Effect of age, sex, and season on the prevalence of Linguatula serrata *infestation in mesenteric lymph nodes of goats slaughtered in Tabriz, Iran*

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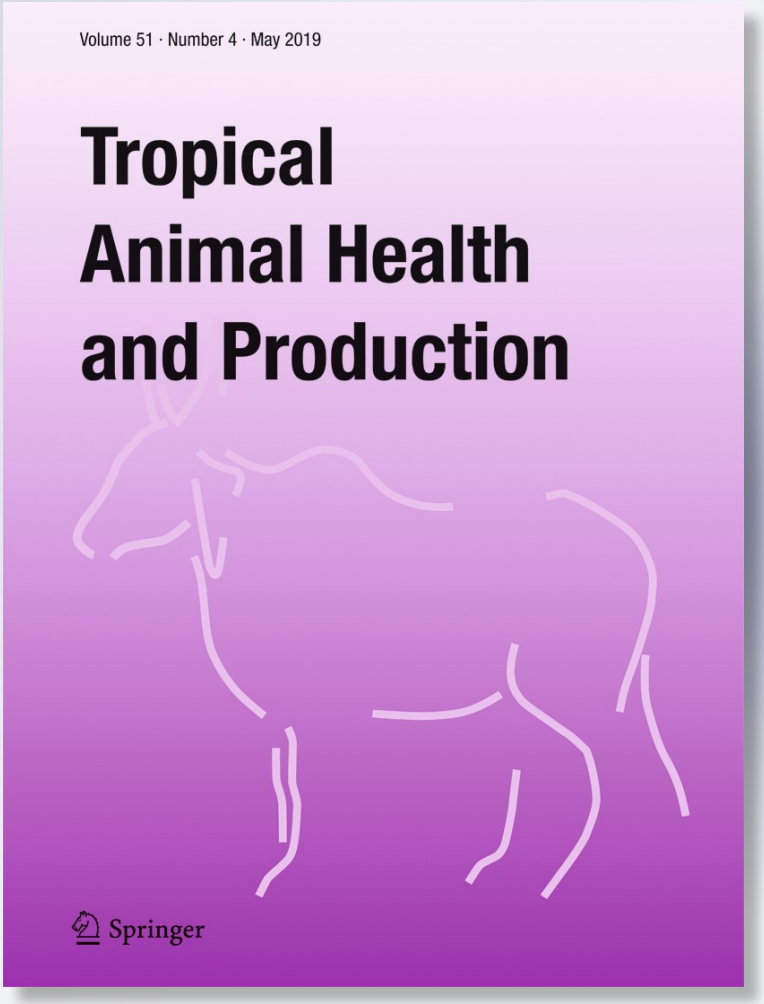

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REGULAR ARTICLES

Effect of age, sex, and season on the prevalence of *Linguatula serrata* infestation in mesenteric lymph nodes of goats slaughtered in Tabriz, Iran

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Abstract

This research was conducted to determine the prevalence and intensity of infection with nymphs of *Linguatula serrata* in mesenteric lymph nodes of the goats from northwest of Iran. Moreover, the intensity of infection of mesenteric lymph nodes was compared based on gross appearance and the morphological changes in these nodes. In addition, the effects of age, sex, and season on the prevalence of *Linguatula serrata* were assessed. In this study, mesenteric lymph nodes ($n = 16{,}284$) were randomly collected from 2132 goats slaughtered in the abattoir of Tabriz, Iran, from September 2013 to September 2017. The samples were categorized based on their gross appearance (color and consistency) and then were cut into small pieces and immersed in normal saline (0.9% NaCl) solution and left for 5 to 6 h to allow nymphs to come out from the tissue. The results showed that 1241 out of total 2132 (58. 2%) goats and 9282 out of total 16,284 mesenteric lymph nodes (57%) were infested with the nymphal stage of *Linguatula serrata*. It was also revealed that the infestation rate was age-dependent: as the goats grow older, the infestation increases. Further, the prevalence of *Linguatula serrata* nymphs in the mesenteric lymph nodes in various seasons was not significant ($P > 0.05$). The prevalence of infestation rate in female goats was significantly higher than of male goats ($P < 0.05$). Besides, the infestation rate in the black-colored lymph nodes (75.88%) was significantly ($P < 0.05$) higher than those of hemorrhagic nodes (54.94%) and normal-colored nodes (22.65%). Moreover, the infestation rate of nymphs in the soft lymph nodes (83.91%) was significantly ($P < 0.05$) higher than those in normal (21.85%) and hard (32.43%) lymph nodes. Given the fact that the *Linguatula serrata* is a zoonotic parasite; thus, the inspection process should be meticulously done in an abattoir, especially in areas where residents consume raw or under-cooked liver and/or visceral organs of herbivores.

