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Original Article

Morphological and Morphometrical Description of *Trichostrongylus* Species Isolated from Domestic Ruminants in Khuzestan Province, Southwest Iran

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ABSTRACT

Backgrounds: Genus *Trichostrongylus* (Nematoda: Trichostrongylidae) is one of the most important zoonotic nematodes with wide geographic distribution in the world. The purpose of the present study was to describe morphological and morphometrical characteristics of male *Trichostrongylus* species, currently prevalent in domestic ruminants of Khuzestan Province, southwest Iran.

Methods: Gastro-intestinal organs of 1600 sheep, goats, cattle, and buffalos, slaughtered in Khuzestan Province, southwest Iran, were examined for infectivity with *Trichostrongylus* species. For examination and measurements of helminthes, Azo-carmin staining was performed, followed by camera lucida drawings of morphological characters and measurements of morphometrical criteria with a calibrated microscope. Using valid nematodes systematic keys, almost all the parasites were identified at the level of species.

Results: Overall, 114 animals were found infected with at least one species of *Trichostrongylus*. Considering morphological characteristics of male *Trichostrongylus*, six species were identified including *T. colubriformis*, *T. vitrinus*, *T. probolorus*, *T. capricola*, *T. longispicularis* and *Trichostrongylus* sp..

Conclusion: Although, compared to the previous decades, currently *Trichostrongylus* is much less prevalent in the domestic ruminants of the study area, but still different species occur in these animals.

Keywords: *Trichostrongylus*, Morphology, Morphometry, Ruminant, Iran

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Introduction

Trichostrongylid nematodes (*Nematoda: Trichostrongylidae*) are parasites of digestive tract of many animals. There are several species in ruminants in different countries throughout the world. Due to the impact of these parasites on livestock health and production, they are considered as one of the major veterinary importance. Some species can also infect human, mainly people living in close association with infected animals.

In Iran, various species of *Trichostrongylus* have been reported from different hosts including wild herbivores (1-3) domestic animals (4,5), and human (5). For most species occurring in domestic ruminant there are also reports on human infectivity (5, 6). So far, nine species have infected human in Iran (6).

Khuzestan Province, southwest Iran has previously been an area with high prevalences of *Trichostrongylus* species both in livestock (5) and in human (7). Although, compared to the previous decades rate of infectivity in human is currently much lower (8), however, information is lacking about the species of *Trichostrongylus* infecting human population and cross-transmission of these nematodes among different animal hosts and human.

Therefore, in order to identify and describe the species of *Trichostrongylus* currently occurring in domestic animals in this province, the authors carried out a study on slaughtered livestock, both with the traditional techniques and PCR-based technology. The part presented herein is the morphological and morphometrical description of the species of *Trichostrongylus* found during the study period.

Materials and Methods

Sampling

By referring to different abattoirs of Khuzestan Province, southwest Iran, including Izeh, Shadegan, Ahvaz, Behbahan, Andimeshk, Masjedsoleiman, Shush, Ramhormoz and Susangerd, overall gastrointestinal of 1600 slaughtered ruminants were collected, during 2007-2008. The animals comprised 600 sheep, 600 goats, 250 cattle, and 150 buffalos. All these animals were examined for infectivity with *Trichostrongylus* nematodes, following washing the contents of small intestines through a 100mesh sieve and observation by stereomicroscope. Every helminth recovered from the contents was cleaned with normal saline and then cleared in lactophenol for sex determination.

Staining, Morphological and Morphometrical Examinations

Male *Trichostrongylus* were separated from those of females, prior to staining in Azocarmine dye. Morphological characters of helminthes were drawn by a light microscope, equipped with camera lucida drawing tube. Morphometrical indices were also measured by a microscope with calibrated eyepiece. For each species, a minimum of 10 male specimens were measured. Photography of the specimens was performed using a digital camera equipped microscopy. Using valid nematodes systematic keys (9, 10) almost all the parasites were identified at the level of species.

Results

Overall, 114 out of the 1600 ruminants (7.1%) were found infected with at least one *Trichostrongylus* species. Table 1 shows *Trichostrongylus* species identified in each ruminant. For all animal hosts *T. colubriformis* was the dominant species.

Fig. 1 is correspondent with the digital pictures of spicules and gubernaculum of each *Trichostrongylus* species. Fig. 2 illustrates camera lucida drawings of spicules, gubernaculum and caudal bursa of each *Trichostrongylus* species. Follows a brief description of each species which considered key points for species identification in systematic references (9, 10) is presented.

Trichostrongylus probolorus (Fig. 1a & 2a)

The spicules are more massive than those of other species are. They are dark brown, with two triangular projections on the ventral side. The two spicules are approximately identical in length. The gubernaculum is shiny dark brown.

Trichostrongylus capricola (Fig. 1b & 2b)

Spicules are thicker at the proximal end. The distal ends of the spicules are less sharp compared with that of *T. vitrinus*, and, with-

out triangular shape, as in *T. colubriformis*. The gubernaculum is light brown, and simple in shape.

