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Original Article

Prevalence and Seasonality of Adult and Arrested Larvae of Gastrointestinal Nematodes of Sheep from Mashhad City, Northeastern Iran

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Abstract

Background: This study was conducted to determine the prevalence and intensity of nematode infections in sheep located in northeastern Iran from Apr 2018 to Mar 2019.

Methods: Gastrointestinal nematodes of 300 sheep were inspected. The season of slaughter, anatomic location where the parasite was located, the animal's sex, infection prevalence and intensity were recorded. Seasonal differences in arrested larvae numbers also were assessed using Cochran's Q test.

Results: Overall, 4,331 adult nematode specimens were collected. Among the examined sheep, 53% (159/300) were infected with one or more nematode species. Among infected sheep, 42.8% were infected with a single species of nematode, 26.4% were infected with two species of nematodes, and 30.8% were infected with three or more species of nematodes. *Marshallagia marshalli* (13.3%) was the most common nematode recovered from the abomasums of infected sheep, while *Trichostrongylus vitrinus* (4.6%) was commonly recovered from the small intestines, and *Trichuris ovis* (25.6%) was commonly recovered from the large intestines. In total, 463 arrested larvae were found in the abomasums of 7.5% of infected sheep and 104 arrested larvae were found in the small intestines of 8.8% of infected sheep. A significantly higher numbers of arrested larvae were found in summer compared to autumn ($P<0.001$).

Conclusion: Intestinal parasites continue to be a problem for sheep in northeastern Iran and additional control measures need to be explored.



Introduction

Gastrointestinal nematode (GIN) infection is a problem for small and large animals globally (1). Domesticated small ruminants, and particularly sheep, are considered an important source of protein for a large part of the world. GIN can lead to serious health problems in small ruminants by causing morbidity and mortality (2). Sheep grazing on contaminated pastures puts the animals at risk for infection with the 3rd stage larvae of GINs (3).

Gastrointestinal nematodes of the family *Trichostrongylidae* are some of the most important parasites of small ruminants worldwide (4). *Ostertagia* spp., *Haemonchus contortus*, *Trichostrongylus* spp., *Nematodirus* spp., and *Marshallagia marshalli* are the main GINs of sheep raised in temperate climates (1, 5). Additionally, *M. marshalli* is believed to be the main cause of parasitic gastroenteritis in ruminants from Iran (6). Several previous investigations were carried out to determine the prevalence of these nematodes in sheep and goats in different parts of Iran (7, 8).

The arrested larval stage of GINs makes control of these parasites challenging, especially when dealing with parasites belonging to the *Trichostrongylidae* family (9). The seasonal onset of hypobiosis (arrested larval development) is associated with decreasing temperature and photoperiod, suggesting that these factors may play a role in stimulating the free-living stages of the nematodes (10). There are few studies evaluating prevalence of sheep GINs and larvae dormancy in infected sheep in Iran (11).

We aimed to determine the species, prevalence, and seasonality of adult nematodes and hypobiotic larvae in slaughtered sheep in Khorasan Razavi Province, Iran during a 12-month period.

Materials and Methods

Sampling

From Apr 2018 to Mar 2019, 300 sheep from two slaughterhouses (60 from the Torqabeh abattoir and 240 from the Tapeh Salam abattoir) in Mashhad, the capital city of Khorasan Province, Iran were selected and examined for GI parasites. A sample size of 250 animals was calculated based on a nematode prevalence of 30.8% found in a 2020 study evaluating sheep slaughtered at an abattoir in Mazandaran Province in northern Iran (12), using an alpha of 0.05 and a precision of 0.06. In order to ensure that appropriate sample size was reached, the number of sheep selected for evaluation was increased to 300. The study did not require ethics approval.

Approximately 400 and 1,500 sheep are slaughtered daily at Tapeh Salam and Torqabeh, respectively. For this study, 25 animals (approximately 21 from Tapeh Salam and 4 from Torqabeh) were evaluated monthly. Carcasses were selected via a systematic random sampling (13), with 2-3 carcasses evaluated from the post-mortem line each Monday and Thursday during the study period. All sheep were raised under traditional production methods and originated from the rural areas around Mashhad. All GINs were collected and sent to the Parasitology Laboratory at the Agricultural Jihad Center, University of Applied Science and Technology for processing.