Keywords *Linguatula serrata* · Goat · Mesenteric lymph nodes · Tabriz · Iran

Introduction

The abattoir of Tabriz is an industrial abattoir, in which more than 100 cattle *(Bos taurus)*, 500 sheep, and goats, and a few buffaloes *(Bubalus bubalis)* are slaughter every day. *Linguatula serrata* is a widespread zoonotic parasite which in adulthood inhabits the upper respiratory system, nasopharyngeal tract, and frontal sinuses of dogs, foxes, cats, and other carnivores as the definitive hosts (Aldemir 2004; Oryan et al. 2008; Rezaei et al. 2011), while in its immature stages, it is found in the mesenteric lymph nodes, liver, lungs and spleen of the herbivorous and other ruminants which serve as the intermediate hosts (Gul et al. 2009; Hami et al. 2009; Ravindran et al. 2008; Tajik and Sabet Jalali 2010; Tavassoli et al. 2007a; Youssef and Hadizadeh 2010). Eggs are discharged from the nasopharyngeal secretion of the definitive hosts and then herbivores ingest them where eggs finally hatch into larvae. The larvae spread throughout the internal organs of intermediate hosts and finally enter the mesenteric lymph nodes (MLNs), where they develop into infective nymphs (Rezaei et al. 2012). The definitive host becomes infected after

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eating the infected viscera of intermediate hosts (Rezaei et al. 2011). In humans, the Linguatulosis displays itself as a nasopharyngeal or visceral disease, usually contracted after consuming the infected under-cooked liver, lungs or other organs of the intermediate hosts. The nymphs leave their cysts and move toward the esophagus and the nasopharynx, where they anchor to the mucosal epithelium with prominent hooks. In this case, humans act as the definitive host and the nymphs and/or mature parasites concentrate in hosts' nasopharyngeal tract and frontal sinuses, causing a range of symptoms, from mild inflammation of the upper respiratory tract to temporary conductive deafness (Khalil et al. 2013; Yagi et al. 1996; Yilmaz et al. 2011). Since the infestation poses a serious danger to human health and, particularly since raw meat is consumed regularly in Tabriz, further studies are needed to know this parasite and the ways of its transmission. Several studies have explored the prevalence rate of *L. serrata* in goats (Dehkordi et al. 2014; Gharekhani et al. 2016; Hashemnia et al. 2016; Nourollahi Fard et al. 2010; Rezaei et al. 2011; Tabaripour et al. 2017; Tavassoli et al. 2007b; Yakhchali and Tehrani 2013). The recent studies tend to emphasize the prevalence rate of parasites; however, no information is available on the roles played by age, sex, season, status of the infested lymph nodes, and the frequency and severity of the infestation by *L. serrata* in MLNs of goats. Therefore, the present paper describes the prevalence rate of nymphal stages of *L. serrata* in MLNs of goats slaughtered over a 4-year period in Tabriz abattoir, northwest of Iran and possible effects of some factors such as season, age, and sex on parasitic infestation.

Materials and methods

Area of study

This study was undertaken in Tabriz County in East Azerbaijan Province in the northwest of Iran with geographical coordinates of 38° 4′ N and 46° 18′ E and a population of almost 1.5 million (Fig. 1). This city lies between Eynali and Sahand mountains and is characterized by a humid continental climate with regular seasons. The annual rainfall is around 320 mm. The climate is temperate during the hot summer months while the winters are long and bitter cold with a temperature plummeting to − 10 °C. Goats are kept mainly by villagers in rural areas and sometimes in the outskirt of the city.

