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Prevalence and Morphometric Comparison of *Trichostrongylus* spp. among Sheep and Goats from Kashan Abattoir, Central Iran

Mohsen Arbabi[1], Alimohammad Bakhshi[1], Hossein Hooshyar[1*], Reza Ghasemikhah[2], Mahdi Delavari[1], Mojtaba Sehat[3]

[1]Department of Medical Parasitology, Kashan University of Medical Sciences, Kashan, Iran; [2]Department of Medical Parasitology, Arak University of Medical Sciences, Arak, Iran; [3]Department of Community Medicine, Kashan University of Medical Sciences, Kashan, Iran

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***Correspondence**
Email: hooshyar4@yahoo.com
Tel: +983155540021
Fax: +983155541112

ABSTRACT

Introduction: Trichostrongyloiasis is a prevalent infection in humans and some animals worldwide. Morphology is a reliable tool for identifying *Trichostrongylus* species. This study aimed to determine the prevalence of *Trichostrongylus* infection in livestock and compare the morphometric characteristics of the species in sheep and goats referred to Kashan Abattoir, Iran, in 2018. **Methods:** This cross-sectional study was performed on 130 goats and 154 sheep. The small intestine was collected from the slaughterhouse; the samples were opened and examined, and the genus and species of worms were identified based on morphological features reflected in diagnostic keys. Five morphometric indices, including body length and width, copulatory bursa width, shape length of the spicule, and gubernaculum length, were measured in 70 worm isolates. The data were analyzed using the ANOVA test in SPSS 18 software. **Results:** Of 284 livestock (130 goats and 154 sheep) examined, 26 (9/15%) were infected with *Trichostrongylus.* The prevalence of infection in goats and sheep was 12.3% and 6.5%, respectively. The most frequent species were *Trichostrongylus colubriformis* (48.7%), followed by *Trichostrongylus vitrinus* (25.7%). *Trichostrongylus capricula* and *Trichostrongylus probolurus* had an incidence of 12.8%. *T. probolurus* showed a higher length of spicule and gubernaculum, while *T. vitrinus* showed a wider copulatory bursa compared to the other species (P <0.001). **Conclusion:** The prevalence of *Trichostrongylus* infection in this region was remarkable. Morphometric and morphological methods are practical tools in differentiating male *Trichostrongylus* species. However, in addition to morphometric studies, molecular methods are required to identify female worms, larvae, and eggs accurately.

INTRODUCTION

Trichostrongylus nematodes are the causative agent of trichostrongyliasis in humans and animals such as cattle, sheep, goats, deer, and rabbits. These nematodes are globally distributed [1]. Sheep and goats play a critical role in maintaining the parasite cycle as the main reservoirs [2]. However, other animals can also act as reservoirs for the parasite [2]. So far, at least ten species of *Trichostrongylus* have been identified that can infect humans, causing various clinical signs such as anemia, weight loss, and intestinal disorders [3-4].

Epidemiological studies indicate a global prevalence of *Trichostrongylus* infections in animals. However, human infections are more commonly reported in Middle Eastern and Asian countries, including Iran, Iraq, India, Korea, Japan, and China. Various species of *Trichostrongylus* are assumed to infect over five and a half million people in these countries [3, 5-6]. Despite reported cases, there is limited information on the prevalence of human and animal infections with the *Trichostrongylus* species in Iran.

Infection with this group of nematodes is of great veterinary importance. Some species belonging to the *Trichostrongyloidea* superfamily, such as *Trichostrongylus colibriformis* and *Haemonchus*, can

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cause more severe symptoms and even be fatal in livestock. These parasites cause significant economic losses by reducing the growth rates of livestock and livestock and decreasing the production of livestock products such as milk, meat, and wool. Studies in Australia indicate that roughly 1 billion dollars are spent annually on controlling parasitic diseases in sheep and cattle, including trichostrongyliasis. It is estimated that the global expenditure on such diseases is around 10 billion dollars [7-9].

