The highest infection rate was reported as *Eimeria* spp. 74.5 and 78.5% followed by *Haemonchus* spp. 9.5 and 18 %, *Strongyloides* 8 and 19.5 %, *Moneizia* spp. 4.5 and 5.5%, *Ostertagia* spp. 1.5 and 3.5%, *Trichostrongylus* spp. 1.3 and 3.5%, *Toxocara* spp. 1.5 and 2% and *Trichuris* spp. 0.5 and 2 %, the lowest infection rate was *Nematoduris* spp. 0.5 and 0.0% and coinfection 26.5 and 39 % in goat and sheep, respectively (Table I).

The gender wise prevalence of GIT parasite in male and female was recorded as 84 and 74%, respectively. The highest infection rate was reported *Eimeria* spp. 76 and 74 % followed by *Haemonchus* spp. 16 and 10%, *Strongyloides* spp. 14 and 10 %, *Moneizia* spp. 4 and 4%, *Oestertagia* spp. 1.6 and 2.0 %, *Trichostrongylus* spp. 1.6 and 2.0%, *Toxocara* spp. 1.6 and 2 %, *Trichuris* spp. 1.6 and 2 % and lowest was *Nematoduris* spp. 1.6 and 0.0% in male and female, respectively (Table I). The data was also analyzed for the infection rate in pregnant and non-pregnant small ruminants and our findings indicated that, the highest infection rate was reported *Eimeria* spp. 76.0 and 79.6% followed by *Haemonchus* spp. 5 and 14.2 %, *Strongyloides* spp., 8.0 and 10.2%, *Moneizia* spp. 1 and 6.4%, *Oestertagia* spp. 4.0 and 1.4%, *Trichostrongylus* spp. 2 and 3.8%, *Toxocara* spp. 0.0 and 1.4%, *Trichuris spp* 0.0 and 1.4% and *Nematoduris spp* 0.0 and 1.4 % and coinfection 18 and 38.6% in pregnant and non-pregnant animals of small ruminants, respectively (Table I).

The age-wise prevalence of GIT parasite in small ruminants were 92, 89, 80.0 and 52 % in the age of <6 month, <1 year, < 2years, and > 2 years, respectively. The results revealed that, the protozoal (*Eimeria*) infection was higher and gastrointestinal nematode infection was less in the early age as compared to old age whereas gastrointestinal nematode infections is increasing as the age of animals increases. The infection rate was recorded as *Eimeria* spp. 92.04, 89.1, 77 and 45.8%, followed by *Haemonchus* spp. 4.54, 7.27, 16 and 14.58 %; *Strongyloides* spp. 3.40, 7.27, 16.0 and 14.58 %; *Moneizia* spp. 3.40, 7.27, 4.0 and 4.16%; *Ostertagia* spp. 0.0, 1.8, 3 and 2.08%; *Trichostrongylus* spp. 0.0, 0.0, 3 and 4.16%; *Toxocara* spp. 2.27, 1.8, 1 and 0.0%; *Trichuris* spp. 0.0, 1.8, 0.0 and 2.08% and lowest prevalence was recorded as *Nematoduris* spp. 0.0, 0.0, 1 and 0.0% in the age of <6 month, <1 year, < 2year and > 2 year, respectively (Table I). The Koka breed of sheep was found more infected (84 %) as compared to Kacchi breed (80 %) and in the case of goat breeds, Pateri was found more prone to GIT parasites (80 %) followed by Kamori (50 %), Tapri (74.8 %), and Jattan (79.16%).

The prevalence of GIT parasite according to the feed of animals were 98, 94, 65.4, 34, 50 and 78 % in wild grasses, wild grass cum chopped fodder, Babul (*Accacia nilotica*) leaves, Babul pods, Janter (*Sesbania bispinosa*) and Burseem (*Trifolium alexandrinum*), respectively. The results shown that the protozoal (*Eimeria*) infection was reported in all the feeds whereas the GIT helminths infections were reported in all the feed except babul which suggests that babul has anthelmintic properties. The prevalence of GIT parasite according to feeding method in small ruminants were 92, 69, 91 and 64 % in open grazing, ground feeding, open grazing cum ground feeding and stall feeding, respectively. The prevalence of GIT parasite according to the animal housing in small ruminants were 76, 81, 84 and 69 % in open, cottage, open cum cottage and cemented animal housing, respectively (Table II).

H. contortus *internal transcribed spacer 2* (ITS-2) *gene and phylogenic analysis*

The amplicon of *ITS-2* gene of *H. contortus* was successfully amplified and the product of PCR was approximately 248bp, which was visualized by agarose gel stained with ethidium bromide (Fig. 1). The sequence results were further confirmed by Blastx

Table I. Animal factors associated with gastrointestinal parasites.