Trichostrongylus colubriformis (Fig. 1c & 2c)

Spicules are slightly unequal in lengths, with a structure similar a small boat with a thick outgrowth capping the root proximally. The gubernaculum, is seen laterally, in the form of a wavy curve with two bends.

Trichostrongylus longispicularis (Figs 1d & 2d)

Spicules are thicker at the proximal end than distal end. The gubernaculum is light brown in color, with simple shape.

Trichostrongylus vitrinus (Figs 1e & 2e)

The spicules are small and straight with sharply tapering at distal extremities. The gubernaculum is of the usual form.

Trichostrongylus sp. (Figs 1f & 2f)

The spicules and gubernaculum are almost similar to those of *T. colubriformis*. The spicules are light brown, with characteristic shape resembling ladies long boots; one slightly bigger than the other (average: 146 vs. 152 μ m). The gubernaculum is light brown boat-shaped.

Table 1: *Trichostrongylus* species identified in each domestic ruminant slaughtered in Khuzestan Province, Iran

Species of <i>Trichostrongylus</i>	Sheep	Goat	Cattle	Buffalo
<i>T. colubriformis</i>	+	+	+	+
<i>T. vitrinus</i>	+	+	+	+
<i>T. probolorus</i>	+	+	-	-
<i>T. longispicularis</i>	+	+	-	-
<i>T. capricola</i>	+	+	-	+
<i>Trichostrongylus</i> sp.	+	-	-	-

Table 2: Morphometrical measurements of the males of different species of *Trichostrongylus* identified in domestic ruminants of Khuzestan Province, Iran

Species Parameter	<i>T. probolorus</i>	<i>T. capricola</i>	<i>T. colubri formis</i>	<i>T. longispicularis</i>	<i>T. vitrinus</i>	<i>Trichostrongylus</i> sp.
Length (mm)	4.3-5.55	4.3-4.9	4-6	4.6-5.23	4.8-6.52	4.85
Max. width (µm)	78-112	80-100	89-112	117-123	80-123	113
Spicule length (µm)	125-142	130-149	120-148	178-198	157-182	146-152
Gubernaculum length (µm)	72-80	70-80	64-76	79-105	80-95	75
Distance of excretory pore from the anterior end (µm)	163-172	155-165	163-172	158-170	171-177	183

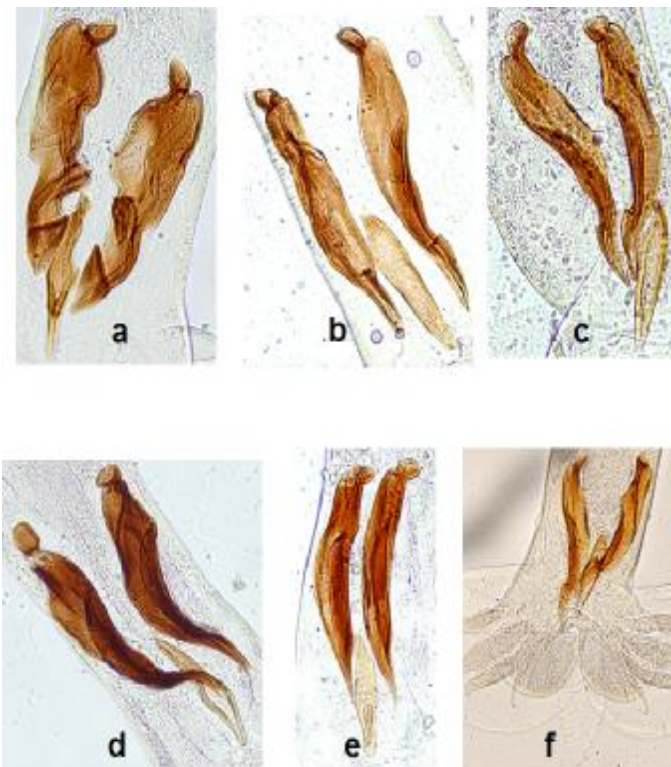


Fig. 1: Digital pictures of spicules and gubernaculum of different species of *Trichostrongylus* identified in domestic ruminants of Khuzestan Province, Iran: **a.** *T. probolorus* **b.** *T. capricola* **c.** *T. colubriformis* **d.** *T. longispicularis* **e.** *T. vitrinus* **f.** *Trichostrongylus* sp. (Source: Authors)

