



Pathological characteristics of *Linguatula serrata* (aberrant arthropod) infestation in sheep and factors associated with prevalence in Iran

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ABSTRACT

Linguatula serrata is a cosmopolitan zoonotic parasite in which carnivores and herbivores serve as final and intermediate hosts, respectively. The aim of this study was to compare the *L. serrata* nymphal infection rate and intensity of infection (mean number of nymphs $\pm$ standard error) to the appearance and pathological changes of mesenteric lymph nodes (MLNs) infected with *L. serrata* using sheep slaughtered in Tabriz, Iran. In addition, the effect of age, sex, and season on the prevalence of *L. serrata* infection was evaluated. For this purpose, over a four-year period, 31,078 MLNs from 3199 sheep were examined, with 4972 (15.99%) MLNs infected representing 518 (16.20%) sheep. Collected MLNs were categorized by color as normal, red or black and by consistency as normal, soft or hard. *L. serrata* were found in 8.88% of normal-colored MLNs, 14.45% of red (hemorrhagic) MLNs and 44.57% of black-colored MLNs, with the difference being significant for infection and infection intensity ($P < 0.0001$). In regards to MLN consistency, 7.98% of normal, 31.52% of soft and 5.42% of hard lymph nodes were found to be infected with the infection rate and intensity in soft nodes being significantly different ($P < 0.0001$). Pathological changes in MLNs infected with *L. serrata* nymph with normal color and consistency had calcification of the *L. serrata* nymph, granulomatous inflammation around the nymph and some neutrophils. Granulomatous inflammation around the *L. serrata* nymph, haemosiderophage, macrophage and lymph node depletion from lymphocytes were observed in MLNs infected with *L. serrata* nymph with soft consistency. In addition, MLNs infected with *L. serrata* nymph with hard consistency and black color contained neutrophils in the capsule's wall, caseous necrotic mass and *L. serrata* surrounded by a thick capsule. In regards to prevalence, age, sex and season (autumn) were significant ($P < 0.0001$, $P < 0.01$, respectively). These study results suggest that targeted meat inspection and targeted animal interventions could be used to decrease human exposure to *L. serrata* and animal infection.

1. Introduction

Linguatula serrata Fröhlich, 1789 is a zoonotic aberrant arthropod of the class Pentastomida (Hendrix, 1998) which uses dogs, and to a lesser extent, foxes, cats and other carnivores as the final host and herbivores as the intermediate host. It is distributed worldwide but occurs more in warm subtropical and temperate regions. The adult form inhabits the upper respiratory system, nasal airways and frontal sinuses of the final host (Aldemir et al., 2014; Nourollahi et al., 2010). Eggs are released to the environment via nasopharyngeal secretions and consumed by intermediate herbivore hosts. The larvae, after emerging from the eggs, spread throughout the internal organs of the intermediate host and eventually reach the mesenteric lymph nodes (MLNs), liver, lungs and spleen, where they develop into infective nymphs (Rezaei et al., 2012;

Tavassoli et al., 2018). The final host becomes infected by eating the infected viscera of intermediate hosts. In the stomach of the final host, nymphs are released from the cysts and move toward the esophagus and the nasopharynx, where they anchor to the mucosal epithelium with prominent hooks. Humans display Linguatulosis as nasopharyngeal or visceral disease. When under-cooked infected lungs, liver, or other organs of the intermediate herbivore host are consumed, the nymph and/or mature parasite localizes in the nasopharyngeal tract and frontal sinuses causing a broad spectrum of symptoms, ranging from mild inflammation of the upper respiratory tract to temporary conductive deafness (Khalil et al., 2013; Yagi et al., 1996; Yilmaz et al., 2011). The visceral form develops in humans when egg contaminated water or vegetables are consumed. Symptoms depend on the organ system involved and result from the death of the nymphs or their