GIT parasites	Animal Factor																	
		Animal wise prevalence		Gender wise prevalence		Animal status wise prevalence		Age wise prevalence				Breed wise prevalence					
												Sheep		Goat			
	Prevalence % (n=800)	Goat (n= 600)	Sheep (n= 200)	Male (n= 250)	Female (n= 550)	Pregnant (n= 300)	Non pregnant (n=500)	<6 months (n=88)	<1 Year (n=220)	<2 Years (n=300)	>2Year (n=192)	Kooka (n=100)	Kacchi (n=80)	Pateri (n= 150)	Kamori (n=100)	Tapri (n= 250)	Jattan (n= 120)
Eimeria spp	75.5	74.5	78.5	76.0	74.0	76.0	79.6	92.04	89.1	77.00	45.8	80.0	76.25	80.0	50.0	74.8	79.16
Hemonchus spp	11.0	9.5	18.0	16.0	10	5.0	14.2	4,54	7.27	16.0	14.58	23.0	13.75	12.0	5.0	7.6	12.5
Strongloides spp	11.0	8.0	19.5	14.0	10	8.0	10.2	3.40	7.27	16.0	14.58	23.0	17.5	10.0	0.0	8.8	4.16
Moniezia spp	5.0	4.5	5.5	4.0	4.0	1.0	6.4	3.40	7.27	4.0	4.16	6.0	3.75	6.0	0.0	4.4	4.16
Oestertagia spp	2	1.5	3.5	1.6	2.0	4.0	1.4	0.0	1.8	3	2.08	4.0	3.75	2.0	0.0	1.6	0.0
Trichostrongulus spp	2	1.3	3.5	1.6	2.0	2	3.8	0.0	0.0	3	4.16	4.0	3.75	2.0	0.0	1.6	0.0
Toxocara spp	5	1.5	2.0	1.6	2	0.0	1.4	2.27	1.8	1.0	0.0	4.0	0.0	2.0	0.0	1.6	0.0
Trichuris spp	1	0.5	2.0	1.6	2	0.0	1.4	0.0	1.8	0.0	2.08	4.0	0.0	2.0	0.0	0.0	0.0
Nematoduris spp	0.5	0.5	0.0	1.6	0.0	0.0	1.4	0.0	0.0	1.0	0.0	0.0	0.0	2.0	0.0	0.0	0.0
Coinfections spp	29.5	26.5	39.0	31.6	28.0	18.0	38.6	23.86	29.54	34.66	27.08	46.0	33.75	36.0	6.0	25.8	25.0

Table II. Managemental factors associated with gastrointestinal parasites.

GIT parasites	Managemental factors													
	Feed type							Feeding method			Housing system			
	Wild grasses	Wild grass cum chopped fodder	Babul leaves	Babul pods	Janter	Burseem	Open grazing	Ground feeding	Open grazing cum ground feeding	Stall feeding	Open	Cottage	Open cum cottage	Cemented
Eimeria spp	23.5	21.5	14.0	8.0	20.0	19.16	21.53	16.66	21.5	14.0	17.81	22.5	22.0	18.57
Hemonchus spp	6.5	3.0	2.0	0.0	1.25	1.66	3.07	2.5	4.5	2.0	3.27	2.5	3.0	1.14
Strongloides spp	5.5	3.0	1.0	0.0	1.25	1.66	1.53	1.66	5.5	2.0	2.54	3.75	4.0	2.85
Moniezia spp	1.5	1.5	1.0	0.0	1.25	1.66	1.9	0.83	1.5	2.0	0.72	2.5	3.0	1.14
Oestertagia spp	0.5	1.0	0.0	0.0	0.0	0.0	0.76	0.41	0.5	0.0	0.36	1.25	1.0	0.0
Trichostrongulus spp	0.5	1.0	0.0	0.0	0.0	0.0	0.0	0.41	1.5	0.0	0.36	1.25	0.0	1.14
Toxocara spp	0.5	0.5	0.0	0.0	0.0	0.0	1.9	0.41	0.0	0.0	0.36	1.25	1.0	0.0
Trichuris spp	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.83	0.0	0.0	0.36	0.0	1.0	0.0
Nematoduris spp	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	1.0	0.0
Coinfections spp	15.5	12.5	1.0	0.0	2.5	0.83	6.92	4.58	13.5	3.0	6.0	22.5	14.0	5.71

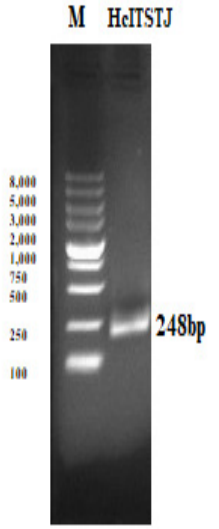

Fig. 1. PCR product of *ITS-2* gene of *H. contortus* on agarose gel.