In Iran, as in many other countries, nematodes belonging to the *Trichostrongyloidea* superfamily are a significant cause of parasitic disease, especially for small ruminants [10]. Some 52 million sheep and 26 million goats live in various climatic regions throughout the country [11]. One of the most significant and severe health problems in these animals is resistance to anthelmintic drugs. Recent studies in Iran confirm the increased prevalence of anthelmintic drug resistance, especially for *Trichostrongylus* species affecting sheep and goats [12-13].

Accurate identification of nematode species, including *Trichostrongylus* species in domestic animals, is one of the most critical challenges in epidemiology and control studies for treating drug-resistant parasites [14]. Despite many morphological similarities observed in the egg and larval stages of various *Trichostrongylus* species, relying solely on these characteristics to differentiate between them is not practical or efficient. A comparative study of adult worms is necessary for accurate identification [15-16].

Considering the economic and veterinary importance of *Trichostrongylus* species in domestic animals in Iran, coupled with limited data on the morphological characteristics and prevalence of these nematodes, this study was conducted on *Trichostrongylus* nematodes isolated from sheep and goats slaughtered in Kashan abattoir.

MATERIAL AND METHODS

Sampling. This cross-sectional study was conducted randomly on 284 animals (130 goats and 154 sheep) slaughtered at the Kashan industrial slaughterhouse in central Iran. Upon recording the information of slaughtered animals in the information form, the small intestines of sheep and goats were collected in special containers with lids and promptly transported to the research laboratory in the parasitology department.

Following the complete opening of the intestine with specialized scissors, *Trichostrongylus* worms were isolated from the intestinal contents. Then the worms were identified under a camera-lucida microscope using morphological features reflected in diagnostic keys [3, 16].

Morphometric method. Seventy *Trichostrongylus* isolates were selected for the morphometric study. The worms were washed three times in PBS, then the species of *Trichosrongylus* were identified according to standard keys and criteria [17-18]. This study measured five morphometric indices, including worm size (length and width in the widest part), spicule length, gubernaculum length, and copulatory bursa width, using micrometers and X40 objective, and the results were recorded.

Statistical analysis. The collected results were analyzed using the SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Chi-square tests were used to compare the observed descriptive results statistically. Kruskal Wallis Test and ANOVA were used to compare the mean morphometric indices in the studied species, with a significance level of less than 0.05. The confidence interval was separately determined according to the hosts.

Ethics. All experimental and animal housing procedures were supervised by the Institutional Animal Care and Use Committee at the Kashan University of Medical Sciences, Kashan, Iran. The Research Ethics Committee of Kashan University of Medical Sciences approved the ethics code IR.KAUMS.MEDNT.REC.1396.112.

RESULTS

Of 284 animals studied, 130 were goats (45.8%), and 154 were sheep (54.2%). 26 livestock (9.15%) were infected with at least one species of *Trichostrongylus* (C.I=9.15 ± 3.35). The prevalence of infection with *Trichostrongylus* nematodes was 12.3% in goats and 6.5% in sheep. There was no significant relationship between the type of host and *Trichostrongylus* infection. Table 1 shows the prevalence of infection in different hosts.

Table 1. Prevalence of *Trichostrongylus* infection in goats and sheep

Infection Host	Positive		Negative		Total	
	No	%	No	%	No	%
Goats	16	12.3	114	87.7	130	100
Sheep	10	6.5	144	93.5	154	100
Total	26	9.15	258	90.85	284	100
Statistical comparison *P*=0181 C.I= 9.15±3.35						

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Of the 70 *Trichostrongylus* isolates studied morphometrically, 39 (55.7%) were male, and 31 (44.3%) were female. The morphometric analysis identified four species, namely *T. colubriformis, T. vitrinus, T. capricola,* and *T. probolurus* (Fig. 1). *Trichostrongylus colubriformis* (19 out of 39; 48.7%) and *T. vitrinus* (10 out of 39; 25.7%) were found to be the dominant species, while the least frequent species were *T. capricola* and *T.* probolurus, both with a prevalence of 12.8% (5 out of 39). In this study, *T. probolurus* was not detected in sheep (Table 2).