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migration. If only a few parasites are present, *L. serrata* may mimic hepatic or pulmonary malignancy clinically and on radiological assessments. Patients may develop abdominal pain, chronic cough, or night sweats (Mateva et al., 2013; Pampiglione et al., 2001; Tappe and Buttner, 2009). In heavy infections with *L. serrata* death may occur due to secondary septicemia, pneumonia, or severe enterocolitis (Ette et al., 2003; Ma et al., 2002). While many human infections are asymptomatic with the disease an incidental finding during routine medical consultation or at autopsy (Taba Taba et al., 2012), the infection can pose serious danger to human health, particularly in regions where raw meat such as mutton is consumed. Recent case reports of human infection from Iran (Anaraki Mohammadi et al., 2008; Athari, 2013; Hamid et al., 2012; Janbakhsh et al., 2015; Maleky, 2001; Siavoshi et al., 2002; Yazdani et al., 2014), North America (Baird et al., 1988), India (Bhende et al., 2014), Austria (Koebsler et al., 2011), Bulgaria (Mateva et al., 2013), Egypt (Khalil, 1972) and Nigeria (Vanhecke et al., 2016) highlight the importance of this parasite. A few studies have been conducted on the prevalence of *L. serrata* in sheep and pathological MLN changes in Iran and other countries (Aydenizoz et al., 2012; Azizi et al., 2015; Gharekhani et al., 2017; Gul et al., 2009; Hashemnia et al., 2018; Kheirabadi et al., 2015; Miclaus et al., 2008; Tabaripour et al., 2017; Tavassoli et al., 2007; Yakhchali and Tehrani, 2011; Yektaseresht et al., 2017). However, a better understanding of transmission patterns and MLN changes is still needed to develop intervention methods such as meat inspection guidelines, to break the life cycle and decrease human infection. In Iran, *L. serrata* is known to occur in sheep making it an ideal location to study factors related to prevalence and pathology of MLNs (Azizi et al., 2015; Dehkordi et al., 2014; Gharekhani et al., 2017; Youssefi et al., 2012). Therefore, the purpose of this study was to assess pathological changes in MLNs and determine the prevalence of nymphal *L. serrata* infection in sheep during different seasons.

2. Materials and methods

2.1. Study Area

This study was conducted in Tabriz County in East Azerbaijan Province in northwestern Iran with a population of almost 1.5 million and geographical directions of 38° 4' North and 46° 18' East. This city, located between Sahand and Eynali mountains, has a humid continental climate with regular seasons. The climate is temperate during the summer months and the winters are long with temperatures reaching -10 °C. Summer is categorized as the months of July, August and September, autumn as October, November and December, winter as January, February and March and spring as April, May and June. The annual average rainfall is 320 mm (Organization, 2019). Daily temperature and rainfall data are collected by the Meteorological Organization of Iran and compiled monthly. These data were used within this study for determining seasonal rainfall and mean minimum and maximum temperatures for each season and year (Table 1). Flocks of sheep, as well as herds of cattle and goats, are kept mainly by villagers in rural areas and sometimes in the outskirts of the city. While many sheep are slaughtered at home, the Tabriz slaughterhouse serves as the main facility in the region with more than 100 cattle, 500 sheep and goats, and a few buffalo slaughtered every day. All sheep at the abattoir come from within the region.

2.2. Sampling

During a 4-year period (September 2014–September 2017) on three to four days of each week, 31,078 MLNs were collected from 3199 sheep after slaughter at the Tabriz facility. Sheep were selected randomly during each visit. After the first sheep was identified and followed from pre-slaughter through processing, the next available sheep was selected for sampling. From each sheep 6–15 MLNs were collected randomly. Sex and age (based on dentition) of each sampled sheep was

recorded.

2.3. Mesenteric lymph node examination

The MLNs were examined macroscopically and categorized based on color (normal, red/hemorrhagic or grey/black) and consistency (normal, soft or hard/calified) (Gracey, 1986). For histopathological examination, fresh tissue samples from MLNs were fixed in 10% buffered formalin solution and then embedded in paraffin wax, sectioned at 5 µ and stained with Hematoxylin and Eosin (H&E). MLNs were examined for nymphal stages of *L. serrata*, after recording the gross appearance and collections for histopathology. The samples were cut into small pieces (approximately 2 × 2 mm) and then incubated in saline (0.9% NaCl) for 5–6 h at room temperature to allow nymphs to be released from the tissue. Nymphs were collected from the saline by forceps. After recovering nymphs, they were flattened, dehydrated in ascending grades of ethyl alcohol, cleared in creosote and examined under a stereomicroscope (10x and 40x) (Hajipour, 2012). The total number of nymphs per lymph node was recorded. A sheep was categorized as infected if at least one nymph was seen after incubation or during histopathologic examination. Within MLNs color and consistency categories, infection intensity was calculated as the mean number of *L. serrata* nymphs ± standard error.