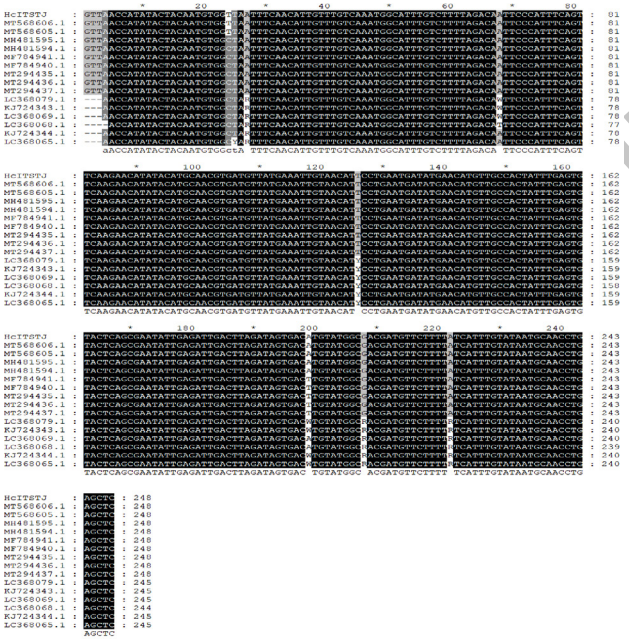

Fig. 2. Multiple alignment of nucleotides sequence *H. contortus ITS-2* gene. The nucleotides sequence of *H.contortus* Internal Transcribed Spacer 2 (ITS-2) Tandojam (HcITSTJ) aligned with other genes reported in the NCBI database as MT568606.1 MT568605.1 (100%), MH481595.1(100%), MH481594.1 (99.60%), MT294437.1 (99.19%), MT294436.1 (99.19%), MT294435.1 (99.19%), MF784941.1 (99.19%), MF784940.1 (99.19%), LC368069.1 (97.55%), KJ724344.1 (97.55%), LC368068.1 (97.55%), LC368079.1 (97.14%), LC368065.1 (97.14%) and KJ724343.1 (97.14%).

(https://blast.ncbi.nlm.nih.gov/Blast.cgi) and results shows that amplified product belongs to *H. contortus* internal transcribed spacer 2 (*ITS-2*) gene.

The data was further analyzed for genotype identification; the nucleotide sequences of a fragment of ITS-2 were aligned and compared to other sequences by Clustalx (1.81) and Genedoc software for identification of genotype-based on sequences. The GC nucleotide percentage of of ITS-2 gene was from 33.6-33.9%. Fifteen sequences of various genotype of *H. contortus* ITS-2 gene were retrieved from *NCBI* gene bank and aligned with our data (HcITSTJ). Findings of bioinformatics data revealed that HcITSTJ Tandojam was closely related to MT568606.1, MT568605.1 and MH481594.1 of a sheep genotype (Fig. 2). Multiple alignment results with different genotypes found as MT568606.1 (100%) MT568605.1 (100%), MH481595.1 (100%), MH481594.1 (99.60%), MT294437.1 (99.19), MT294436.1 (99.19), MT294435.1 (99.19%), MF784941.1 (99.19), MF784940.1 (99.19), LC368069.1 (97.55%), KJ724344.1 (97.55%), LC368068.1 (97.55), LC368079.1 (97.14), LC368065.1 (97.14) and KJ724343.1 (97.14). The phylogenetic tree showed that, HcITSTJ was closely associated with HcITS nucleotide sequences obtained from NCBI and the numbers on the branches represent the bootstrap values for 1000 replicates. MEGA 5.10 software was used to generate a phylogenetic tree (Fig. 3). In calculation by using bootstrap consensus, the sequences which is greatly homologous to HcITSTJ were 100% with sheep MT568606.1, MT568605.1 and MH481594.1 genotype of Myanmar (Burman and Ktistakis) and Ghana, respectively.

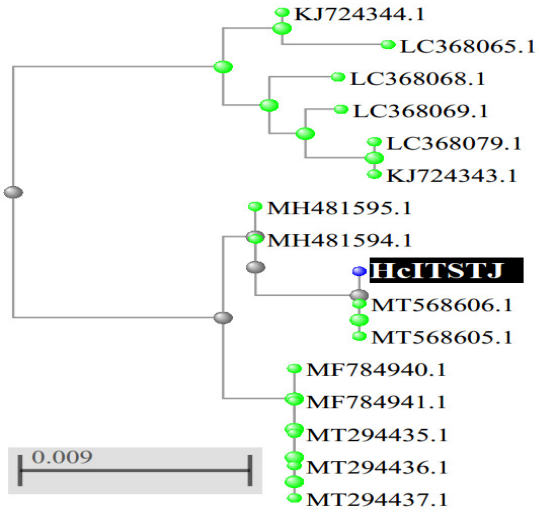

Fig. 3. Phylogenetic tree structure by N.J algorithm for goat isolates (denoted by HcITSTJ) based on the ITS-2 gene of *H. contortus* with the reference sequence.

DISCUSSION

Gastrointestinal parasitic infection is one of the major problems, which affects the productivity of goats and sheep. Losses caused by the GIT parasite invariably depend on the management, prevalence, nature and intensity of infection. The infection rate was higher in sheep as compared to goat is due to their feeding behavior. Sheep is the grazing animals whereas goats are the browsing animals. The infection rate which was reported in the present study is lower than the reported by the Raza *et al*. (2007) which may be due to seasonal variations and the period of study and higher than the reported by Akhter *et al*. (2011) which may be due different species of GIT parasites. Al-Shaibani *et al*. (2008), Gadahi *et al*. (2009), Qayyum *et al*. (2010) and other researchers have already reported various species of GIT parasites in various parts of the world. The infection rate which was reported in the present study is lower than the reported by Akhter *et al*. (2011), Al-Shaibani *et al*. (2008), Gadahi *et al*. (2009), and Raza *et al*. (2014). However, another researcher also reported other helminths in addition to those recorded in this study. It may be interesting to note that, no work has been done up to date on the prevalence of protozoal (*Eimeria*) in small ruminants in Sindh, Pakistan.

