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■ Prevalence of *Linguatula serrata* infection in domestic ruminants from Meshkin Shahr, Iran

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ABSTRACT. *Linguatula serrata* is an aberrant cosmopolitan parasite, which inhabits the canine respiratory system (final host). The domestic ruminants such as sheep, goats, cattle and buffaloes (intermediate hosts) play the important role for transmission of parasite to humans and animals. The current investigation aimed to investigate the prevalence of *L. serrata* infection in domestic ruminants in Meshkin Shahr, Northwest of Iran. In cross-sectional study between March 2013 and March 2014, the mesenteric lymph nodes (MLNs) of 753 sheep, 403 goats, 657 cattle, and 341 buffaloes were sampled in Meshkin Shahr slaughterhouse, randomly. All of samples were examined for *L. serrata* infection using parasitology methods. The prevalence rate was reported 26.30% in goats, 18.32% in sheep, 14.30% in cattle, and 13.19% in buffaloes ($P<0.05$). In all animals, a significant difference were found between infection rate and different seasons ($P<0.05$), opposite to different age groups. Also, there was significant differences between infection rate and gender in cattle ($P=0.024$, OR=1.6) and buffaloes ($P=0.034$, OR=2), unlike to sheep and goats ($P>0.05$). This study was demonstrated of linguatulosus in domestic ruminants from Northwest of Iran for first time. According to our findings, Meshkin Shahr is a new endemic region for *L. serrata* infection in Iran. Since the linguatulosus is a zoonotic parasitic disease, preventive measures to break the parasite's life cycle and reduce the risk of infection in humans and animals is highly recommended.

Keywords: *Linguatula serrata*, domestic ruminants, Meshkin Shahr, Iran

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INTRODUCTION

Linguatula serrata (Pentastomida: Linguatulidae) also known as “tong-worm” is a cosmopolitan, zoonotic and obligate endoparasite. The parasite lives in the nasopharyngeal region of final hosts; dogs, foxes, felines, and other carnivores are the main final hosts. Various herbivores, including cattle, buffaloes, sheep, and goats, serve as the best intermediate hosts for nymph stages (Soulsby, 1982).

Eggs produced by the female parasite exit the definitive host through its faeces and nasopharyngeal secretions. They are then swallowed by grazing ruminants and hatch into larvae in their small intestine. In order for the infective nymphs to develop, the larvae need to travel to the intermediate host's mesenteric lymph nodes (MLNs), liver, and lungs (Tajik et al., 2008). Final hosts are infected by ingestion of herbivores's infected viscera. Likewise, the consumption of contaminated food is the main source of infection with *L. serrata* in humans. While *L. serrata* commonly inhabits the nasal airways of canids and felines, it may occur in nasopharyngeal, visceral, or ocular forms in humans (Siavashi et al., 2002).

The parasite causes a syndrome, known as halzoun or Marrara syndrome, in humans. Nasopharyngitis, violent coughing, asphyxiation, edematous congestion of gums, tonsils, and eustachian tube, aural pruritus, deafness, frontal headache, sneezing, lacrimation, coryza, yellow nasal discharge, facial edema, vomiting, and breathing blockage are the bold symptoms (Yazdani et al., 2014). Human infection with *L. serrata* has been reported from various parts of the world and some regions of Iran (Maleky, 2001; Siavashi et al., 2002; Yazdani et al., 2014).

Extensive research has also evaluated the prevalence of *L. serrata* infection in dogs (Oryan et al., 2008), camels (Haddadzadeh et al., 2009; Radfar et al., 2010), buffaloes (Tajik and Jalali, 2010), sheep (Nourollahifard et al., 2011; Azizi et al., 2015; Kheirabadi et al., 2015), cattle (Youssefi and Hadizadeh-Moalem, 2010), and goats (Nourollahifard et al., 2010a; Kheirabadi et al., 2014) in different regions of Iran. There are no published data on linguatulosus in animals from Northwest of Iran. Therefore, the present study aimed to determine the prevalence of MLNs infection with the nymphal stage of *L. serrata* in cattle, buffaloes, sheep, and goats slaughtered in Meshkin Shahr, Northwest of Iran.

MATERIALS AND METHODS

Sampling

In different seasons, a total of 657 cattle, 753 sheep, 403 goats, and 341 buffaloes were sampled randomly in Meshkin Shahr slaughterhouse during March 2013 to March 2014. The animals were categorized in different age groups (<2 years, 2-4 years, and >4 years) and gender.

Parasitology

In order to confirm the presence or absence of *L. serrata* nymphs, the MLNs of animals were examined in two steps. In the first, individual lymph nodes were thinly sliced and maintained in normal saline for five-six hours to allow nymphs to exit the tissue. The recovered nymphs were flattened, dehydrated by passing through a series of ascending grades of ethyl alcohol, and cleared with creosote. They were then observed under a stereomicroscope to determine their nymphal stage. In the second step, the negative samples were digested in 200 ml of digestion solution (5 g of pepsin and 25 ml of hydrochloric acid solved in 1,000 ml distilled water). They were incubated at 37° C for 24 hours. During this time, nymphs came out and floated in the solution. The suspensions were transferred to Petri dishes and examined under a stereomicroscope (Razavi et al., 2004; Alborzi et al., 2013).