Sampling

During a 4-year period (September 2013–September 2017), 2132 goats and 16,284 mesenteric lymph nodes in three age groups (< 1, 1–2 and > 2 years old) were randomly selected at the industrial abattoir of Tabriz, northwest of Iran. Age animals were estimated using the eruption of permanent incisor teeth criterion as described by Curasson (1947).

The sampled lymph nodes were placed in separate pots containing normal saline and were transferred to the parasitology laboratory of Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.

Preparation method

The mesenteric lymph nodes of the goats were examined macroscopically and since no definite morphological criteria was available to classify the infected lymph nodes, the samples were ranked based on the changes observed in color and consistency of lymph nodes. According to Gracey (1986), the initial stages of the infection appear to be yellow to green and are soft, while the succeeding lesions are gray and calcified (hard). After recording the gross appearance, mesenteric lymph nodes were examined for the nymphal stage of *L. serrata*. The samples were cut into small pieces before being soaked in normal saline (0.9% NaCl) solution for 5–6 h to allow nymphs to come out from the tissue. After being recovering, the nymphs were flattened, dehydrated in ascending grades of ethyl alcohol and cleared in creosote and

Fig. 1 Location of abattoir in East Azerbaijan province where *Linguatula serrata* were collected from mesenteric lymph nodes of goats in 2014–2017

examined under a stereomicroscope. Lymph nodes were sorted based on their color (normal, red, or hemorrhagic and black) and consistency (normal, soft, and hard).

Statistical analysis

Associations between host factors (age, genus, and season) and prevalence rate of infestation were evaluated using the chi-square test with SPSS software version 16.2. One-way analysis of variance followed by pairwise comparisons using the Bonferroni correction was used to test the difference between mean numbers of parasites (intensity of infection) in lymph nodes with different colors and consistency. Here, when $P < 0.05$, the differences were considered significant.

Results

The results are summarized in Tables 1 and 2. Based on the research data, out of 2132 goats, 1241 (58. 2%) were infested with the nymphal stage of *L. serrata*. It was found that the infestation rate was age-dependent: as the goats grow older, the infestation increases. Further, it significantly varies over different seasons and across sexes. The results clearly indicate that the infestation rate among females was significantly ($P < 0.05$) higher than among male animals. In addition, 9282 (57%) out of a total of 16,284 MLNs of goats had the parasite's nymphs. Significant differences were observed between various lymph nodes as the highest infestation rate belonged to the black-colored lymph nodes (75.88%) while the hemorrhagic lymph nodes (54.94%) and normal-colored lymph nodes (22.65%) stood in the second and third ranks, respectively (Fig. 2). In addition, the highest infestation rate was recorded in soft lymph nodes (83.91%), a rate significantly higher than those of normal (21.85%) and hard (32.43%) lymph nodes.