The most prevalent gastrointestinal nematode (GIN), which was recorded in this study was *Haemonchus contortus* in sheep and goats. *Haemonchus contortus* was highly reported nematode in Pakistan by Ayaz *et al*. (2013), Khan *et al*. (2010), Lashari and Tasawar (2011) and in various parts of the world by Ansari *et al*. (2008), Pathak and Pal (2008). It may be due to the fact that, these nematodes can take advantage of favorable environments with short generation intervals relative with other nematodes (Farooq *et al*., 2012; Ali *et al*., 2023).

In addition, it is also reported that a regional variation in the various species of parasites which may be associated due to climatic conditions, geographical distribution and host factors require for the development of parasitic stages. Age, sex, breed of the host, grazing habits, level of education and economic capacity of the farmers and usage of anthelmintic drugs are the variety of factors which are discussed by Ayaz *et al*. (2013).

In this study, males were found more prone to GIT parasites as compared to females in sheep and goats. The results are in agreement with the previous studies by Ayaz *et al*. (2013) and Lashari and Tasawar (2011). It is also reported that male sheep are more susceptible to gastrointestinal nematodes as compared to female. Similar results were also reported after puberty by Saddiqi *et al*. (2011). It was reported that the difference in the prevalence of GI nematodes in sheep may be due to the inhibitory effect of androgen and the stimulatory effect of estrogen on the immune response. In this study, this could be the reason for the higher infection of GIT parasite in male as compared to female in sheep.

Normally, females are more prone to GIT parasites due to hormonal differences, pregnancy, parturition and different stress conditions. The present results may be due to practicing stallfeeding of females during pregnancy, feed, and animal housing which leads to lesser exposure of contaminated pasture (Azhar *et al*., 2023; Ayaz *et al*., 2013; Iqbal *et al*., 1993; Khan *et al*., 2010) have also reported that female are more prevalent than male. In this study, there was a random sampling of about four months (January-April) therefore; the effect of season on the infection rate of different GIT parasites could not be reported. It is also suggested that some species of GI parasite like *Ostertagia* spp. grow well in cool and moist conditions (Lateef *et al*., 2005).

It might be a unique finding that the infection rate of different GIT parasite was higher in nonpregnant as compared to pregnant in. The effect of the reproductive cycle on worm burden in animals has been reported, it has an important significance. It is also reported that there is a progressive increase in the egg per gram (EPG) and number of helminths in the peri and post-parturient period. It is attributed to various reasons including periparturient relaxation in immunity (PPRI), seasonal variation, host factors, activation of hypobiotic larvae parturition stress, nutritional stress, etc. (Memon *et al*., 2024; Ayaz *et al*., 2013). It was also reported that the prevalence of GIT nematodes is due to the inhibitory effect of androgen and the stimulatory effect of estrogen on the immune response (Khalafalla *et al*., 2011).

It may be interesting to note that, the protozoal (*Eimeria*) infection was higher and gastrointestinal nematodes infection was lesser in the early age (<6 months) as compared to young (>6months) and adult age (>1 year) whereas the gastrointestinal nematodes infections are increasing as the age of animal increases. The results of my studies are somewhat in agreement with the previous studies by Khanum and Yeasmin (2015). In kids and lambs, there was a high prevalence of *Eimeira* spp. and it caused diarrhea in early age of small ruminants. Results revealed that there was a high infection rate of nematodes in young ones and adults (>6 months to <2 years) as compared to older ones (>2 years), it may be associated with lesser resistance due to lesser exposure to different species of helminths as compared to older once which are high resistance due to more exposure of different species of helminths. Tasawar *et al*. (2010), Qamar *et al*. (2009) and other researchers have already reported various species of GIT parasites in relation to the age at various

parts of the world.

The results of breed-wise prevalence of the GIT parasite are in agreement to the previous studies by Al-Shaibani *et al*. (2008). The result of the present study shows that there is an influence of breed on the prevalence of haemonchosis. He reported that the Kooka breed of sheep is more susceptible to the GIT parasite as compared to the Kacchi breed. No systemic work has been done on the breed wise prevalence of gastrointestinal parasite in small ruminants up to date in Sindh. The results shown that the protozoal (*Eimeria*) infection was reported in all the feeds whereas the gastrointestinal helminths (GIH) infections were reported in all the feeds except babul pods (*Acacia nilotica*) which may be the interesting findings that, babul pods (*Acacia nilotica*) have anthelmintic properties. Anthelmintic properties of babul (*Acacia nilotica*) were reported by Badar *et al*. (2011).