Collection of nematodes from the gastrointestinal tract

Esophagi

The esophagi from sheep were examined specifically for *Gongylonema* spp., where adult worms embed in the esophageal mucosa or submucosa, and their anterior ends protrude into the lumen.

Abomasums, small intestines, and large intestines

The abomasums and small and large intestines were opened separately, and their contents and mucosa were washed with water through a 100µm mesh sieve to remove all parasites. The contents of the abomasums, small intestines, and large intestines were diluted and examined under a stereomicroscope. Each recovered nematode was cleaned with saline solution (NaCl 0.9%) and fixed in 70% alcohol. The nematodes were then cleared in lactophenol and identified under a microscope using published keys, with female nematodes classified at the genus level and male nematodes classified at the species level (14).

Arrested larva

The mucosa of the collected abomasums and small and large intestines were digested by incubation at 37 °C for 24 h in pepsin hypochloric acid solution (10g pepsin, 30mL hypochloric acid and 1000ml distilled water) (15). The suspension was sieved through 40 µm mesh and the larvae counted. The different organs were processed separately.

Statistical analysis

The overall frequency of infection, as well as frequency of infection by species and anatom-

ic location, were expressed as proportions with 95% confidence intervals (CIs). The Cochran's Q test was used to evaluate seasonal differences in arrested larvae numbers. Analyses were performed using SPSS ver. 20 (IBM Corp., Armonk, NY, USA). Findings were considered statistically significant at $P<0.05$.

Results

Prevalence and burden of adult worms

Overall, 53.0% (159/300) of sheep were found to be harboring at least one species of GIN. Of the infected sheep, 42.8% (68/159) were infected with a single species of nematode, 26.4% (42/159) were infected with two species of nematodes, and 30.8% (49/159) were infected with three or more species of nematodes. Information on the animal's sex and age was available for 79 (52.6%) and 159 (100%) of sheep, respectively. Of the sheep with known sex, 31.6% of males (25/79) (all under 18 months of age) had at least one adult nematode and 60.6% of females (134/221) (all above 2 years of age) had at least one adult nematode. Out of 300 sheep (25 per month), GIN infections were most prevalent in October (Fig. 1).

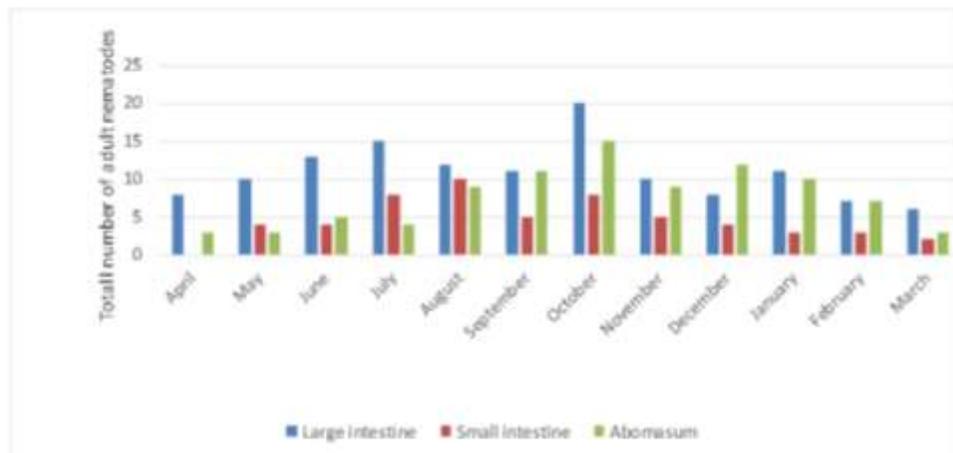


Fig. 1: Total number of GI adult nematodes from 300 sheep (25 per month) evaluated at two abattoirs in Khorasan Province (Apr 2018 through Mar 2019)

The large intestines were the most commonly parasitized region of the GI tract, while the largest number of nematodes was recovered

from the evaluated abomasums (Table 1, Fig. 2).