2.4. Statistical analysis

Relation between prevalence of infection and host factors (age, sex, and season) were evaluated by the Chi square test with SPSS software version 16.2. To determine if MLN color or consistency was different depending on the number of nymphs, a one-way analysis of variance was used followed by pairwise comparisons with a Bonferroni correction for multiple comparisons. Differences were considered significant when $P < 0.0001$ or $P < 0.01$.

3. Results

Approximately 490,944 sheep were slaughtered at the Tabriz facility during the time period of this study with an estimated 0.65% of all sheep slaughtered examined. Based on the data obtained during four years of study, 518 out of 3199 sheep and 4972 out of 31,078 MLNs of sheep were infected with the nymphal stage of *L. serrata* (see Tables 2 and 3). There was a significant correlation between the study year and the rate of infection, which was higher in 2016 than in other years. Also, more sheep had *L. serrata* infected MLNs in the autumn than in other seasons ($P < 0.01$). Age had a significant effect on the infection rate of this parasite ($P < 0.0001$) and infection was more common in female sheep than male sheep ($P < 0.0001$). Nymphal *L. serrata* were found in 8.88% of normal-colored MLNs, 14.45% of hemorrhagic (red) MLNs and 44.57% of black-colored MLNs (see Table 3). The rate of infection in black-colored MLNs was significantly more than that in hemorrhagic and normal-colored ones ($P < 0.0001$) and the infection rate in hemorrhagic MLNs was significantly different from normal-colored MLNs ($P < 0.0001$). Infection based on MLN consistency was 7.98% of normal, 31.52% of soft and 5.42% of hard ones. Infection rate in soft MLNs was significantly higher than those of normal and hard MLNs ($P < 0.0001$). There also was a significant difference ($P < 0.0001$) between the infection rate in normal and hard MLNs.

In all years of the study, the intensity of infection (mean number of nymphs ± standard error) in black-colored lymph nodes was significantly ($P < 0.01$) more than intensity of infection in hemorrhagic lymph nodes and the intensity of infection in hemorrhagic lymph nodes was significantly ($P < 0.01$) more than in normal-colored lymph nodes. In terms of consistency, the intensity of infection in soft lymph nodes was significantly ($P < 0.01$) more than normal and hard ones. No significant differences were observed in mean number of nymphs between normal and hard lymph nodes ($P > 0.01$). Pathological

Table 1

The mean temperature and precipitation in Tabriz based month in.2014–2017.

Monthly precipitation (mm)												
Year/Month	Winter			Spring			Summer			Autumn		
	January	February	March	April	May	June	July	August	September	October	November	December
2014	14.8	28.5	25.8	38.6	46.3	0.1	18.8	1	2.4	34.8	68.3	33.3
2015	12.3	4.8	22	26.8	3	0.9	0.2	22	6.2	22.7	29	15.1
2016	24.3	7.5	40.9	66.7	53.9	37.8	3.1	3	4.6	82.7	14.6	48.9
2017	1.6	5.5	11.3	21.1	15.6	14	0	15	0.3	7.7	23.6	28.6

Mean monthly temperatures (°C)												
Year/Month	Winter			Spring			Summer			Autumn		
	January	February	March	April	May	June	July	August	September	October	November	December
2014	0.2	3.2	8.4	13.1	19.3	24.2	27.2	28.4	22.9	12.6	5.5	2.9
2015	−2	1.1	6.6	12.5	17.6	25	28.5	28.4	22.6	14.8	5.6	−1.3
2016	0.9	4.4	7.3	12	17.7	22.3	26.3	26.9	20.9	13.2	4.9	−3.3
2017	1.9	2.3	2.9	11.8	20.9	26.5	28.7	28.7	24.7	14	7.3	2.1

changes in MLNs of normal color and consistency but infected with *L. serrata* included calcification of the *L. serrata* nymph, granulomatous inflammation around the nymph and some neutrophils (Fig. 1). Granulomatous inflammation around the *L. serrata* nymph, haemosiderophages, macrophages and lymph node depletion from lymphocytes were observed in MLNs infected with *L. serrata* nymph with soft consistency (Fig. 2). In addition, histopathology of MLNs infected with *L. serrata* nymph with hard consistency and black color revealed neutrophil in the capsule's wall, caseous necrotic mass and *L. serrata* surrounded by a thick capsule (Fig. 3).