It was also reported that different extract of *Acacia nilotica* has inhibitory effect on the egg-hatching of nematodes (Kahiya *et al*., 2003). The protozoal (*Eimeria*) infection was reported in all the feeding method whereas gastrointestinal (GI) helminths infections were reported higher in all the feeding method as compared to stall-feeding. These findings suggested that coccidiosis was reported in all the feeding methods because it is an infectious disease; the cyst of *Eimeria* does not require any significant development outside the host whereas GI helminths require development outside the host (Chikwanda *et al*., 2013). It was reported that due to practicing stall-feeding of females during pregnancy, feed, and animal housing which led to lesser exposure of contaminated pasture and having lesser EPG (Ayaz *et al*., 2013; Abbasi *et al*., 2020).

Molecular characterization is an essential tool, which is mainly used for the confirmation of different species of nematodes and phylogenetic investigation. It gives us information about variations in genetics, understanding transmission patterns, spread of the drug resistance gene of *H. contortus* within or among the population and give us information to propose a strategy to control the internal parasite effectively (Gasser *et al*., 2008). ITS-2 is most commonly used for the identification of cestodes, trematodes and nematodes (Králová-Hromadová *et al*., 2012). Up to fifteen sequences of various genotype of *H. contortus* ITS-2 gene such as MT568606.1, MT568605.1, MH481595.1, MH481594.1, MT294437.1, MT294436.1, MT294435.1, MF784941.1, MF784940.1, LC368069.1, KJ724344.1, LC368068.1, LC368079.1, LC368065.1 and KJ724343.1 were found by using the DNA amplification and sequencing (HcITSTJ) (Hussain *et al*., 2014; Khanal, 2018; Suchawan *et al*., 2020).

The present study showed that the molecular analysis of the internal transcribed spacer 2 gene of *H. contortus* produces a sequence of 248bp for each sample analyzed. Similar results were also identified in Pakistan (Hussain *et al*., 2014). The GC content of *H. contortus* ITS-2 was ranging from 33.6-33.9%. The results are agreement to the previous studies in which the GC content was ranging from 32.5-33.8% (Dey *et al*., 2019). The alignment obtained sequence was compare with fifteen different (already identified) genotypes present in GeneBank, which showed 100% homology with the sheep MT568606.1, MT568605.1 and MH481594.1 genotype of Myanmar (Burman and Ktistakis) and Ghana, respectively. The molecular characterization of *H. contortus* was the first time identified to recognize the genotype of prevalent *H. contortus* in small ruminants. The DNA sequencing of *H. contortus* internal transcribed spacer tandojam (HcITSTJ) was analyzed and the results of the present study showed that the prevalent *H. contortus* is sheep strain with accession number (MT568606.1, MT568605.1, MH481595.1) (Squire *et al*., 2018).

CONCLUSION

It is concluded that, gastrointestinal parasitic infection is endemic in Tandojam and its adjoining village in small ruminants. However, it is suggested that small-holder farmer should improve their management by strategic use of anthelmintic drugs, improving feed, feeding method and housing system of small ruminants to control the gastrointestinal parasites infections in small ruminants. Based on our findings it is also suggested that the anthelmintic potential of babul pods needs to be further explored.

DECLARATIONS

Acknowledgement

Authors would like to express our gratitude to Miss Irum Shehnaz for her assistance throughout this research

Funding

The study was funded by the Higher Education Commission (HEC)-Pakistan for this research under Indigenous Scholarship Phase II Batch VI, Pin NO: 520-141902-2AV6-142 (50093543).

IRB approval

The research work was approved by the Board of Studies at Department of Veterinary Parasitology, Faculty of Animal Husbandry and Veterinary Science, Sindh Agriculture Tandojam, Pakistan (November 2019).

Ethical statement

The experiment was approved by the ethical committee of the Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tandojam before the practical execution of this research work.

Novelty statement

Gastrointestinal parasites significantly reduce the growth and productivity of small ruminants. *Eimeria* spp. and *Haemonchus contortus* are the most prevalent gastrointestinal protozoa and nematodes in small ruminants, respectively. There is a strong association between feed and feeding method of an animal in the prevalence of gastrointestinal parasites in small ruminants. The sheep were found more infected as compared to the goat and females were more prone to infection. Sequence analysis indicated that HcITSTJ was closely related to isolates of Pakistan, Myanmar, and Ghana.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Abbasi, A.G., Abro, S.H., Kamboh, A.A., Kalhoro, D.H., Mazari, M.Q., Arain, M.B. and Depar, S.H., 2020. Epidemiological studies on bacterial respiratory infections in commercial poultry of district Hyderabad, Sindh, Pakistan. *Pure appl. Biol.*, **9**: 1253-1265. https://thepab.org/index.php/journal/article/view/1285, https://doi.org/10.19045/bspab.2020.90131

Achi, Y., Zinsstag, J., Yao, K., Yeo, N., Dorchies, P. and Jacquiet, P., 2003. Host specificity of *Haemonchus* spp. for domestic ruminants in the savanna in northern Ivory Coast. *Vet. Parasitol.*, **116**: 151-158. https://doi.org/10.1016/S0304-4017(03)00258-9

Adedapo, A.A., Otesile, A.T. and Soetan, K.O.J.P.B., 2007. Assessment of the anthelmintic efficacy of an aqueous crude extract of vernonia amygdalina. *Pharmac. Biol.,* **45**: 564-568. https://doi.org/10.1080/13880200701498978

Akhter, N., Arijo, A., Phulan, M., Iqbal, Z. and Mirbahar, K., 2011. Prevalence of gastro-intestinal nematodes in goats in Hyderabad and Adjoining Areas. *Pak. Vet. J.*, **31**: 287-290.

