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Trichostrongyloid nematodes in ruminants of northern Iran: prevalence and molecular analysis

Hedayat Hosseinnezhad¹, Meysam Sharifdini^{1*}, Keyhan Ashrafi¹, Zahra Atrkar Roushan², Hamed Mirjalali³ and Behnaz Rahmati¹

Abstract

Background: This study was carried out to investigate the prevalence and analyze the molecular characteristics based on the internal transcribed spacer (ITS) 2 region of the ribosomal RNA (RNA) gene of trichostrongyloid nematodes in different ruminants from Guilan province, northern of Iran.

Methods: The gastrointestinal tracts of 144 ruminants including 72 cattle, 59 sheep, and 13 goats were collected from an abattoir in Guilan province during July to September 2018. After isolation the helminths, male specimens were identified based on morphological parameters. PCR and partial sequencing of the ITS2 fragment were conducted. After phylogenetic analysis, the intraspecific and interspecific differences were calculated.

Results: The prevalence of total infections with the nematodes was 38.9, 74.6 and 84.6% among cattle, sheep and goats, respectively. Eleven species of trichostrongyloid nematodes including *Haemonchus contortus*, *Marshallagia marshalli*, *Trichostrongylus axei*, *T. colubriformis*, *T. vitrinus*, *Ostertagia trifurcata*, *Teladorsagia circumcincta*, *Marshallagia occidentalis*, *O. lyrata*, *O. ostertagi*, and *Cooperia punctate* were recovered from the ruminants. The most prevalent trichostrongyloid nematodes in cattle, sheep and goats were *O. ostertagi* (26.4%), *M. marshalli* (64.4%) and *T. circumcincta* (69.2%), respectively. Phylogenetic tree was discriminative for Trichostrongylidae family, while phylogenetic analysis of the ITS2 gene represented low variations and no species identification of Haemonchidae and Cooperiidae families.

Conclusions: This study suggests the high prevalence and species diversity of trichostrongyloid nematodes in different ruminants, indicating the importance of implement antiparasitic strategies in north regions of Iran. As well, this study showed that the ITS2 fragment is not a discriminative marker for Haemonchidae and Cooperiidae families, and investigation of other genetic markers such as mitochondrial genes would be more valuable for better understanding of their phylogenetic relationships.

Keywords: Trichostrongyloid nematodes, Ruminants, ITS2, Molecular analysis, Iran

Background

Helminthic parasites cause long-term chronic infections associated with significant morbidity and mortality rates in both human and animal hosts. These infections lead to reduced productivity of ruminants in many countries [1]. There are several genera and an enormous number of species in super family Trichostrongyloidea, which are primarily parasites of the gastrointestinal tract of the

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animals with a worldwide distribution. Trichostrongyloid nematodes such as *Trichostrongylus* (Trichostrongylidae), *Haemonchus* (Haemonchidae), *Ostertagia* (Haemonchidae), *Nematodirus* (Molineidae), *Marshallagia* (Haemonchidae) and *Cooperia* (Cooperiidae), mostly live in abomasum and the first part of the small intestine in ruminants [2]. Actually, infection with these parasites leads to loss of meat, wool, and milk production and decreased feed intake, weight gains and growth rate [3]. These parasites also can occur serious or even fatal manifestations in ruminants and other grazers. Among the super family, *H. contortus* is one of the most pathogenic blood sucking nematodes, which causes severe anemia, hypoproteinemia, edema, and even death in ruminants [4]. Transmission of these nematodes is mostly through the ingestion of infective larvae in contaminated pasture and water [5]. The epidemiology of gastrointestinal helminths in domestic animals serves to improve prevention and control strategies, evaluation of risk factors, and decrease in production losses of the animals. In addition, these studies help to determine probable sources of animal and human infections [1, 6–8].

Trichostrongylus species are the potential zoonotic nematodes, which are prevalent in humans from some regions of the world, particularly in the Middle East, Far East, and some African countries [9–11]. Human trichostrongylosis has the highest infection rates and largest variety of species in Iran compared to other countries in the world due to close contact with ruminants in rural and nomadic populations [9]. Recent studies illustrated

that Guilan province in north of Iran is one of most important endemic regions of human trichostrongylosis in Iran [7, 12–14]. Other genera of this super family including *H. contortus*, *M. marshalli*, *N. abnormalis*, *O. ostertagi* and *T. circumcincta* have been rarely reported in humans from different parts of world, especially in Iran [7, 9, 10, 15].

Recently, PCR based methods are used to identify species and phylogenetic analysis of different trichostrongyloid nematodes [16–19]. These studies have shown that the internal transcribed spacer 2 (ITS2) and the cytochrome c oxidase subunit 1 (*Cox1*) genes are useful targets for species differentiation and analyzing of the genetic variations [19–21]. The aim of this study was to determine the prevalence of the trichostrongyloid nematodes obtained from slaughtered indigenous ruminants in Guilan province, northern Iran, and to investigate of their genetic diversity using the ITS2 region of the rRNA gene.