Discussion

The results of the present study showed that *L. serrata* infestation rate in MLNs of goats was 58.2% which exceeded the previously reported rate by other surveys in Iran such as the study in northwest of Iran (50.75%) (Rezaei et al. 2011), Kerman (49.1%) (Nourollahi Fard et al. 2010), Kermanshah (25.1%) (Hashemnia et al. 2016), Hamadan (31.4%) (Garedaghi 2011), Hamadan (24%) (Dehkordi et al. 2014), and Mazandaran (16.8%) (Tabaripour et al. 2017). However, it was comparatively smaller than other previously reported rates by Tavassoli et al. (2007b) with 68%, Yakhchali et al. (2009) with 59.78% and Youssefi et al. (2012) with 69.15%. More recent studies showed that the infestation rates of parasites in MLNs of goats was significantly higher than that of cattle's MLNs (Youssefi et al. 2012; Alborzi et al. 2013; Castro et al. 2015; Gharekhani et al. 2016; Hashemnia et al. 2016; Parraguez et al. 2017;Tabaripour et al. 2017), camels (Nourollahi Fard et al. 2012; Rezaei et al. 2012; Bamorovat et al. 2014; Farjanikish and Shokrani 2016; Attia et al. 2017), sheep (Azizi et al. 2015, Kheirabadi et al. 2015; Gharekhani et al. 2016; Hashemnia et al. 2016; Tabaripour et al. 2017; Yektaseresht et al. 2017), and buffalo (Tajik and Sabet Jalali 2010; Rezaei et al. 2011; Alborzi et al. 2013; Sudan et al. 2014) reported in previous studies. The reason is not clear but it may be related to heterogeneous foraging habitats of goats, geographical climate; or greater exposure to dogs which serve as definitive hosts. Close contact between dogs and intermediate hosts like goats can also play an important role in *L. serrata* transmission. On the other hand, goats grazing ahead of the flocks can be another reason for the observed increase in the risk of infection (Tavassoli et al. 2007b; Hajipour 2012; Hajipour 2016). Probably, another reason for the high infestation rate of lymphs in goats can be attributed to the fact that the parasite genetically has different strains in different ruminant species (Tavassoli et al. 2014). In their study on *L. serrata* isolated from different farm animals of Iran, Tavassoli et al. (2014) found molecular differences among the parasite by amplifying and sequencing of 18S rRNA. Based on the reported results, the lowest diversity of nucleotide sequences (98.8%) was observed in sheep, cattle, and dogs, while the highest one (100%) was recorded in camels and goats. The excretory-secretory products of *L. serrata* nymphs such as proteases contribute to the destruction of intermediate host tissues and play an important role in the pathogenesis of the parasite (Alcala-Canto et al. 2007; Hajipour 2012). Metalloprotease is the E/S release in the nymphal stage that is the result of *L. serrata* isolated from MLNs of goats (Hajipour 2012), whereas the protease detected in E/S release from the nymphal stage of *L. serrata* isolated from MLNs of sheep is serine protease (Alcala-Canto et al. 2007). These differences may be a reason for the high prevalence infestation of *L. serrata* nymphs in goats. The results of the studies conducted by Yakhchali et al. (2009), Rezaei et al. (2011), and Dehkordi et al. (2014) indicate that the prevalence of *L. serrata* nymphs in female goats was significantly ($P < 0.05$) higher than in male goats, a finding which is similar to the results of the present study. However, Gharekhani et al. (2016), Hashemnia et al. (2016), and Nourollahi Fard et al. (2011) showed that although infestation rates of *L. serrata* among females were greater than in male goats, it was not significant at the same age groups of male and female ($P > 0.05$) probably due to the higher mean age of females than those of males as these females can live longer before being slaughtered. Our results showed that although there was not a significant difference in the infestation rates over different

Table 1 The prevalence of *Linguatula serrata* nymph in goats slaughtered on based age, sex, and season during 2014–2017