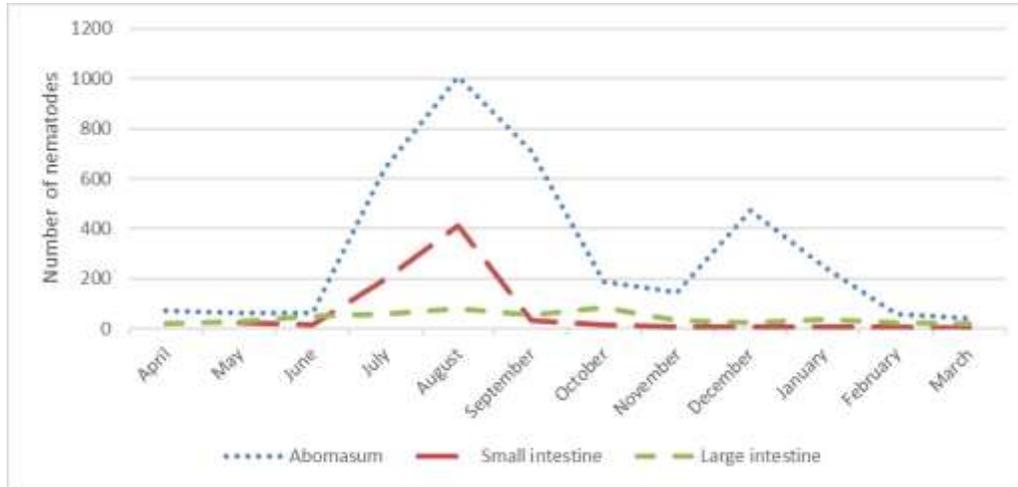


Fig. 2: Number of adult nematodes collected from different regions of the gastrointestinal tract of 300 sheep (25 per month) from two abattoirs in Khorasan Province (April 2018 through March 2019).

Esophagi

No worms were found in the esophageal contents of the evaluated sheep.

Abomasums

Postmortem examinations revealed that the most prevalent helminth in the abomasums was *M. marshalli*, with a prevalence of 13.3% (40/300).

Small intestines

Trichostrongylus vitrinus (14/300; 4.6%) was the most common species found in the small intestines (Table 1).

Large intestines

Trichuris ovis (77/300; 25.6%) was the most common species found in the large intestines (Table 1, Fig.3).



Fig. 3: Male *Trichuris discolor*, *T. ovis*, and *T. skrjabini* parasites isolated from sheep in this study

Table 1: Prevalence and intensity of gastrointestinal adult nematode infections in sheep (n=300) from two abattoirs in Khorasan Province (Apr 2018 through Mar 2019)

Parasite genus	Parasite species	Number of nematodes	Infection prevalence		Abundance (interquartile range)
			No. of sheep	% [95%CI]	
<i>Ostertagia</i>	<i>T. circumcincta</i>	521	55	18.3 [16.1-20.5]	3 (1-6)
	<i>O. trifurcate</i>	13	6	2.0 [1.9-2.1]	1.5 (1-3)
	* <i>Ostertagia</i> spp.	1,083	66	22 [20.25-23.75]	8 (4-16)
	<i>O. occidentalis</i>	85	24	8.0 [6.5-9.5]	2 (1-4)
<i>Haemonchus</i>	<i>H. contortus</i>	6	2	0.7[0.5-0.9]	3 (1-3)
	* <i>H. contortus</i>	9	2	0.7 [0.4-1]	4 (1-4)
<i>Marshallagia</i>	<i>M. marshalli</i>	610	40	13.3 [13-13.6]	7 (3-20)
	* <i>M.marshalli</i>	792	45	15 [14.7-15.3]	9 (3-25)
<i>Parabronema</i>	<i>P. skrjabini</i>	54	8	2.6 [2.5-2.7]	7 (2-11)
	* <i>Parabronema</i> spp.	78	11	3.6 [2-5.2]	7 (2-10)
<i>Nematodirus</i>	<i>N. spathiger</i>	4	4	1.3 [0.9-1.7]	1 (1-1)
	* <i>Nematodirus</i> spp.	6	5	1.6 [1.1-2.1]	1 (1-1.5)
<i>Trichostrongylus</i>	<i>T. colubriiformis</i>	8	7	2.3 [2-2.6]	1 (1-2)
	<i>T. vitrines</i>	204	14	4.6 [3-6.2]	1 (1-3)
	* <i>Trichostrongylus</i> spp.	336	33	11 [9.1-13.1]	2 (1-4)
<i>Cooperia</i>	* <i>Cooperia</i> spp.	4	2	0.7 [0.4-0.]	2 (1-2)
<i>Trichuris</i>	<i>T. discolor</i>	42	35	11.6 [9.7-13.5]	1 (1-1)
	<i>T. ovis</i>	114	77	25.6 [22-23.2]	1 (1-2)
	<i>T. skrjabini</i>	26	21	7.0 [0.3-1.1]	1 (1-1.5)
	<i>T. barbetonensis</i>	10	8	2.6 [1.5-3.7]	1 (1-2)
	* <i>Trichuris</i> spp.	326	107	35.6 [31-40.2]	3 (2-4)