Methods

Study area and samples collection

Guilan province is located in southwest coast of the Caspian Sea in the northern Iran (Fig. 1). It covers an area of 14,711 km² (between 36° 36' and 38° 27' N, and between 48° 43' to 50° 34' E), and has temperature humid climate with mean annual rainfall of 1359 mm. The average seasonal temperature in Guilan province is 7.5°C in winter, 18.5°C in spring 24°C in summer, and 13.5°C in autumn. The average relative humidity is about 80%,

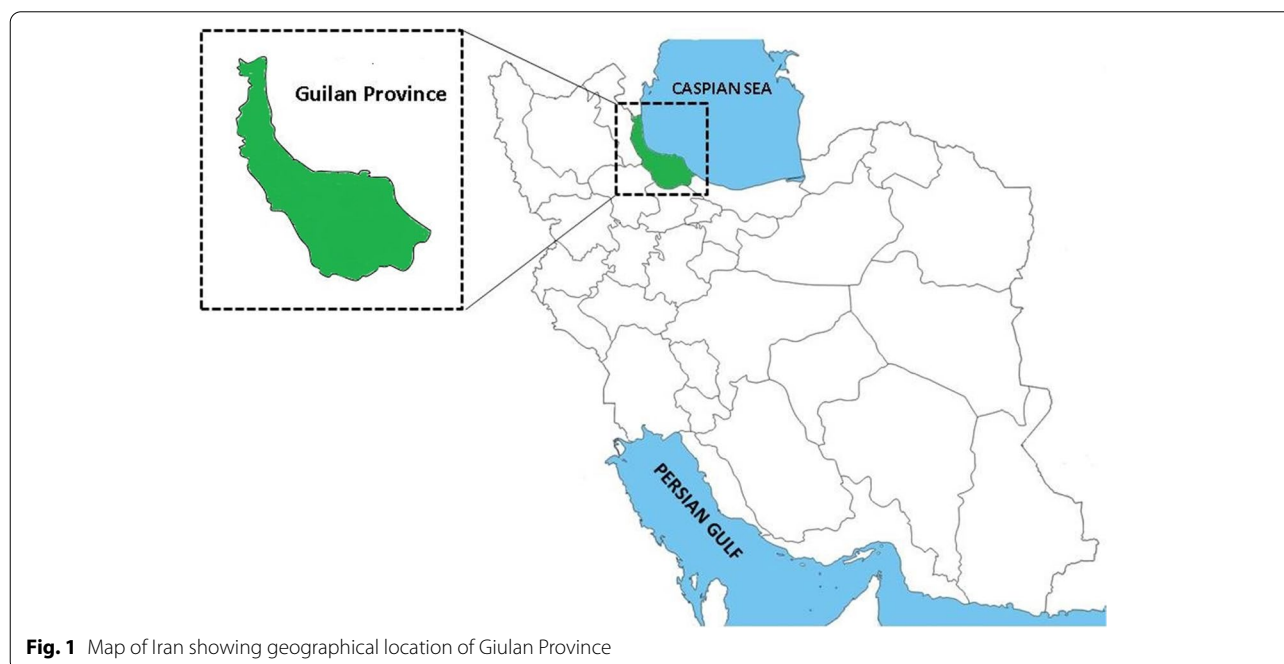


Fig. 1 Map of Iran showing geographical location of Guilan Province

which reaches to maximum in autumn and winter, and decreases in summer and spring [22]. In current study, a total of 144 ruminant gastrointestinal tracts from 72 cattle, 59 sheep, and 13 goats were collected from Talesh abattoir of Guilan province, northern of Iran, during July to September 2018. Animals all were local breed and samples were randomly collected from 10 to 15 animals in each sampling time (1 day in each week) for 12 weeks. The contents of abomasum and duodenum were washed using tap water runs through 20, 40, and 100 mesh sieves and examined for infectivity with trichostrongyloid nematodes by stereomicroscope. The obtained specimens were preserved in 70% ethanol for parasitological and molecular examinations. For morphological identification, the male nematodes were stained with Azocarmine, and identified at the level of species using valid nematodes systematic keys [23, 24].

DNA extraction and polymerase chain reaction (PCR) amplification

Total genomic DNA was extracted from one male worm of each species of trichostrongyloid nematodes in every kind of ruminants ($n=21$) using a commercial DNA extraction kit (Yekta Tajhiz Azma, Tehran, Iran), according to the manufacturer's instructions.

The ITS2 region was amplified using the primer pairs NC1 (5'-ACGTCTGGTTCAGGGTTGTT-3') and NC2 (5'-TTAGTTTCTTTTCCTCCGCT-3') [25]. PCR conditions comprised an initial denaturing step of 95°C for 6 min followed by 35 cycles of denaturation at 94°C for 45 s, annealing at 60°C for 90 s and extension at 72°C for 60 s, and 72°C for 5 min as a final extension. The amplification products were separated on 1.5% agarose gel electrophoresis, and visualized with UV transilluminator (UVITEC, Cambridge, UK). Later, the PCR products were sent to a domestic sequencing company (Codon genetic company, Tehran, Iran) for sequence determination via the Sanger method.

Phylogenetic analysis

The sequence results were edited and trimmed using Chromas version 2.01 (Technelysium Pty Ltd., Brisbane, Queensland, Australia), and compared to the GenBank submitted sequences using the BLAST programs and databases (<http://www.ncbi.nlm.nih.gov/>). The sequences of the ITS2 gene were submitted to the GenBank database (accession numbers: MN845160-MN845180). Phylogenetic analysis was performed with sequences obtained in the present study along with the reference sequences, which were deposited in the GenBank database, using Maximum-Likelihood algorithm and Tamura-3-parameter model in the MEGA 6.0 software.

Bootstrap value was determined based on 1000 replications for evaluation of the phylogenetic tree reliability.

Statistical analysis

Statistical analyses were performed using SPSS software version 18 (SPSS Inc., Chicago, Illinois, USA), and statistical tests including chi-squared (χ^2) and Fisher's exact tests were used to evaluate the association between trichostrongyloid nematode infections with different kinds of ruminants and sex of animals. A value less than 0.05 was considered statistically significant.