Year			Prevalence infestation rate (no. %)					
	Season	Number of animals	No. of infected animals	Age groups (years)			Sex	
				1 > $n = 28$	1–2 $n = 29$	2 < $n = 43$	Male $n = 43$	Female $n = 57$
2014	Spring	100	48	0	14 (48.27%)	34 (79.06%)	13	35
	Summer	100	42	1 (3.57%)	12 (41.37%)	29 (67.44%)	5	37
	Autumn	100	85	22 (78.57%)	23 (79.31%)	40 (93.02%)	21	64
	Winter	100	65	11 (39.28%)	17 (58.62%)	37 (86.04%)	7	58
	Total	400	240(60%)	$n = 112$ 34 (30.35%)*	$n = 116$ 66 (56.89%)*	$n = 172$, 140 (81.39%)*	$n = 172$ 46 (26.74%)*	$n = 228$, 194 (85.08%)*
				1 > $n = 35$	1–2 $n = 42$	2 < $n = 43$	Male $n = 70$	Female $n = 50$
2015	Spring	120	41 (34.1%)	0	21 (50%)	20 (45.51%)	19	22
	Summer	120	82 (68.3%)	12 (34.28%)	28 (66.66%)	42 (97.67%)	45	37
	Autumn	120	70 (58.3%)	15 (42.85%)	23 (54.76%)	32 (74.41%)	31	39
	Winter	120	65 (54.16%)	33 (94.28%)	9 (21.42%)	23 (53.48%)	40	25
	Total	480	258 (53.75%)	$n = 140$ 60 (42.85%)*	$n = 168$ 81 (48.21%)*	$n = 172$, 117 (68.02%)*	$n = 280$, 135 (48.21%)*	$n = 200$, 123 (61.50%)*
				1 > $n = 38$	1–2 $n = 46$	2 < $n = 26$	Male $n = 65$	Female $n = 45$
2016	Spring	110	44 (40%)	16 (42.10%)	16 (34.78%)	12 (46.15%)	21	23
	Summer	110	36 (32.72%)	3 (7.89%)	13 (28.26%)	20 (76.92%)	20	16
	Autumn	110	76 (69.09%)	25 (65.78%)	31 (67.39%)	20 (76.92%)	41	35
	Winter	110	54 (49.09%)	3 (7.89%)	25 (54.34%)	26 (100%)	28	26
	Total	440	210 (47.72%)	$n = 152$ 47 (30.92%)	$n = 184$ 85 (48.85%)	$n = 104$ 78 (75%)*	$n = 260$ 110 (42.30%)	$n = 180$ 100 (55.55%)
				1 > $n = 60$	1–2 $n = 82$	2 < $n = 61$	Male $n = 129$	Female $n = 74$
2017	Spring	203	109 (53.69%)	22 (36.66%)	42 (51.21%)	45 (73.77%)	44	65
	Summer	203	95 (46.79%)	18 (30.00%)	38 (46.34%)	39 (63.93%)	48	47
	Autumn	203	187 (92.11%)	49 (81.66%)	79 (96.34%)	59 (100%)	117	70
	Winter	203	142 (69.95%)	40 (66.66%)	57 (69.51%)	45 (73.77%)	77	65
	Total	812	533 (65.64%)	$n = 240$, 129 (53.75%)	$n = 328$, 216 (65.85%)*	$n = 244$, 188 (77.04%)*	$n = 516$, 286 (55.42%)*	$n = 296$, 247 (83.44%)*
2014–2017		2132	1241 (58.20%)	1 > $n = 644$ 270 (41.92%)	1–2 $n = 796$ 448 (56.28%)*	2 < $n = 692$ 523 (75.57%)*	Male $n = 1228$ 577 (46.98%)*	Female $n = 904$ 664 (73.45%)*

$P < 0.05$, statistically significant difference in comparison to parasite control in the same time interval

Table 2 Intensity of infection and relative frequency of *Linguatula serrata* nymphs in mesenteric lymph nodes of goats based on their color and consistency during 2014–2017

Year	MLNs/appearance	Lymph nodes gross appearance and relative frequency (no., %)					
		Color			Consistency		
		Normal	Hemorrhagic	Black	Normal	Soft	Hard
2014	MLNs (no.) $n = 3200$	653	524	2023	427	1798	
	Infected of MLNs (no.)	131(20.06%)*	402(76.71%)*	1450(71.67%)	106 (24.82%)*	1620(90.10%)*	257(26.35%)*
	Range of no. of nymphs	1–15	1-60	1-87	1–13	1-90	1-8
	Total of MLNs infected		1983 (61.96%)			1983 (61.96%)	
2015	MLNs (no.) $n = 4320$	947	1256	2117	1103	2230	987
	Infected of MLNs (no.)	227 (23.97%)*	607(48.32%)*	1230(58.10%)*	222 (20.12%)*	1401(62.82%)*	441(44.68%)*
	Range of no. of nymphs	1–17	1-50	1-90	1–20	1-87	1-10
	Total of MLNs infected		2064 (47.77%)			2064 (47.77%)	
2016	MLNs (no.) $n = 3080$	1225	155	1700	1156	1397	527
	Infected of MLNs (no.)	235 (19.18)*	50(32.25%)*	1200(70.58)*	210 (18.16)*	1030(73.72%)*	245(46.48%)*
	Range of no. of nymphs	1–20	1–75	1–100	1–15	1–90	1–12
	Total of MLNs infected		1485 (48.21)			1485 (48.21)	
2017	MLNs (no.) $n = 5684$	2000	484	3200	1070	3117	1497
	Infected of MLNs (no.)	500 (25.00%)*	270(55.78%)*	2980(93.12%)*	283 (26.44%)*	3117(100%)*	350(5.91%)*
	Range of no. of nymphs	1–23	1-90	1-120	1–10	1-95	1-10
	Total of MLNs infected		3750 (65.97%)			3750 (65.97%)	