*Female worms that could not be identified at the species level

Prevalence and burden of arrested larvae

Overall, 463 arrested larvae were found in the abomasums of 4.0% (12/300) of sheep and 104 arrested larvae were found in the small intestines of 4.6% (14/300) of sheep. There was a significant difference in the number of arrested larvae in the abomasums found in the summer versus autumn, with a median of 18.5 (interquartile range: 6-85) larvae per sheep in the summer and a median of 12.5

(interquartile range: 5-12) larvae per sheep identified in the autumn ($P<0.001$) (Fig. 4). There was also a significant difference in the number of arrested larvae found in the small intestines in the summer versus autumn, with a median of 9.5 (interquartile range: 2-21) larvae per sheep in the summer and a median of 1 (interquartile range: 1-3) larvae identified per sheep in the autumn ($P<0.001$).

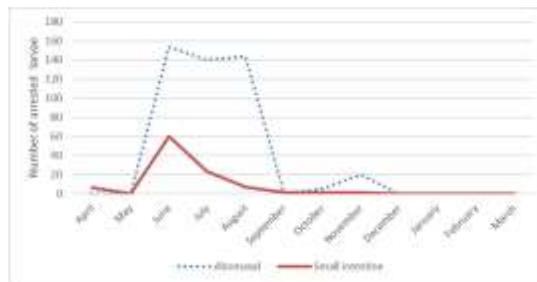


Fig. 4: Number of arrested nematode larvae collected from the abomasums and small intestines of 300 sheep (25 per month) from two abattoirs in Khorasan Province (Apr 2018 through Mar 2019)

Discussion

Iran ranks fourth in the world in sheep husbandry after Australia, China, and India, with approximately 51.7 million adult sheep in 2020 (https://en.wikipedia.org/wiki/Sheep_farming). High prevalence of parasitic diseases can result in significant losses to the country's livestock industry. Parasite distribution varies depending on climate and seasonal changes (16). In Iran, the warm season lasts from May to Sep, with an average daily high temperature above 30 °C. In July, the warmest month of the year, the average high temperature is about 37 °C. In the current study, sheep had a higher prevalence of adult nematodes in the autumn when the temperature is cooler (between 22 and 25 °C).

Gongylonema pulchrum lies in a zipper-like fashion in the wall of the esophagus or upper alimentary tract of sheep, goats, cattle, pigs, zebus, buffalo, and less frequently in horses, camels, donkeys, wild boars and humans (17). The presence of *G. pulchrum* has been documented in a variety of domestic and wild mammals in Iran, with some of the highest prevalence values reported in cattle (18, 19). In previous studies, up to 21% of domesticated sheep and 5.6% of wild sheep from Iran were infected with *G. pulchrum* (20, 21). However, the prevalence was lower in northern Iran, with 4.5% of sheep infected (7, 17). In the current study, conducted in the northeastern part of the country, *G. pulchrum* was not observed. This concurs with findings from another study conducted in northern Iran where infection with this species was also not observed in the esophagi of 584 evaluated sheep and goats (22).