Table 2. Frequency of male *Trichostrongylus* species according to the host.

Host	**Sheep**	**Goats**	**Total**
Species	No (%)	No (%)	No (%)
T. colubriformis	8 (20.5)	11(28.2)	19 (48.7)
T. vitrinus	4 (10.3)	6 (15.4)	10 (25.7)
T. capricola	3 (7.7)	2 (5.1)	5 (12.8)
T. probolurus	0	5 (12.8)	5 (12.8)
Total	15 (38.5)	24 (61.5)	39 (100)

T. vitrinus had the largest length of the adult male worm, while *T. probolurus* displayed the largest copulatory bursa. Table 3 compares the five morphometric indices studied for these species.

Table 3. Morphometric characteristics of male worms in different *Trichostrongylus* species.

species Morphometric index (µm)	***T. colubriformis* (no:19) X±SD*** **	***T. vitrines* (no:10) X±SD**	***T. capricola* (no:5) X±SD**	***T. probolurus* (no:5) X±SD**	**Comparison groups (*P* .value)**
Length of the worm	5800.63±69.73	6640.30±60.04	5970.00±14.83	6920.00±18.23	*P*<0.001*
Width of the worm	1060.61±10.97	1020.74±80.84	1070.00±90.75	1200.40±10.14	*P*<0.018**
Spicule length	1410.06±70.63	1580.34±50.64	1400.80±70.46	1570.80±80.17	*P*<0.001**
Gobernaculum length	810.32±8.62	880.10±7.36	800.80±7.82	840.00±8.22	*P*=0187 **
Copulatory bursa width	1970.21±25.75	2840.93±39.23	2430.80±20.55	1810.60±32.02	*P*<0.001**

P<0.05
*Kruskal Wallis Test
**ANOVA significant differences
*** average ± standard deviation

No sheep harbored *T. probolurus*. Morphometric analysis of five indices in sheep and goats revealed *T. vitrinus* as the largest adult worm with the most extended spicule length among the identified species (Fig. 1). Table 4 presents morphometric indices for male worms in both goats and sheep.

Table 4. Comparison of morphometric indices in male *Trichostrongylus* species based on the host.

Host	**Sheep**			**Goats**			
Species Indicator (µm)	***T. colubriformis* (no:8) X±SD**	***T. vitrinus* (no:4) X±SD**	***T. capricola* (no:3) X±SD**	***T. colubriformis* (no:11) X±SD**	***T. vitrinus* (no:6) X±SD**	***T. capricola* (no:2) X±SD**	***T. probolurus* (no:5) X±SD**
Worm length	5540.38±23.93	6400±41.28	6050±70.64	5990.74±20.43	6470.17±19.89	5850±50	6920.30±80.15
Worm width	1050.6±40.26	1010.36±60.44	1000±1.01	1070.41±30.21	1030.67±20.54	1170±20.5	1200.40±1.06
Spicule length	1370.30±20.14	1560.85±20.17	1380±40.36	1440.02±70.50	1590.33±20.68	1450±50	1570.30±30.65
Gubernaculum length	800.30±20.11	860.75±40.31	810.33±60.36	820.28±10.31	890±20.90	800±1.01	840.30±30.61
Copulatory bursa width	1940.38±10.01	2750.32±24.47	2450±16.07	1990.26±70.51	2910.33±14.20	2420±80	1810.60±14.41

DISCUSSION

Trichostrongylus species are responsible for causing trichostrongyliasis in humans and animals worldwide, including in Iran. Accurately identifying species within this genus through morphometric measurements has always been challenging. By deploying the morphological and morphometric methods, the present study identified four *Trichostrongylus* species, namely *T. colubriformis*, *T. capricula*, *T. vitrinus*, and *T. probolurus*, in goats and sheep located in the Kashan region.