4. Discussion

The results of the present study showed that *L. serrata* infection rate in MLNs of sheep from this region was higher than previously reported rates in other surveys from Iran which found 6.8–15% of sheep to be

infected (Azizi et al. (2015); Dehkordi et al. (2014); Gharekhani et al. (2017); Tabaripour et al. (2017); Shekarforoush et al. (2004) and Kheirabadi et al. (2015)) and other countries such as Turkey (5.4%) (Aydenizoz et al., 2012) and Romania (11%) (Miclaus et al., 2008). However, the prevalence found in this study was low according to other reported results in previous studies within Iran (18.3–52.5%) (Hashemnia et al., 2018; Nourollahi et al., 2011) and India (18.3%) (Sudan et al. (2014)). The reason for the difference of prevalence is not clear but it might be related to heterogeneous forage habitats of sheep, geographical climate and annual variation or low exposure to dogs, the definitive hosts. There was a significant difference in prevalence of *L. serrata* among the years of study with the highest in 2016 and the lowest 2015. As with the prevalence in other locations, the variation in prevalence in this study was likely due to differences in annual rainfall and temperature. *L. serrata* egg survival and development is higher at temperatures of 10–27 °C with relative humidity and moisture also

Table 2The prevalence of *Linguatula serrata* nymphs in mesentery lymph nodes in sheep by season, age and sex from 2014 to 2017.

Year	Season	Number animals	No. infected animals (%)	Number of infected animals (%)				
				*Age groups (years)			*Sex	
				< 1	1-2	> 2	Male	Female
2014	Spring	201	21(10.5)	1(0.5)	7(3.5)	13(6.5)	7(3.5)	14(7.0)
	Summer	200	35(17.5)	4(2.0)	12(6.0)	19(9.5)	8(4.0)	27(13.5)
	Autumn	200	44(22.0)	4(2.0)	9(4.5)	31(15.5)	11(5.5)	33(16.5)
	Winter	200	39(19.5)	10(5.0)	12(6.0)	17(8.5)	11(5.5)	28(14.0)
	Total	801	139(17.35)	19(2.3)	40(5.0)	80(10.0)	37(4.6)	102(12.7)
2015	Spring	201	9(4.5)	0	2(1)	7(3.5)	2(1.0)	7(3.5)
	Summer	200	20(10.1)	0	5(2.5)	15(7.5)	5(2.5)	15(7.5)
	Autumn	200	36(18.0)	1(0.5)	25(12.5)	10(5)	11(5.5)	25(12.5)
	Winter	199	30(15.0)	2(1)	8(4.0)	20(10.0)	8(4.0)	22(11.0)
	Total	800	95(11.9)	3(0.4)	40(5.0)	52(6.5)	26(3.25)	69(8.6)
2016	Spring	201	30(14.9)	0	7(3.5)	23(11.4)	8(3.9)	22(11.0)
	Summer	200	40(20.0)	5(2.5)	13(6.5)	22(11.0)	7(3.5)	33(16.5)
	Autumn	200	61(30.5)	5(2.5)	16(8.0)	40(20.0)	16(8.0)	45(22.5)
	Winter	199	49(24.6)	9(4.5)	17(8.5)	23(11.6)	8(4.0)	40(20.1)
	Total	800	180(22.5)	19(2.4)	53(6.6)	108(13.5)	39(4.9)	140(17.5)
2017	Spring	201	20(10.0)	3(1.5)	7(3.5)	10(5.0)	8(6.66)	12(15)
	Summer	199	19(9.5)	2(1.0)	5(2.5)	12(6.0)	6(7.50)	13(12.5)
	Autumn	199	35(17.6)	5(2.5)	10(5.0)	20(10.0)	12(12.5)	23(25)
	Winter	199	30(15.1)	5(2.5)	10(5.0)	15(7.5)	9(14.20)	21(16.30)
	Total	798	104(13.0)	15(1.9)	32(4.0)	57(7.1)	35(4.4)	69(8.6)
2014-2017				< 1	1-2	> 2	Male	Female
		3199	518(16.2)	n = 700	n = 1171	n = 1328	n = 1565	n = 1636
				56(8.0)	165(14.1)	297(22.4)	138(8.7)	380(23.2)

* *L. serrata* prevalence was significantly higher in female sheep and in sheep > 2 years of age ($P < 0.0001$).