Transmission of *G. pulchrum* to livestock occurs primarily through ingestion of infected coprophagous insects, including dung beetles (23). A possible reason for the absence of *G. pulchrum* infection in the current study is the sheep-raising system in Iran. The Iranian

sheep industry is based on free grazing. Therefore, the probability of a sheep swallowing an infected beetle is low.

In the current study, *M. marshalli* was the most prevalent nematode species found in the abomasums of sheep. In another study, *Teladorsagia circumcincta* (19.3%) and *M. marshalli* (12.2%) were the most common nematode species found in the abomasums of slaughtered sheep across the three climatic zones of Iran (24). The prevalence of *M. marshalli* in sheep from northern and northwestern Iran was previously 34.0% and 9.0%, respectively (25, 26). In Iran's neighboring country of Turkey, *T. circumcincta* was recorded as the most prevalent nematode species. In comparison to data from Turkey, there were overall lower numbers of nematodes per infected sheep found in the current study (27). This difference may be due to recent droughts in Iran, resulting in unfavorable conditions for free-living L3 larvae.

In the current study, three species of the genus *Trichostrongylus* were found in the small intestines, with *T. vitrinus* (4.6%) being the most prevalent. This is the same species found to be the most common in studies conducted in the provinces of Isfahan and Tabriz (26, 28). In other studies, carried out in southwestern and northern Iran, *T. colubriformis* was the dominant species (7, 29). Finally, in the present study, four species of *Trichuris* were identified in the large intestines, of which *T. ovis* was the most prevalent. This finding is similar to values obtained from Iran and other countries in the region and beyond (30-32). *Trichuris vondwei* (28.2%) was the most prevalent species in northwestern Iran, while *T. ovis* was found in 3% of goat large intestines. Recently, *T. infundibolus* and *T. globulosa* were observed for the first time in Iran (33).

Post-mortem examination for nematode infection involves conducting a total worm count, followed by the morphological identification of adult and/or larval stages collected

from the GI contents. This method is the best way to understand the prevalence of parasites, especially for some Trichostrongylidae species of ruminants that undergo hypobiosis in the host (34).

When conditions in the external environment are unfavorable for the development and survival of nematodes, eggs are not produced by worms (35). Climatic factors, host immune status, and farm management are believed to affect arrestment levels (36).

Severe freezing, as seen in western Iran, and scorching heat, as seen in eastern and southern parts of Iran, promote this situation (11, 37). There were higher numbers of arrested larvae found in the summer versus autumn, which reflects the impact of high temperatures (38, 39). In addition to seasonal change, acquired immunity in the host has an impact on incidence of arrested larvae (11). Acquired immunity, which is dependent on genotype, animal age, and nutritional status, plays a vital role in host defense mechanisms against GINs. In addition, pregnancy can impact immune status (40-42). This may result in higher prevalence of helminth infection in female animals (12, 42).

Most outbreaks of disease caused by nematodes occur in the autumn in northeastern Iran (36), while high numbers of arrested larvae can be found in the summer. Since, in this study, *M. marshalli* was the most common nematode identified in the autumn, this species represented a large proportion of the detected arrested larvae. In contrast to *Ostertagia* spp. that tends to arrest during the colder months (43, 44), hypobiosis of *M. marshalli* larvae often occurs in warm and dry weather (45, 46). Certain nematodes, including *T. vitrines*, *T. colubriformis*, and *M. marshalli* are considered zoonotic. Therefore, their control and prevention will always be an area of concern for endemic and hyperendemic regions. Unfortunately, there have been no studies conducted on the human prevalence of zoonotic GI helminths in Khorasan Province.

Conclusion

More than half of the sheep evaluated in this study were infected with GINs. Hypobiosis of larvae often occurs in the summer in north-eastern Iran where the weather is warm and dry. Identified nematodes, including *T. vitrines*, *T. colubriformis*, and *M. marshalli* are considered zoonotic. Therefore, their control and prevention will always be an area of concern for endemic regions. Unfortunately, there have been no studies conducted on the human prevalence of zoonotic GI helminths in Khorasan Province. Therefore, this should be a priority for future research.

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Conflict of interest

The authors declare that there is no conflict of interest.

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