Akkari, H., Jebali, J., Gharbi, M., Mhadhbi, M., Awadi, S. and Darghouth, M.A., 2013. Epidemiological study of sympatric *Haemonchus* species and genetic characterization of *Haemonchus contortus* in domestic ruminants in Tunisia. *Vet. Parasitol.*, **193**: 118-125. https://doi.org/10.1016/j.vetpar.2012.12.014

Ali, W., Memon, M.A., Khoso, A.S., Tunio, S., Ayoob, A., Arain, M.B. and Hussain, M., 2023. *Biological characteristics and immunological role of mesenchymal stem cells for improvement of health.*

Al-Quaisy, H.H., Al-Zubaidy, A.J., Altaif, K.I. and Makkawi, T.A., 1987. The pathogenicity of haemonchosis in sheep and goats in Iraq: 1. Clinical, parasitological and haematological findings. *Vet. Parasitol.*, **24**: 221-228. https://doi.org/10.1016/0304-4017(87)90043-4

Al-Shaibani, I., Phulan, M., Arijo, A. and Qureshi, T., 2008. Epidemiology of ovine gastrointestinal nematodes in Hyderabad district, Pakistan. *Pak. Vet. J.*, **28**: 125-130.

Ansari, J.Z., Haq, A., Yousaf, M., Ahmad, T. and Khan, S., 2008. Evaluation of different medicinal plants as growth promoters for broiler chicks. *Sarhad J. Agric.*, **24**: 323-330.

Ayaz, M.M., Raza, M.A., Murtaza, S. and Akhtar, S., 2013. Epidemiological survey of helminths of goats in southern Punjab, Pakistan. *Trop. Biomed.*, **30**: 62-71.

Azhar, M., Gadahi, J.A., Bhutto, B., Tunio, S., Vistro, W.A., Tunio, H. and Ram, T., 2023. Babesiosis: Current status and future perspectives in Pakistan and chemotherapy used in livestock and pet animals. *Heliyon*, **9**. https://doi.org/10.1016/j.heliyon.2023.e17172

Badar, N., Iqbal, Z., Khan, M.N. and Akhtar, M.S., 2011. *In vitro* and *in vivo* anthelmintic activity of *Acacia nilotica* (L.) Willd. ex Delile bark and leaves. *Pak. Vet. J.*, **31**.

Bowman, D.D., 2014. *Georgis parasitology for veterinarians-E-book*. Elsevier Health Sciences.

Brasil, B.S., Nunes, R.L., Bastianetto, E., Drummond, M.G., Carvalho, D.C., Leite, R.C., Molento, M.B. and Oliveira, D.A., 2012. Genetic diversity patterns of *Haemonchus placei* and *Haemonchus contortus* populations isolated from domestic ruminants in Brazil. *Int. J. Parasitol*. **42**: 469-479. https://doi.org/10.1016/j.ijpara.2012.03.003

Burman, C. and Ktistakis, N.T., 2010. Autophagosome formation in mammalian cells. *Semin. Immunopathol.*, **32**: 397-413. https://doi.org/10.1007/s00281-010-0222-z

Cerutti, M., Citterio, C., Bazzocchi, C., Epis, S., D'amelio, S., Ferrari, N. and Lanfranchi, P., 2010. Genetic variability of *Haemonchus contortus* (Nematoda: Trichostrongyloidea) in alpine ruminant host species. *J. Helminthol.*, **84**: 276-283.

https://doi.org/10.1017/S0022149X09990587

Chikwanda, A., Mutisi, C., Sibanda, S., Makuza, S., Kusina, N. and Chikwanda, D., 2013. The effect of housing and anthelmintic treatment on pre-weaning kid death rate and infestation with gastrointestinal helminthes. *Appl. Anim. Husb. Rural Dev*., **6**: 32-35.

Dey, A.R., Zhang, Z., Begum, N., Alim, M.A., Hu, M. and Alam, M.Z., 2019. Genetic diversity patterns of Haemonchus contortus isolated from sheep and goats in Bangladesh. *Infect. Genet. Evol*., **68**: 177-184. https://doi.org/10.1016/j.meegid.2018.12.021

Farooq, Z., Mushtaq, S., Iqbal, Z., Akhtar, S.J.I.J.o.A. and Biology, 2012. Parasitic helminths of domesticated and wild ruminants in Cholistan desert of Pakistan. *Int. J. Agric. Biol.,* **14**: 1814–9596.

Gadahi, J., Arshed, M., Ali, Q., Javaid, S. and Shah, S., 2009. Prevalence of gastrointestinal parasites of sheep and goat in and around Rawalpindi and Islamabad, Pakistan. *Vet. World*, **2**: 51.