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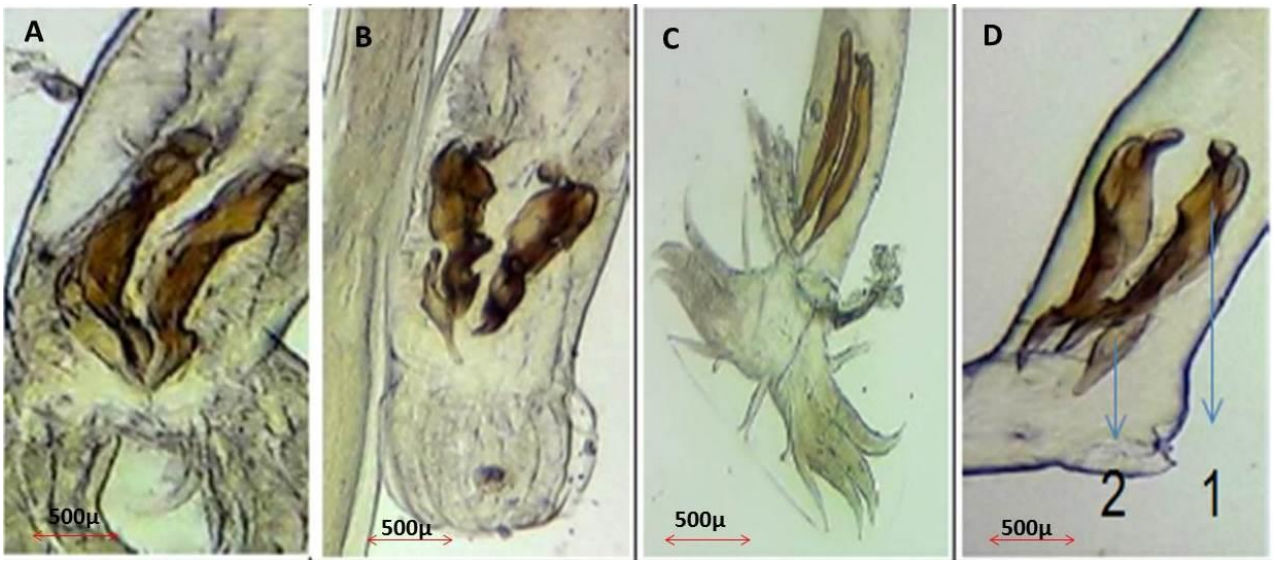

Fig. 1. Comparison of the copulatoryy bursa and spicule morphology among four species of male *Trichostrongylus* worms.
1: Spicule 2: Gubernaculum A: *T. capricola* , B: *T. probolurus* , C: *T. vitrines*, D: *T. colubriformis*

Various studies in Iran have reported different *Trichotyrongylus* species among domestic animals., including *T. colubriformis* and *T. vitrines* [19-23], *T. axei* [19], *T. capricula* [19, 21], *T. probolurus* [19-23], *T. longispicularis* [21], *T. orientalis* [21, 24], *T. skrjabini* [21] and, *T. hamatus* [23], *T. lerouxi* [25] in Mazandaran, East Azarbaijan, Khorasan Razavi and Khuzestan provinces, among herbivores such as goats [19, 21], sheep [19-21] cattle and buffaloes [19, 21], and camels [22-23]. *In Iran, T. colubriformis and T. orientalis have been reported as the predominant species responsible for human infections* [26].

In this study, *Trichostrongylus* spp. infection rate was 9.15%, comparable to a previous report of 7.1% among domestic ruminants in Khuzestan province in 2011 [20]. However, another study reported a much higher total prevalence of infection of 32.8% in sheep from Tabriz in 2012 [21]. This difference in prevalence rates may be attributed to variations in climatic and geographical conditions and the utilization of animal fertilizers to improve agricultural farms in different regions of the country. Using animal fertilizers to enhance agriculture and gardens has been identified as a possible facilitator of *Trichostrongylus* spp. infection transmission in both humans and animals [27]. In the Northern provinces of Iran, domestic livestock such as sheep, goats, and cattle often graze freely in the environment, thereby elevating the risk of contamination of vegetables and agricultural products with helminth eggs via animal manure [26].