MLNs, mesenteric lymph nodes, *$p < 0.05$, statistically significant difference in comparison to parasite control in the same time interval

seasons ($P > 0.05$), infestation rates in autumn were higher than in other seasons, a finding not similar to the results of the studies carried out by Nourollahi Fard et al. (2010). Tabaripour et al. (2017) reported that there was no significant difference between seasons in terms of the infestation rate of parasite and in summer was higher than in other seasons. The period from the ingestion of eggs discharged from nasopharyngeal secretions of the definitive host to the formation of the nymphal stage of *L. serrata* in the grazing herbivores takes 6 months. Thus, if the infected eggs are swallowed by intermediate hosts in spring, *L. serrata* nymphs will emerge in the autumn. In the areas studied in these researches, suitable weather conditions in the late spring coincide with the time of grazing, a situation that is most likely responsible for an infestation of in ruminants. Interestingly, we found that the rate and intensity of infestation were greater in lymph nodes with altered color and consistence: the black-colored (75.88%), hemorrhagic (54.94%), and soft (83.91%) lymph nodes were significantly more frequently and more severely infested. This finding suggests that events such as calcification probably reduce the chance of the survival of nymphs in old nodes (Tavassoli et al. 2007a).

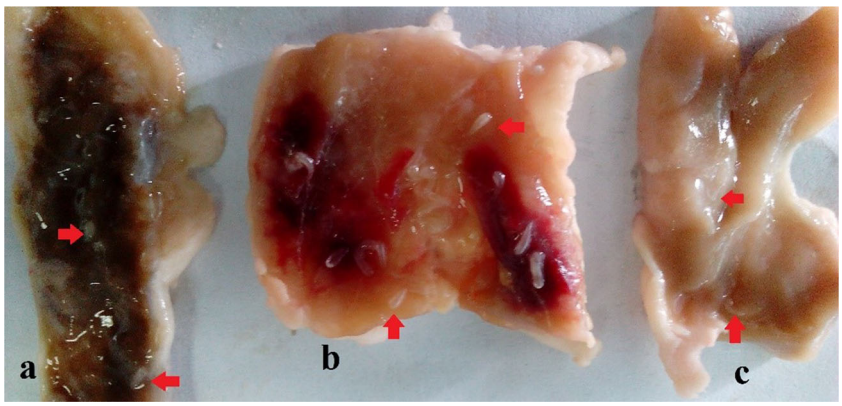

Fig. 2 Mesenteric lymph nodes infection with *Linguatula serrata* nymphs in goats based on gross appearance changes; black-colored lymph nodes (**a**), hemorrhagic lymph nodes (**b**), normal lymph nodes (**c**), (nymphs are shown by the arrow)

In conclusion, the high prevalence of infestation observed in goats is critical due to the zoonotic nature of the parasite and the considerable threat to humans' and other animals' health. Considering the results, it could be concluded that the prevalence of *L. serrata* in goats in Tabriz is high that could play an important role in the epidemiology of linguatulosis. Further, the observed gross changes in color and consistency of lymph nodes in this study could be used as an effective indicator of the infected nodes. As such, these nodes can be used to identify infested nodes and to terminate the life cycle of the parasite. Further studies could be focused on genetic analyses of *L. serrata* from different animals due to the knowledge of genetic diversity among *L. serrata* isolated from different hosts can provide information for understanding the evolution of the parasite in different regions of the world. This can also help to resolve taxonomic discrepancies. However, we believe that analyzing more specimens collected from different hosts and regions would assist in answering the questions concerning the possible dispersal routes of the isolates and their evolution.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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