Results

Overall, 83 out of the 144 ruminants (57.6%) were found infected with different genera of trichostrongyloid nematodes. The prevalence of total infections with the nematodes was 38.9% (28/72), 74.6% (44/59) and 84.6% (11/13) among cattle, sheep and goats, respectively. There are significant differences between the infection with trichostrongyloid nematodes and different kinds of ruminants ($P<0.0001$). Eleven species of trichostrongyloid nematodes including *H. contortus*, *M. marshalli*, *T. axei*, *T. colubriformis*, *T. vitrinus*, *O. trifurcata*, *T. circumcincta*, *M. occidentalis*, *O. lyrata*, *O. ostertagi*, and *C. punctate* were recovered from the ruminants (Figs. 2, 3, and 4).

The most prevalent trichostrongyloid nematodes in cattle and sheep were *O. ostertagi* (26.4%) and *M. marshalli* (64.4%), respectively. Also, the most predominant nematodes in goats were *T. circumcincta* (69.2%) and *M. marshalli* (53.8%). The prevalence rate of these parasites is shown in Table 1. Thirty-one of all ruminants (21.5%) were males and 113 (78.4%) were females. Of these, 18 (58.1%) of the males and 65 (57.5%) of the females were infected with the trichostrongyloid nematodes. No statistically significant difference was seen between total infection and the sex ($P>0.05$). Also, there was not any significant difference between sex and the infection rates of each animal ($P>0.05$) (Table 2).

The genetic divergence within the specimens of *H. contortus*, *M. marshalli*, *T. axei*, *T. colubriformis*, *T. vitrinus* and *O. ostertagi* obtained from this study was 0%. However, intra-species distance for *O. trifurcata* and *O. circumcincta* isolates obtained in current study was 2.7 and 0.6%, respectively. The mean intra-species distance rate within species of *T. axei*, *T. colubriformis*, *T. vitrinus*, *O. ostertagi*, *M. marshalli*, *T. circumcincta*, *M. occidentalis*, *O. lyrata*, *H. contortus* and *C. punctate* obtained in this study and those available in the GenBank amounted to 0.2, 0.1, 0.4, 0.4, 0.3, 1.8, 0.2, 1.5, 0.3 and 1.2%, respectively.

The phylogenetic analysis of the Haemonchidae family illustrated that our *M. marshalli* (MN845179, MN845180 and MN845178) and *M. occidentalis* (MN845171)

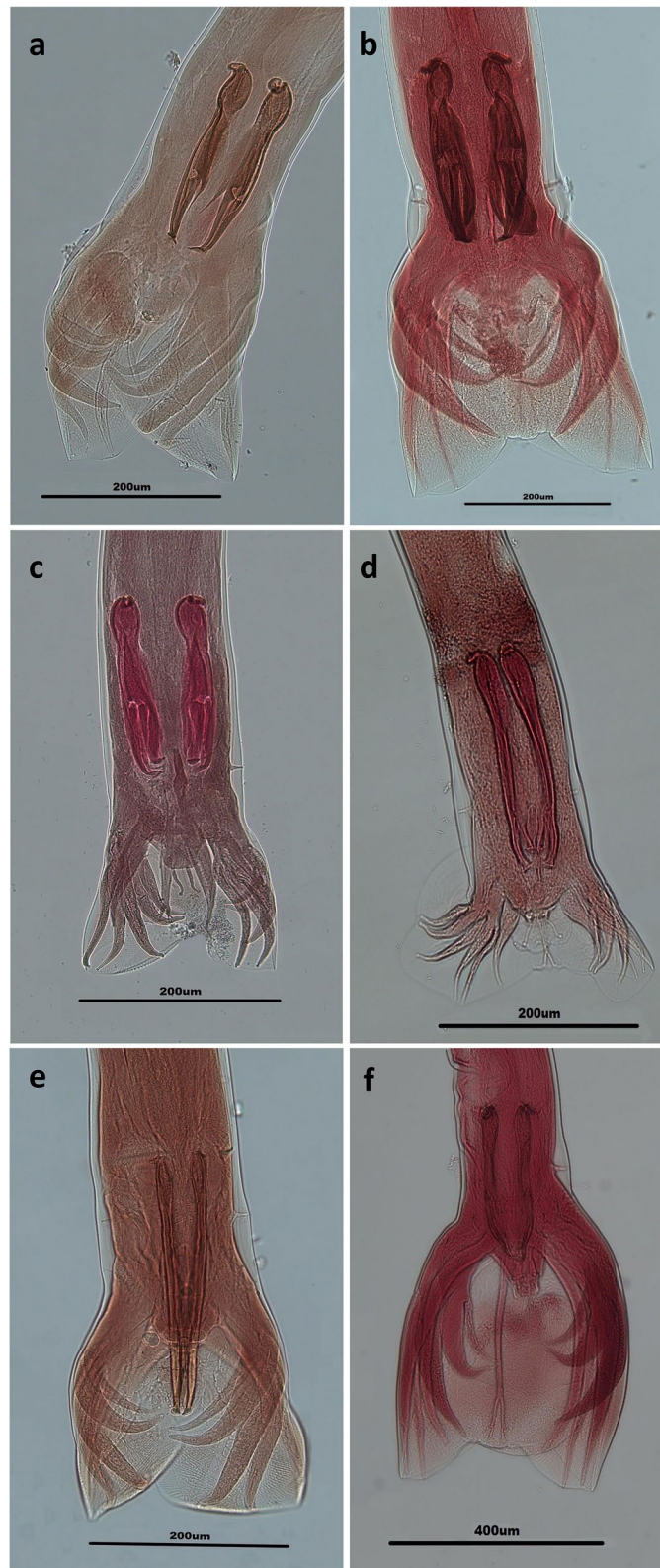


Fig. 2 Light microscope view of *Marshallagia* and *Ostertagia* species isolated from ruminants in Guilan province, northern Iran. Posterior ends of males of *Ostertagia trifurcata* (a), *Marshallagia occidentalis* (b), *Ostertagia lyrata* (c), *Ostertagia ostertagi* (d), *Teladorsagia circumcincta* (e) and *Marshallagia marshalli* (f)