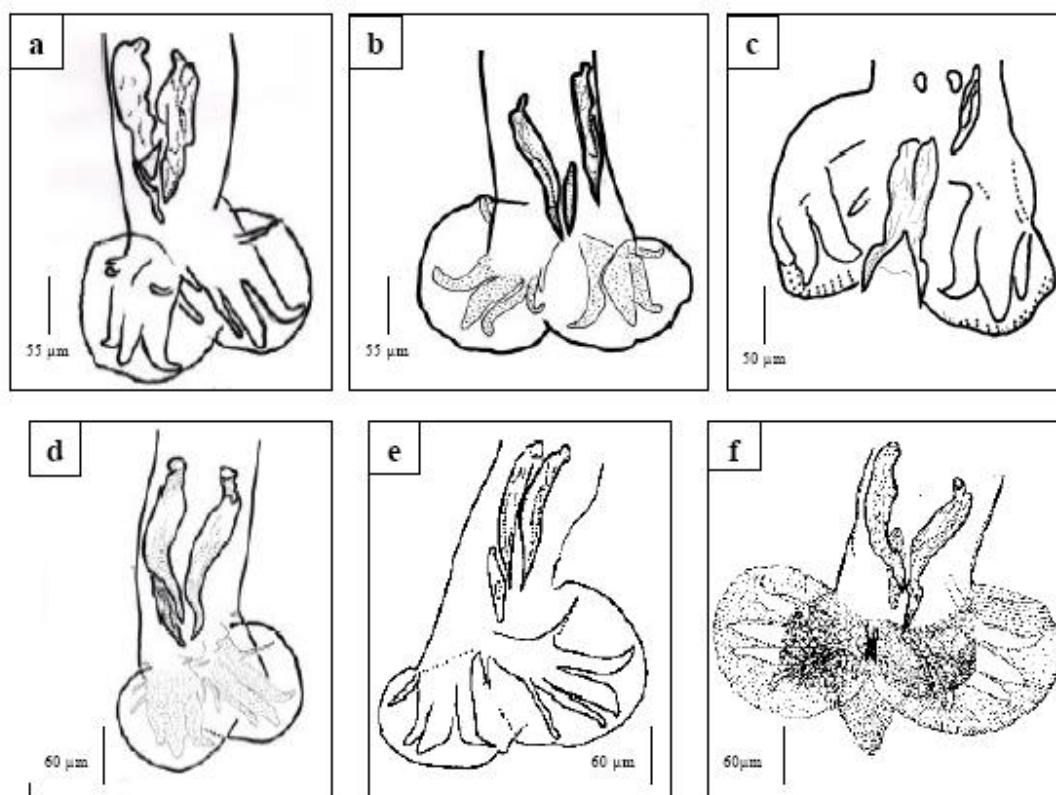


Fig. 2: Camera lucida drawings of spicules, gubernaculum and caudal bursa of different species of *Trichostrongylus* identified in domestic ruminants of Khuzestan Province, Iran: **a.** *T. probolorus* **b.** *T. capricola* **c.** *T. colubriformis* **d.** *T. longispicularis* **e.** *T. vitrinus* **f.** *Trichostrongylus* sp. (Source: Authors)

Discussion

In this study, the overall rate of infection with *Trichostrongylus* species in the livestock slaughtered in Khuzestan abattoirs was found much lower than those of previous report back to 1975 (5). However, current results indicate that various species are still present in this area including *T. probolorus*, *T. capricola*, *T. colubriformis*, *T. longispicularis*, *T. vitrinus*, and *Trichostrongylus* sp.

The most variation of *Trichostrongylus* species in the present study was found in sheep, with occurrence of all these six species in

this host. This higher variation of species in sheep is in agreement with previous result on trichostrongylid species indicating the presence of all seven identified species in Khuzestan Province in this host (5). This is partly due to the higher number of sheep examined in both studies.

The dominant decrease in the prevalence of *Trichostrongylus* in livestock of Khuzestan Province is clearly reflected in the prevalence of infection in human population in this area, too. In a recent study carried out on

the prevalence of intestinal parasites in tribal parts of this province, *Trichostrongylus* infection was reported 0.5% (8), while previously there has been report of human infectivity with this nematode up to 71% in rural areas of this province (7).

Whether still different species of *Trichostrongylus* are circulating in human in this area, as with the domestic ruminants, is not known. According to the reports back to the previous decades, in most areas of the country surveyed, the species found in domestic animals were also recovered in human inhabitant, indicating its zoonosity (5, 6, 11). However, the predominance of species in human and various animals in a same area might be the same, as in nomadic tribe of Zard Kuh, central Iran that *T. capricola* had been common (11); or might be different as in the Khuzestan Province (12). This issue can be because of different susceptibility of each host to various species of *Trichostrongylus*, and human life style, as well as importance of man and animal close contact in each area.

The two previous prevalent species of *Trichostrongylus* in human in the study area were *T. orientalis* and *T. colubriiformis* (12). At present, it is not clear which species is dominant in human in this area. Application of DNA-based technology for determination of species, specially for female worms and eggs that morphologically are indistinguishable, is improving and so far it has been effectively used in different studies throughout the world (13-15). In Iran, the use of molecular assays for identification of trichostrongylid species has been applied on adult worms, collected from sheep and goat (16), as well as on eggs collected from sheep (17), in Isfahan Province, using PCR-RFLP technique. Nevertheless, characterization of *Trichostrongylus* species in other parts of the country and different hosts will be interesting.

The results of the current study complemented with the result of PCR-based techniques, which have been undertaken by the authors, is a new insight into the identification and characterization of current prevalent *Trichostrongylus* species of livestock in the study area. However, further studies utilizing DNA-based assays, will facilitate differentiation of species in order to understand the clinical importance of each species and cross-transmission of these species between human and animals, especially in areas that dominant species in man and animal hosts are the same.

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