Gasser, R.B., Bott, N.J., Chilton, N.B., Hunt, P. and Beveridge, I., 2008. Toward practical, DNA based diagnostic methods for parasitic nematodes of livestock bionomic and biotechnological implications. *Biotech. Adv*., **26**: 325-334. https://doi.org/10.1016/j.biotechadv.2008.03.003

Ghosh, P.K., Kader, M.A., Riaz, M. and Rahman, M.M.J.V.R.I., 2016. Retrospective study on parasitic diseases of cattle at Mymensingh district of Bangladesh. *Vet. Res. Int.,* **4**: 144-147.

Hussain, T., Periasamy, K., Nadeem, A., Babar, M.E., Pichler, R. and Diallo, A., 2014. Sympatric species distribution, genetic diversity and population structure of Haemonchus isolates from domestic ruminants in Pakistan. *Vet. Parasitol*., **206**: 188-199. https://doi.org/10.1016/j.vetpar.2014.10.026

Iqbal, Z., Akhtar, M., Khan, M.N. and Riaz, M., 1993. Prevalence and economic significance of haemonchosis in sheep and goats slaughtered at Faisalabad abattoir. *Pak. J. Agric. Sa*, **30**.

Jacquiet, P., Cabaret, J., Thiam, E. and Cheikh, D., 1998. Host range and the maintenance of *Haemonchus* spp. in an adverse arid climate. *Int. J. Parasitol*., **28**: 253-261. https://doi.org/10.1016/S0020-7519(97)00185-9

Kahiya, C., Mukaratirwa, S. and Thamsborg, S.M., 2003. Effects of *Acacia nilotica* and *Acacia karoo* diets on *Haemonchus contortus* infection in goats. *Vet. Parasitol*., **115**: 265-274. https://doi.org/10.1016/S0304-4017(03)00213-9

Kanzaki, N. and Futai, K., 2002. A PCR primer set for determination of phylogenetic relationships of *Bursaphelenchus* species within the xylophilus group. *Nematology*, **4**: 35-41. https://doi.org/10.1163/156854102760082186

Khalafalla, R.E., Elseify, M.A. and Elbahy, N.M., 2011. Seasonal prevalence of gastrointestinal nematode parasites of sheep in Northern region of Nile Delta, Egypt. *Parasitol. Res*., **108**: 337-340. https://doi.org/10.1007/s00436-010-2066-9

Khalil-ur-Rehman, K.J., Tunio, M. and Kuthu, Z., 2009. Passive surveillance of gastrointestinal parasites in bufflaoes of Mandi Bahauddin and Gujrat districts of the Punjab. *J. Anim. Pl. Sci*., **19**: 17-19.

Khan, M.N., Sajid, M.S., Khan, M.K., Iqbal, Z. and Hussain, A., 2010. Gastrointestinal helminthiasis: Prevalence and associated determinants in domestic ruminants of district Toba Tek Singh, Punjab, Pakistan. *Parasitol. Res*., **107**: 787-794. https://doi.org/10.1007/s00436-010-1931-x

Khanal, P., 2018. A PCR/sequencing technique to identify species and genotypes of haemonchus from mixed trichostrongyle egg DNA Recovered from Livestock Fecal Samples. https://openprairie.sdstate.edu/etd/2636/

Khanum, H. and Yeasmin, T., 2015. Infestation of helminth parasites in sheep (*Ovis aries*). J. Environ. Bio-Sci., **29**: 1-6.

Králová-Hromadová, I., Bazsalovicsová, E., Oros, M. and Scholz, T., 2012. Sequence structure and intragenomic variability of ribosomal ITS2 in monozoic tapeworms of the genus Khawia (Cestoda: Caryophyllidea), parasites of cyprinid fish. *Parasitol. Res*., **111**: 16211627. https://doi.org/10.1007/s00436-012-3001-z

Kumsa, B., Tolera, A. and Abebe, R., 2008. Vulvar morphology and sympatry of *Haemonchus* species in naturally infected sheep and goats of Ogaden region, eastern Ethiopia. *Vet. Arhiv*, **78**: 331-342.

Lashari, M.H. and Tasawar, Z., 2011. Prevalence of some gastrointestinal parasites in sheep in southern Punjab, Pakistan. Pak. Vet. J., **31**: 295-298.

Lateef, M., Iqbal, Z., Jabbar, A., Nisar, M.K, and Akhter, M,S., 2005. Epidemiology of trichostrongylid nematode infections in sheep under traditional husbandry system in Pakistan. *Int. J. Agric. Biol.,* **7**: 596-600.

Lavín, S., Marco, I., Rossi, L., Meneguz, P.G. and Viñas, L., 1997. Haemonchosis in Spanish ibex. *J. Wildl. Dis*., **33**: 656-659. https://doi.org/10.7589/0090-3558-33.3.656

Memon, M.A., Kumar, C., Khoso, A.S., Vistro, M.K., Arain, S., Panhwar, S.A. and Ali, W., 2024. Potential role of autophagy in the male reproductive system.

Int. J. Sex. Reprod. Hlth. Care, **7**: 28-32.