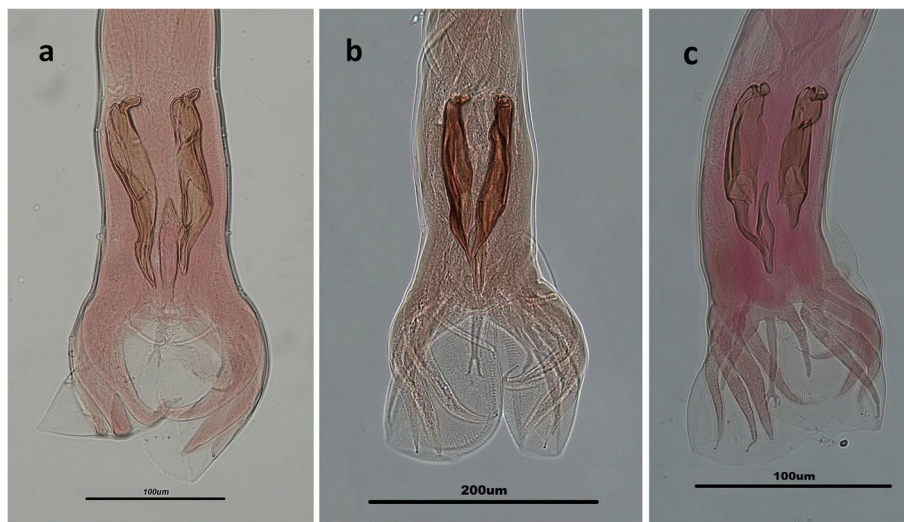


Fig. 3 Light microscope view of *Trichostrongylus* species isolated from ruminants in Guilan province, northern Iran. Posterior ends of males of *Trichostrongylus colubriformis* (a), *Trichostrongylus vitrinus* (b) and *Trichostrongylus axei* (c)

sequences placed together in one branch along with sheep isolates of *M. marshalli* from Iran (MN888760, MK253690 and HQ389231) and Uzbekistan (KX929996 and MT110919), and also sheep isolates of *M. occidentalis* from Iran (MK760917 and KC295417) and Uzbekistan (MT110967), and *M. schumakovitschi* (MT110926) and *M. sogdiana* (MT118024) from Uzbekistan. In addition, one goat isolate of *M. marshalli* from Iran (MK253691), sheep isolates of *M. marshalli* from Uzbekistan (MT110920 and KT428384) and sheep isolates of *M. trifida* (MT118027) and *M. occidentalis* (KX929997) from Uzbekistan clustered separately in this clade. Our sequences of *O. lyrata* (MN845170) and *O. ostertagi* (MN845172 and MN845173) grouped together in one clade along with sheep isolates of *O. ostertagi* (KX929994 and KT428385) and *O. lyrata* (KX929995) from Uzbekistan and cattle isolate of *O. ostertagi* (KX929995) from New Zealand. Two cattle of *O. ostertagi* isolates from New Zealand (KC998715 and KC998717) formed an innermost clade. The tree showed that our *T. circumcincta* isolates obtained from sheep (MN845177) and goat (MN845176), and *O. trifurcata* isolate from sheep (MN845175) clustered with sheep isolates of *T. circumcincta* from the United Kingdom (UK) (JF680984) and Morocco (MH047832). Moreover, our goat isolate of *O. trifurcata* (MN845174) grouped with sheep isolates of *T. circumcincta* from New Zealand (KC998711) and the UK (AY439025). The *H. contortus* isolates (MN845168 and MN845169) clustered with sheep (HQ844231) and giraffe (KM586651) isolates from China and goat isolate from Bangladesh (KU870652). Two sheep sequences

of *H. contortus* from Egypt (KF176320) and Bangladesh (KU870651) located separately in this clade (Fig. 5).

The phylogenetic reconstruction of Trichostrongylidae family showed that our sequences of *T. colubriformis* obtained from sheep (MN845161) and goat (MN845160) clustered with *T. colubriformis* isolated from human (KY355058, KF989494 and KF826913), sheep (JF276021), and goat (JF276020) in Iran. They also grouped with human isolates of *T. colubriformis* from France (HQ174257), Laos (AB503244), and Thailand (KC337070), sheep isolates from New Zealand (KC998728) and Russia (EF427624), goat isolate from Malaysia (KF204576), and cattle isolate from the United States of America (USA) (KP150536). In this clade, two sheep sequences of *T. colubriformis* from Iran (HQ389232) and Ireland (JF680985) located separately. The tree illustrated our sheep (MN845166) and goat (MN845165) isolates of *T. vitrinus* grouped with sheep isolates of *T. vitrinus* from New Zealand (KC998732 and KC998733). Moreover, our *T. axei* sequences isolated from sheep (MN845162), goat (MN845163), and cattle (MN845164) clustered with human isolates from Iran (KY355033 and KF840722) and Thailand (KC337066), sheep isolates from New Zealand (KC998727) and Iran (KJ755059), and cattle from the USA (KP150521) (Fig. 6).