In addition, using sheep and cattle manure in fields is quite common, which can effectively spread *Trichostrongylus* infection to various animals.

The prevalence of Trichostrongylus species in this study was 20.5% and 28.2% for *T. colubriformis*, 10.3%, and 15.4% for *T. vitrinus*, 7.7% and 5.1% for *T. capricula* in sheep and goats, respectively. In this study, *T. probolurus* was detected in 12.8% of goats, while no *T. probolurus* was seen in the sheep samples. The prevalence rates found in this study are consistent with the findings of Shahbazi et al. (2012) in Tabriz, who reported a prevalence of 9.8% for *T. colubriformis*, 16.4% for *T. vitrinus*, and 6.6% for *T. probolurus* [21].

A similar study in Isfahan in 2014 reported a relatively high prevalence of *T. colubriformis* in sheep (28.75%) and goats (15.09%). The same survey also found *T. vitrinus* in 30.36% of sheep and 18.87% of goats [28]. These findings show that the prevalence of *Trichostrongylus* infection is higher in Isfahan. Moreover, a study in Ethiopia found that 26.7% of sheep were infected with *Trichostrongylus* species [29]. In a study in Malesia, however, the highest rate of *Trichostrongylus* infection (79.8%) was found among goats [30]. The distribution and prevalence rate of Trichostrongylus infections in domestic animals appears to be influenced by various factors, including weather conditions, animal contact, sanitation levels, and economic and social circumstances in each region.

Human trichostrongyliasis has been reported in many countries, especially Middle Eastern and Asian countries such as Iran [3-6]. Although many human infections with *Trichostrongylus* species are asymptomatic, some cases may present with symptoms such as abdominal pain, diarrhea, weight loss, and eosinophilia [26, 31].

In the present study, isolating the four more prevalent and zoonotic species of *Trichostrongylus*, including *T. colubriformis* (48.7%), is significant because *T. colubriformis* has been reported as the predominant species in human infections [26, 32].

The results comparing five morphometric indices of *Trichosrtongylus* species isolated from goats and sheep revealed that all of these indices were higher in sheep isolates. This finding agrees with a similar study on the

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morphometric indices of *Trichosrtongylus* species [20]. Another survey on the morphological characterization of the *Trichostrongylus* species isolated from sheep in Tabriz, in northwestern Iran, in 2012 showed similar results for morphometric indices except for the worm's length [21].

Our findings align with most previous studies on the morphometric identification of *Trichostrongylus* in Iran and many other countries. The slight difference in morphological features could be attributed to climate and geographic conditions.

Interestingly, most previous reports concerning *Trichostrongylus* species noticed spicule shape and body length as the primary and essential indices [10, 20-21]. Nonetheless, the morphometric indices are not effective in identifying female worms. Therefore, alternative methods such as analysis of isoenzyme electrophoretic patterns and molecular and PCR-based techniques are necessary to differentiate the species via female worms or eggs of the worm [33-34].

In conclusion, the prevalence of *Trichostrongylus* spp. infection among sheep and goats referred to the Kashan abattoir was relatively high. Of the four isolated species of *Trichostrongylus* identified in goats and sheep in the Kashan region (*T. colubriformis*, *T. capricula*, *T. vitrinus*, and *T. probolurus*), *T. colubriformis* was the most frequent. This finding is significant because *Trichostrongylus* species are zoonotic and can be human pathogens. As a precautionary measure, prevention methods should be implemented to avert human infection and prevent contamination of human water and food with infectious stage eggs of *Trichostrongylus*. Molecular epidemiological studies of *Trichostrongylus* isolates from humans and animals are essential for local and global prevention and control of this zoonotic parasite.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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