Molento, M.B., Fortes, F.S., Pondelek, D.A.S., de Almeida Borges, F., de Souza Chagas, A.C., Torres-Acosta, J.F.d.J. and Geldhof, P., 2011. Challenges of nematode control in ruminants: Focus on Latin America. *Vet. Parasitol*., **180**: 126-132. https://doi.org/10.1016/j.vetpar.2011.05.033

Ndao, M., Pandey, V., Zinsstag, J. and Pfister, K., 1995. Helminth parasites and hypobiosis of nematodes in N'Dama cattle during the dry season in The Gambia. *Vet. Parasitol*., **60**: 161-166. https://doi.org/10.1016/0304-4017(94)00771-4

Pathak, A. and Pal, S., 2008. Seasonal prevalence of gastrointestinal parasites in goats from Durg district of Chhattisgarh. *Vet. World*, **1**: 136.

Prichard, R., 1994. Anthelmintic resistance. *Vet. Parasitol*., **54**: 259-268. https://doi.org/10.1016/0304-4017(94)90094-9

Qamar, M.F., Maqbool, A., Khan, M.S., Ahmad, N. and Muneer, M.A., 2009. Epidemiology of haemonchosis in sheep and goats under different managemental conditions. *Vet. World*, **2**: 413-417.

Qayyum, M., Farooq, U., Samad, H. and Chauhdry, H.J.J.A.P.S., 2010. Prevalence, clinicotherapeutic and prophylactic studies on theileriosis in district Sahiwal (Pakistan). *J. Anim. Pl. Sci.,* **20**: 266-270.

Raza, M., Younas, M. and Schlecht, E., 2014. Prevalence of gastrointestinal helminths in pastoral sheep and goat flocks in the Cholistan desert of Pakistan. J. Anim. Plant Sci., **24**: 127-134.

Raza, M.A., Iqbal, Z., Jabbar, A. and Yaseen, M., 2007. Point prevalence of gastrointestinal helminthiasis in ruminants in southern Punjab, Pakistan. *J. Helminthol*., **81**: 323. https://doi.org/10.1017/S0022149X07818554

Redman, E., Packard, E., Grillo, V., Smith, J., Jackson, F. and Gilleard, J.S., 2008. Microsatellite analysis reveals marked genetic differentiation between *Haemonchus contortus* laboratory isolates and provides a rapid system of genetic fingerprinting. *Int. J. Parasitol*., **38**: 111-122. https://doi.org/10.1016/j.ijpara.2007.06.008

Saddiqi, H.A., Jabbar, A., Sarwar, M., Iqbal, Z., Muhammad, G., Nisa, M. and Shahzad, A.J.P.r., 2011. Small ruminant resistance against gastrointestinal nematodes: A case of *Haemonchus contortus*. *Parasitol. Res.,* **109**: 1483-1500. https://doi.org/10.1007/s00436-011-2576-0

Sharhuu, G. and Sharkhuu, T., 2004. The helminth fauna of wild and domestic ruminants in Mongolia. A review. *Eur. J. Wildl. Res*., **50**: 150-156. https://doi.org/10.1007/s10344-004-0050-3

Squire, S.A., Yang, R., Robertson, I., Ayi, I., Squire, D.S. and Ryan, U., 2018. Gastrointestinal helminths in farmers and their ruminant livestock from the Coastal Savannah zone of Ghana. *Parasitol. Res*., **117**: 3183-3194. https://doi.org/10.1007/s00436-018-6017-1

Suchawan, P., Benjaporn, S., Kanyarat, S., Marchita, C.O., Pattaraporn, T., Kanokwan, S. and Charay, U., 2020. Prevalence and analysis of risk factors for gastrointestinal parasites in beef cattle herds in Aranyaprathet District, Sa Kaeo Province, Thailand. *Jpn. J. Vet. Res*., **68**: 69-76.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol*., **28**: 2731-2739. https://doi.org/10.1093/molbev/msr121

Tasawar, Z., Ahmad, S., Lashari, M.H. and Chaudhary, S.H., 2010. Prevalence of *Haemonchus contortus* in sheep at research centre for conservation of Sahiwal cattle (RCCSC) Jehangirabad District Khanewal, Punjab, Pakistan. *Pakistan J. Zool*., **42**. https://www.zsp.com.pk/pdf/735-739%20(14)%20PJZ-562-08.pdf

Taylor, W., Boomker, J., Krecek, R., Skinner, J. and Watermeyer, R., 2005. Helminths in sympatric populations of mountain reedbuck (*Redunca fulvorufula*) and gray rhebok (*Pelea capreolus*) in South Africa. *J. Parasitol*., **91**: 863-870. https://doi.org/10.1645/GE-436R.1

Urquhart, G., Armour, J., Duncan, J., Dunn, A. and Jennings, F. 1996. *Veterinary parasitology 2nd Edition*. University of Oxford (Longman Scientific and Technical Press, UK).

von Samson-Himmelstjerna, G., Harder, A. and Schnieder, T., 2002. Quantitative analysis of ITS2 sequences in trichostrongyle parasites. *Int. J. Parasitol*., **32**: 1529-1535. https://doi.org/10.1016/S0020-7519(02)00163-7

Zajac, A.M. and Conboy, G.A., 2012. *Veterinary clinical parasitology*. John Wiley and Sons.