The phylogenetic analysis of the Cooperiidae family showed that our *C. punctata* (MN845167) obtained from cattle grouped with cattle isolates of *C. punctata* from New Zealand (KC998744 and KC998745) and cattle isolates of *C. spatulata* from Brazil (MH267786, MH267791 and MH267790) (Fig. 7).

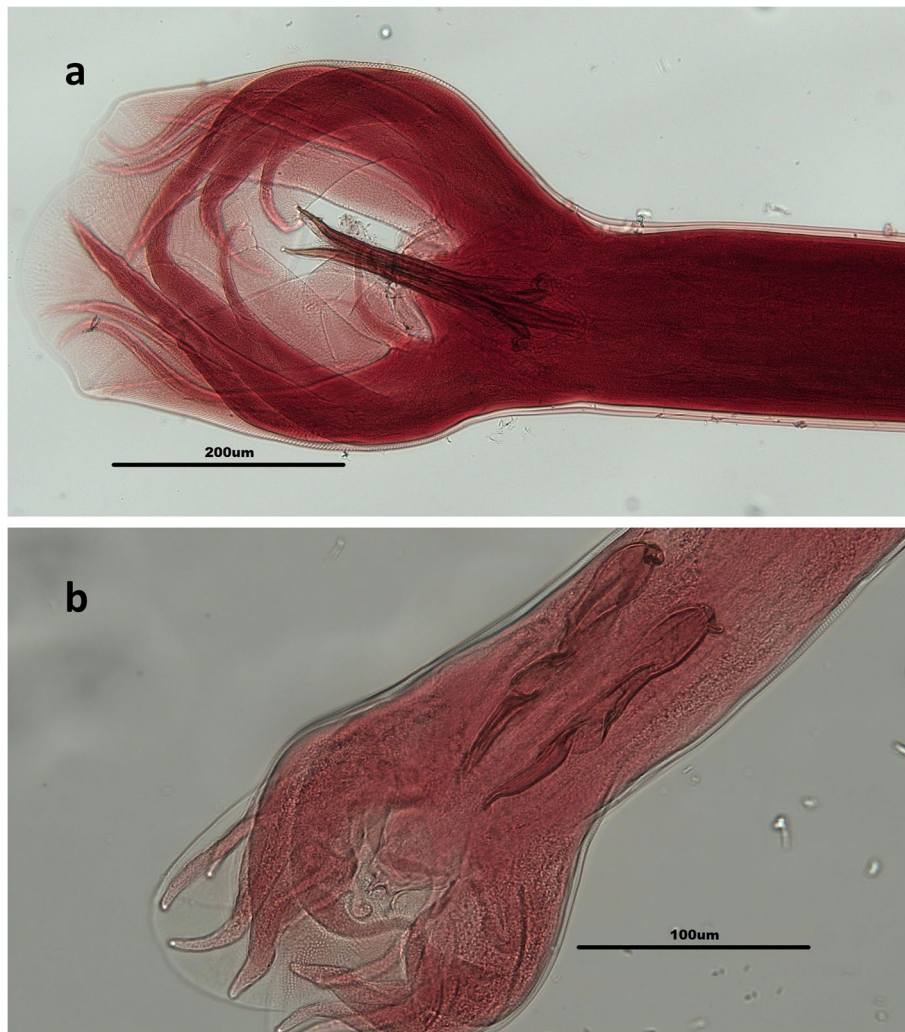


Fig. 4 Posterior ends of males of *Haemonchus contortus* (a) and *Cooperia punctate* (b) isolated from ruminants in Guilan province, northern Iran

Table 1 Prevalence of Trichostrongyloid nematodes recovered from different livestock in Guilan province, northern Iran

Host (144)	Trichostrongyloid nematodes (%)											
	Hc	Mm	Mo	Tc	Ot	OI	Oo	Cp	Ta	Tc	Tv	Co-infection
Sheep (59)	2 (3.4)	38 (64.4)	15 (25.4)	19 (32.2)	1 (1.7)	1 (1.7)	0 (0)	0 (0)	1 (1.7)	1 (1.7)	3 (5.1)	14 (19.4)
Goat (13)	2 (15.4)	7 (53.8)	0 (0)	9 (69.2)	1 (7.7)	1 (7.7)	1 (7.7)	0 (0)	3 (23.1)	2 (15.4)	2 (15.4)	28 (47.5)
Cattle (72)	0 (0)	2 (2.8)	0 (0)	0 (0)	0 (0)	6 (8.3)	19 (26.4)	9 (12.5)	6 (8.3)	0 (0)	0 (0)	8 (61.5)

Abbreviations: Hc *Haemonchus contortus*, Mm *Marshallagia marshalli*, Mo *Marshallagia occidentalis*, Tc *Teladorsagia circumcincta*, Ot *Ostertagia trifurcata*, OI *Ostertagia lyrata*, Oo *Ostertagia ostertagi*, Cp *Cooperia punctate*, Ta *Trichostrongylus axei*, Tc *Trichostrongylus colubriformis*, Tv *Trichostrongylus vitrinus*

Discussion

This study revealed 38.9% cattle, 74.6% sheep, and 84.6% goats were infected with the trichostrongyloid nematodes. The prevalence of abomasal nematodes were reported 44 and 25% of the slaughtered cows in Tabriz, northwestern Iran [26] and Kerman southeastern Iran

[27], respectively. Moreover, the prevalence of trichostrongyloid nematodes was reported 94 and 6.1% of the cattle in Belgium [28] and Ethiopia [29], respectively. Several studies from Iran showed high infection rate of the parasites in sheep and goats. Kordi et al. [27] reported a high infection rate as 100% in sheep and goats