Table 3Intensity of infection and relative frequency of *Linguatula serrata* nymphs in mesenteric lymph nodes from sheep by 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.)	1673	987	590	1472	1116	709
	Infected MLNs(No.)	195(11.65) ^c	147(14.89) ^b	368(62.37) ^a	141(9.58) ^b	559(50.09) ^a	47(6.62) ^c
	Mean no. of nymphs (± SE)	2.14 ± 0.08 ^c	3.64 ± 0.13 ^b	12.67 ± 0.32 ^a	4.57 ± 0.45 ^b	16.52 ± 0.17 ^a	2.36 ± 0.16 ^b
	Total/Total of MLNs infected	n = 3250; 710(21.80)			n = 3297; 747(22.66)		
2015	MLNs(No.)	2813	1302	716	3000	1200	600
	Infected MLNs(No.)	199(7.1) ^c	148(11.52) ^b	142(19.97) ^a	162(5.40) ^b	315(26.25) ^a	15(2.50) ^c
	Mean no. of nymphs (± SE)	2.62 ± 0.07 ^c	5.04 ± 0.24 ^b	11.36 ± 0.23 ^a	4.25 ± 0.17 ^b	18.03 ± 0.58 ^a	2.40 ± 0.31 ^b
	Total/Total of MLNs infected	n = 4831; 489(10.12)			n = 4800; 493(10.20)		
2016	MLNs(No.)	1500	1000	500	1221	1357	422
	Infected MLNs(No.)	145(9.66) ^c	200(20.00) ^b	356(71.20) ^a	140(11.46) ^b	538(39.64) ^a	39(9.24) ^c
	Mean no. of nymphs (± SE)	2.35 ± 0.08 ^c	5.62 ± 0.17 ^b	10.17 ± 0.19 ^a	4.11n ± 0.15 ^b	16.37 ± 0.44 ^a	3.10 ± 0.23 ^b
	Total/Total of MLNs infected	n = 3000; 701(23.37)			n = 3000; 701(23.3)		
2017	MLNs(No.)	2000	1300	700	1500	2000	500
	Infected MLNs(No.)	170(8.5) ^c	168(12.92) ^b	251(35.85) ^a	131(8.66) ^b	376(22.50) ^a	20(4.00) ^c
	Mean no. of nymphs (± SE)	2.87 ± 0.14 ^c	6.01 ± 0.22 ^b	11.55 ± 0.19 ^a	2.34 ± 0.15 ^b	25.35 ± 1.25 ^a	1.75 ± 0.20 ^b
	Total/Total of MLNs infected	n = 4000; 589 (14.72)			n = 4000; 527(13.18)		
2014- 2017	MLNs(No.)	7986	4589	2506	7193	5673	2231
	Infected MLNs(No.)	709(8.88)	663(14.45)	1117(44.57)	574(7.98)	1788(31.52)	121(5.42)
	Total/Total MLNs infected	n = 15,981; 2489(15.57)			n = 15,097; 2483 (16.45)		

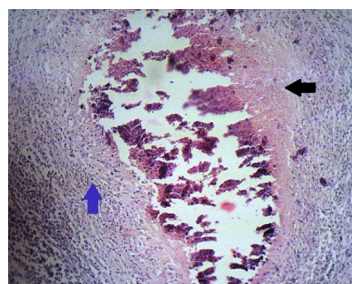
MLNs: Mesenteric lymph nodes. Values with different letters (a–c) are statistically different ($P < 0.01$).* Color and consistency was significant difference between infected and non-infected MLNs ($P < 0.0001$).