Table 2 Prevalence of infection with trichostrongyloid nematodes from different livestock in Guilan province, northern Iran according to sex

Hosts	Males		Femals		Total		P-value
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	
Sheep (59)	8 (80)	2 (20)	36 (73.5)	13 (26.5)	44 (74.6)	15 (25.4)	1
Goat (13)	4 (100)	0 (0)	7 (77.8)	2 (22.2)	11 (84.6)	2 (15.4)	1
Cattle (72)	6 (35.3)	11 (64.7)	22 (40)	33 (60)	28 (38.9)	44 (61.1)	0.783
Total (144)	18 (58.1)	13 (41.9)	65 (57.5)	48 (42.5)	83 (57.6)	61 (42.4)	1

in Kerman. Also, Garedaghi et al. [30] found the infection rates of 81 and 71.2% in sheep and goats of Mazandaran province, respectively. However, on the contrary to the present study, some previous studies in Iran have reported lower infection rate in the small ruminants [31, 32]. Furthermore, Fufa et al. [33] reported the prevalence of abomasum nematodes in sheep and goats, slaughtered in Ethiopia to be 83.6 and 77.6%, respectively.

Our finding showed that the most predominant trichostrongyloid nematode in cattle in the study area was *O. ostertagi* (26.4%). *O. ostertagi* is a common parasitic nematode of cattle in the world, but it can also be found to a lesser extent in sheep, goats, wild ruminants, and horses [34]. Similar to our study, *O. ostertagi* was reported as predominant nematode in cattle in Canada [35]. However, *H. contortus* was reported in cattle with highest prevalence (17.6%) in Tabriz, northwestern of Iran [26]. Abuhay et al. [29] reported *T. axei* (2.86%) was the most predominant abomasal nematodes of cattle slaughtered in Ethiopia.

In present study, *M. marshalli* was identified as the most predominant nematode in sheep (64.4%). Also, the most prevalent nematodes in goats were *T. circumcincta* (69.2%) and *M. marshalli* (53.8%). Similar to our results, Pestechian et al. [36] reported that *M. marshalli* (57.1%) in sheep and *T. circumcincta* (47.2%) in goat were dominant species in Isfahan, central part of Iran. *M. marshalli* was also reported as more prevalent species among both sheep (20.5%) and goats (60%) in Qazvin province, northwestern of the country [37]. In other studies, in Iran, *H. contortus* in goats and *T. circumcincta* in sheep were also found as the frequently reported species in northwest and west of the country, respectively [31, 38]. Aligned with our findings, *M. marshalli* occurred with the highest prevalence among sheep in Canada [39]. In other study in Ethiopia, *Haemonchus* spp. were found as most predominant abomasal nematodes in both sheep and goats [40]. Recently, Ashrafi et al. [7] reported the occurrence of natural human infection by *T. circumcincta* for the first time in Iran and the second time in the world.

In the current study, *Trichostrongylus* species including *T. colubriformis*, *T. axei*, and *T. vitrinus* were detected from the animals. Previously, several studies reported these nematodes as the predominant species of *Trichostrongylus* among different herbivores in most parts of Iran [9]. Recently, infection with some species of *Trichostrongylus* including *T. colubriformis*, *T. axei*, *T. vitrinus*, and *T. longispicularis* have been reported from humans in Guilan province from which *T. colubriformis* was as the most common species [12]. Additionally, Gholami et al. [41] and Sharifdini et al. [17] reported *T. colubriformis* as the most probable common species and *T. axei* in inhabitants of Mazandaran province, north of Iran. Also, *T. colubriformis* was identified as a predominant species among humans in Thailand, France, and Laos [11, 16, 42].

In this study, *O. lyrata* was reported from cattle (8.3%), sheep (1.7%) and goats (7.7%). Until now, *O. lyrata* has not been reported from Iran, and to best of our knowledge this study is the first report of this species in the ruminants. This nematode has mainly reported from cattle in different parts of the world [43–45].

In the current study, there was no significant difference between trichostrongyloid infection and the sex ($P > 0.05$). Our findings are in consistent with Kordi et al. [27], Garedaghi et al. [31] and Amniattalab et al. [46]. Nevertheless, some researchers have observed higher rates of trichostrongyloid nematode infections in female hosts compared to the males [47, 48].

In the last few decades, PCR-sequencing technique was applied to assess the taxonomic identification, diversity, and phylogenetic relationships among trichostrongyloid species. Some investigations showed that the ITS2 region of rRNA gene is useful tool for identification and phylogenetic analysis of trichostrongyloid nematodes [7, 12, 17, 41]. In the present study, no intra-generic differences were apparent between *H. contortus*, *M. marshalli*, *T. axei*, *T. colubriformis*, *T. vitrinus*, and *O. ostertagi* isolated from different domestic animals, but intra- species variations were noted within *O. trifurcate* and *O. circumcincta*



Fig. 5 Phylogenetic tree of isolates of *Ostertagia*, *Marshallagia* and *Haemonchus* genera obtained in this study (▲) and other members of Haemonchidae family as retrieved from GenBank based on the partial ITS2 gene. The tree was constructed based on the maximum likelihood test and the Tamura 3-parameter model in MEGA6. *Nematodirus spathiger* sequence was used as the out group. Bootstrap values lower than 50 were omitted

isolates being 2.7 and 0.6%, respectively. The inter-species differences between species of genera *Ostertagia*, *Marshallagia*, *Trichostrongylus* obtained in our study were 1.00–13.1%, 0.1–1.5% and 2–6.4%.

Our phylogenetic analysis of Haemonchidae family showed that different species of *Marshallagia* including *M. marshalli*, *M. occidentalis*, *M. schumakovitschi*, *M. sogdiana* and *M. trífida* were grouped together in

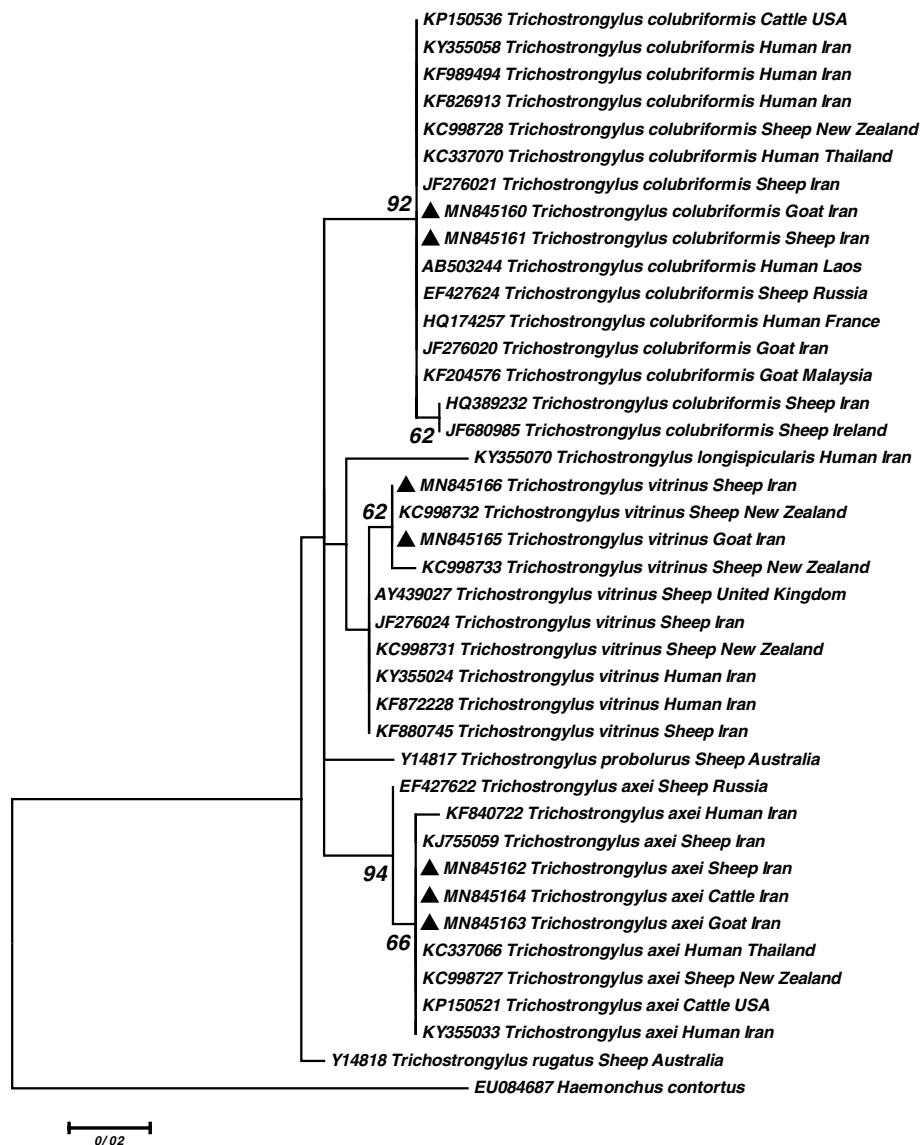
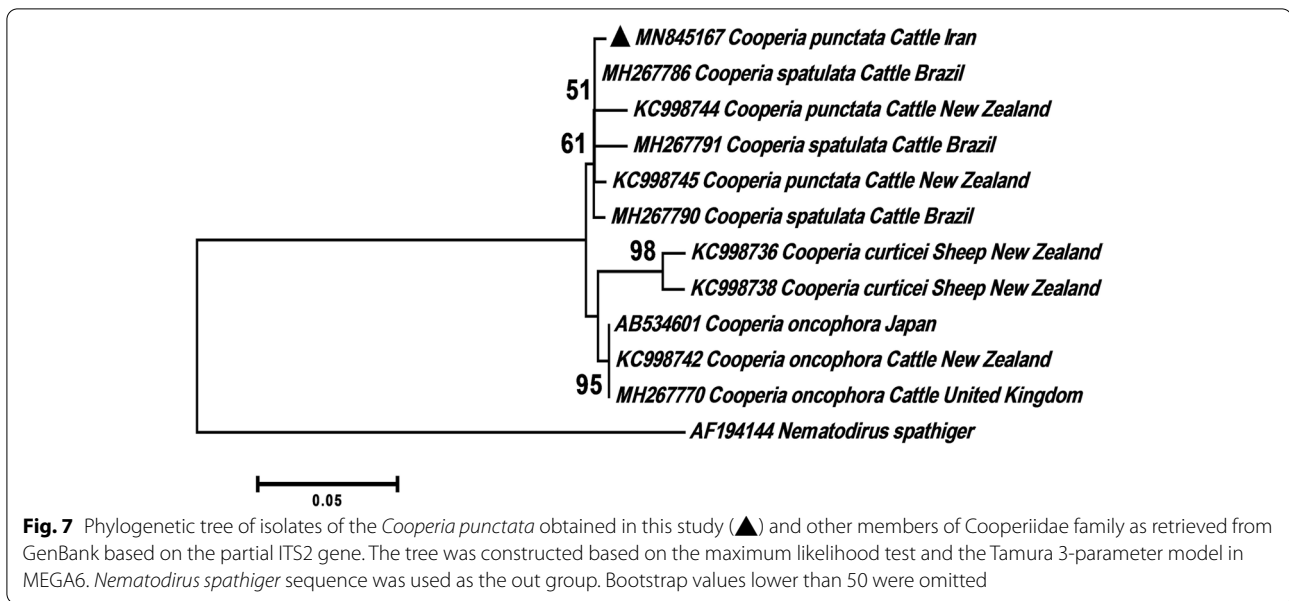