Fig. 1. Coress section of infected mesenteric lymph nodw induced by *L. serrata* nymphs with normal color and consistency (H & E, $\times 800$): Calcified *L. serrata* nymph in lymph node. Granulomatose inflammation around the nymph (black arrow) and some neutrophils are present (Blue arrow). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

considered important (Hajipour, 2012, 2016). During 2016 the mean annual rainfall was 32.33 mm, with several months, especially spring and summer, having much higher rainfall than the other years. In

contrast, 2015 was much drier than other years. The 2016 mean temperature was 13 °C, with the summer months having a lower temperature than the other years in the study time period, potentially expanding the transmission period. No significant difference was observed between the infection rate in different seasons ($P > 0.05$). However, in the autumn the prevalence trended toward being higher than in other seasons which was similar to the results of studies from other regions of Iran (2324). However studies by Tabaripour et al. (2017) and Kheirabadi et al. (2015), who also found seasonal differences, showed higher prevalence in summer and spring, respectively. This seasonal difference is likely due to grazing and exposure periods. If intermediate hosts grazing in spring swallow the infected eggs, *L. serrata* nymphs will be seen in the autumn, since the time from ingestion of eggs to formation of the nymphal stage requires 6 months. In the location of the study reported here, suitable weather conditions for egg development occur in the late spring, coinciding with the time of grazing sheep, resulting in the autumn nymphs in MLNs. These results regarding seasonal and annual changes suggest that weather patterns could be investigated as predictors of infection, which could be used to time treatments or enable increased post-slaughter inspection. Alternatively, if weather patterns can be used as predictors, then targeted

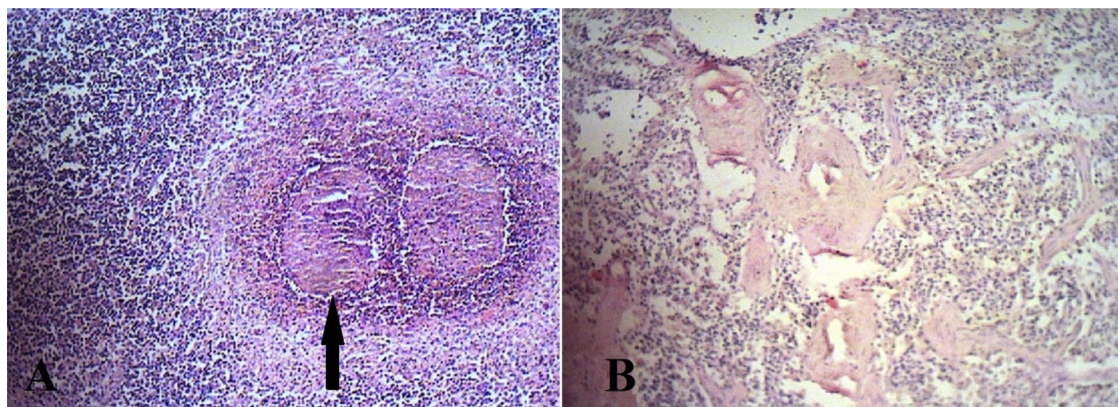


Fig. 2. Cross-section of infected mesenteric lymph node induced by *L. serrata* nymph with soft consistency (H & E): **A:** Granulomatose inflammation around the *L. serrata* nymph (Black arrow shows a parasite), $\times 200$, **B:** Lymph node depletion from lymphocytes, $\times 200$.