Fig. 6 Phylogenetic tree of isolates of the *Trichostrongylus* species obtained in this study (▲) and other members of Trichostrongylidae family as retrieved from GenBank based on the partial ITS2 gene. The tree was constructed based on the maximum likelihood test and the Tamura 3-parameter model in MEGA6. *Haemonchus contortus* sequence was used as the out group. Bootstrap values lower than 50 were omitted

one cluster. Additionally, *M. uzbekistanica* placed separately from other species of *Marshallagia* with strong support. Our finding showed that the ITS2 gene is not suitable for diagnosis and gene diversity of *Marshallagia* species so that *M. marshalli*, *M. occidentalis* obtained in this study had 100% identity. On the other hand, the phylogenetic analysis showed that *O. lyrata* with *O. ostertagi* and *T. circumcincta* with *O. trifurcata* grouped together in one clade. The presented data indicated that the ITS2 sequence is uninformative for phylogenetic inference among the species, because of

the lack of sufficient diversity within the gene. In this line, Zarlenga et al. [21] illustrated that the ITS1 is inappropriate in resolving phylogenetic issues within genus *Ostertagia*. Thus, the application of additional genetic data is necessary for exploring the phylogenetic relationships among the species. *H. contortus* as other genus of Haemonchidae family, was well separated in the tree, with strong support. Our study also revealed *H. contortus* sequences obtained from goat and sheep had 100% similarity. Several studies showed that the ITS2 is a useful target for identification of two species



within the genus *Haemonchus* and the intraspecific variation within *H. contortus* [49–52].

The existence of genetic diversity of the ITS2 fragment among different *Trichostrongylus* species has been confirmed previously [7, 12, 17, 41]. The phylogenetic tree of Trichostrongylidae family represented that all species were clearly separated and they were similar to the species obtained from human and animal subjects in Iran and other countries. Our study also showed that *T. colubriformis*, *T. vitrinus*, and *T. axei* isolates obtained from different animals had 100% similarity. Sequences of *Trichostrongylus* species isolated in present study had 99–100% homology with those obtained from humans and domestic animals in Iran. This finding confirmed that the ruminant and human *Trichostrongylus* species have close phylogenetic relationship. This could explain that human infections in the study area may occur due to close contact with domestic animals and application of domestic animal’s dung as fertilizer in vegetable farms.

Our phylogenetic analysis of the Cooperiidae family showed that isolates of *C. punctata* and *C. spatulata* clustered together in one clade. This finding presented that the ITS2 fragment was unreliable for differentiation at the species level. Recently, Ramünke et al. [53] reported that the ITS2 has minimal nucleotide differences between the *Cooperia* species and is accurate only up to genus level identification. The authors also showed that, in contrast to the morphological characterization, no molecular distinction between *C. punctata* and *C. spatulata* was possible with any of the four gene markers including isotype 1 β -tubulin, the

mitochondrial *Cox2*, ITS, and mitochondrial 12S rRNA genes [53].

Conclusions

The findings of this study showed the high prevalence and species diversity of trichostrongyloid nematodes in different ruminants of northern Iran. Therefore, implement antiparasitic strategies seems to be necessary to increase productivity of the domestic animals. Furthermore, extra epidemiological studies in different seasons and reigns of the country should be conducted to provide more information about the seasonal dynamics of the gastrointestinal helminths. The present molecular analysis of the ITS2 fragment is not sufficient for valid species identification of Haemonchidae and Cooperiidae families and only can differentiate members of Trichostrongylidae family. Providing more genetic markers alongside obtaining specimens from other geographical locations and hosts would be valuable for better understanding of their phylogenetic relationships.

Abbreviations

ITS-rDNA: Internal transcribed spacer ribosomal DNA; Cox1 : Cytochrome c oxidase 1; PCR: Polymerase chain reaction.

Acknowledgements

The authors would like to thank all people who have contributed to this research.

Authors’ contributions

MSH designed the study. HH collected of the samples. HH, BR and MSH carried out morphological species identification. HH, MSH, HM performed the molecular and phylogenetic analysis. MSH, ZAR and KA analyzed the data. MSH and HM wrote the draft of the manuscript. All authors read and approved the final version of the manuscript.

Funding

Not applicable.

Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Declarations**Ethics approval and consent to participate**

The study was approved by the ethics committee of the Research Department of Guilan University of Medical Sciences, under approval number IR.GUMS.REC.1397.176. The samples were collected from slaughtered ruminants from Talesh abattoir of Guilan province, northern of Iran. Formal consent and permission for the research were obtained from both the university and abattoir veterinarians. Moreover, all the samplings were performed with informed consent from animal owners. In this study, no experiment was conducted on live animals. This study was carried out in compliance with the ARRIVE guidelines (<https://arriveguidelines.org/>).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 18 May 2021 Accepted: 23 November 2021

Published online: 04 December 2021

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