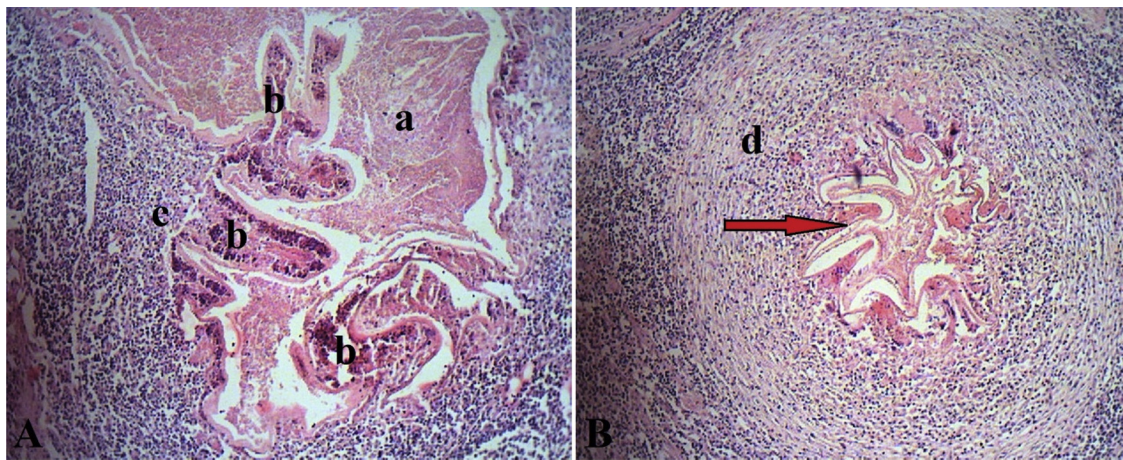


Fig. 3. Cross-section of infected mesenteric lymph node induced by *L. serrata* nymph with hard consistency and black color (H & E, $\times 200$): **A:** Caseous necrotic mass (a), *L. serrata* larva (b) in a cystic space and some neutrophils (c) and are present. **B:** *L. serrata* nymph (Red arrow) with a thick capsule (d) in lymph node. Some neutrophils are present in the capsule's wall. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

education regarding times not to ingest raw meat could be used to assist in preventing human infection. This is similar to what is found with *Fasciola hepatica* for which climate has an impact on the free-living stages of the parasite and its intermediate host, *Lymnaea truncatula*, with the interactions between rainfall and temperature having the greatest influence on transmission (Fox et al., 2011). With *F. hepatica*, *Nematodirus* and other parasites, the understanding of the climatic influence has been successfully modelled to annually predict when exposure is potentially high and inform farmers to aid in treatment decisions (www.nadis.org.uk; www.scops.org.uk).

The finding of a higher prevalence in female sheep than male sheep is in agreement with several studies (Azizi et al., 2015; Dehkordi et al., 2014; Hashemnia et al., 2018; Kheirabadi et al., 2015; Nourollahi et al., 2011); however, studies also have shown that within age groups, female and male sheep do not differ in *L. serrata* prevalence (Rezaei et al., 2011; Yektaseresht et al., 2017). Therefore, the difference seen in various studies including this one is more likely due to female sheep at slaughter being older than a true parasite preference. Our findings revealed that the parasitic infection rate in MLNs of sheep increased with age ($P < 0.05$) which was similar to those obtained in previous studies (Hashemnia et al., 2018; Kheirabadi et al., 2015; Nourollahi et al., 2011; Rezaei et al., 2011). The nymphs develop in approximately 5–6 months, which is in accordance with the lower prevalence in the younger groups. These data are useful in that in areas where raw meat is consumed, people could be encouraged to consume younger versus older animals as a potential means to decrease human exposure. Based on lymph node color, the results reported here showed that intensity of infection and infection rate with *L. serrata* was significantly higher in black-colored lymph nodes than hemorrhagic and normal-colored lymph nodes which was similar to the results of research conducted by Tavassoli et al. (2007). The finding of higher infections in soft lymph nodes also was consistent with the studies conducted by Tavassoli et al. (2007). Lower number of nymphs and frequency of infection in the hard lymph nodes (calcified) in comparison with soft ones may indicate that as lesions become older some pathologic changes, e.g. calcification, occur in the infected nodes and these changes impair survival of the nymphs. Histopathological findings were consistent with those seen in studies conducted by Azizi et al. (2015) where a layer of fibroblastic reactions with a mild underlying inflammatory zone containing mononuclear cells and neutrophil leukocyte infiltration were seen in infected MLNs of sheep. The visible morphological changes in MLNs (soft and black) potentially could be used in meat inspection to identify animals infected with *L. serrata* nymphs and enable condemnation of infected tissues. A study to assess the sensitivity and specificity of using

MLN color and consistency for diagnosis of infection would be needed.

5. Conclusions

In conclusion, presence of such gross changes in the color and consistency of the lymph nodes could be considered as an indication of infection with nymphs of *L. serrata* and condemnation of such lymph nodes is necessary to interrupt the life cycle of parasite.

Declaration of Competing Interest

We declare that we have no conflict of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.prevetmed.2019.104781>.

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