Data analysis

Statistical analysis was performed by using the software package SPSS version 16.0 for windows (SPSS Inc., Chicago, IL, USA). Odds ratios (OR), confidence interval (CI), χ^2 and *p*-value were calculated separately for each variable. *P*-value of less than 0.05 was considered statistically significant.

RESULTS

In the first step of examination, the prevalence of *L. serrata* infection were detected 24.81% (100/403) in goats, 16.07% (121/753) in sheep, 7.91% (52/657) in cattle, and 5.28% (18/341) in buffaloes. Finally, this rate were 26.30% (CI 95%: 22.1-30.5%) in goats, 18.32% (CI 95%: 15.62-21%) in sheep, 14.30%

(CI 95%: 11.7-16.9%) in cattle, and 13.19% (CI 95%: 9.7-16.7%) in buffaloes using digestion method ($P<0.0001$, Table 1). No significant relation was seen between infection rate and age groups in animals ($P>0.05$, Table 1). The infection rate in males was higher than females in cattle ($P=0.024$ and $OR=1.6$) and buffaloes ($P=0.034$ and $OR=2$); unlike to sheep and goats ($P>0.05$, Table 1). In all animals, a significant difference was found in the infection rate and different seasons ($P<0.05$, Table 2), opposite to different age groups ($P>0.05$, Table 1).

DISCUSSION

In Iran, past studies have reported the prevalence of infection with *L. serrata* as 27.8-76.5% in dogs (Meshgi and Asgarian, 2003; Rezaei et al., 2011),

19-68% in goats (Rezaei et al., 2012; Youssefi et al., 2012; Sadeghi-Dehkordi et al., 2014; Kheirabadi et al., 2014), 9-52.5% in sheep (Tavassoli et al., 2007a; Nouroollahifard et al., 2011; Youssefi et al., 2012; Sadeghi-Dehkordi et al., 2014; Azizi et al., 2015; Kheirabadi et al., 2015), and 14.8-69.1% in cattle (Tajik et al., 2006; Nouroollahifard et al., 2010b; Youssefi and Hadizadeh-Moalem, 2010; Rezaei et al., 2011; Youssefi et al., 2012; Alborzi et al., 2013; Nematollahi et al., 2015).

In India, infection rates have been reported 21% in goats and 19% in cattle (Ravindran et al., 2008). This rate was 5.4% in sheep in Turkey (Aydenizöz et al., 2012).

In the previous investigations *L. serrata* nymphs was isolated from the ruminants' viscera (e.g. MLNs, liver, lung, and spleen). MLNs are generally the first

Table 1. The prevalence of *L. serrata* infection in different animal species, gender and age groups in Meshkin Shahr, Iran.

Age groups		No. of examined (prevalence%)			
		Goats	Cattle	Buffaloes	
<2 yr	Male	142(12)	87(24.3)	110(16.4)	62(11.3)
	Female	118(6.8)	59(15.3)	55(16.4)	36(8.3)
	Total	260(9.6)	146(20.5)	165(16.3)	98(10.2)
2-4 yr	Male	153(18.3)	145(13.1)	185(10.3)	122(6.6)
	Female	145(11.7)	49(36.7)	114(8.8)	73(5.5)
	Total	298(15.1)	194(19.1)	299(9.7)	195(6.2)
>4 yr	Male	115(40)	39(59)	105(20.95)	26(61.53)
	Female	80(27.5)	24(66.7)	88(18.2)	22(31.8)
	Total	195(34.9)	63(61.9)	193(19.7)	48(47.9)
Total	Male	485(18.8)	258(24.4)	342(17.3)	185(16.8)
	Female	268(17.5)	145(29.7)	315(11.1)	156(9)
	Total	753(18.3)	403(26.3)	657(14.3)	341(13.2)
P-value	Gender	0.667	0.251	0.024 OR=1.6	0.034 OR=2
	Age group	0.999	0.997	0.991	0.998

Table 2. The prevalence of *L. serrata* infection in MLNs of animals in different seasons in Meshkin Shahr, Iran.

Animals	No. of examined (prevalence%)				P-value	CI 95%
	Spring	Summer	Autumn	Winter		
Sheep	128 (22.7)	119 (22.7)	316 (21.5)	190 (7.4)	<0.0001	15.62-21
Goats	102 (22.5)	109 (11.9)	101 (61.4)	91(8.8)	<0.0001	22.1-30.5
Cattle	127 (9.4)	132 (10.6)	288 (20.1)	110 (9.1)	<0.0025	11.7-16.9
Buffaloes	87 (10.3)	63(15.9)	90 (20)	101 (7.9)	<0.0493	9.7-16.7

body parts infected with *L. serrata*. They also have a higher risk of infection compared to visceral organs (Rezaei et al., 2011; Sadeghi-Dehkordi et al., 2014). Therefore, the present study assessed the prevalence of *L. serrata* infections in the MLNs of goats, sheep, cattle and buffaloes; the obtained infection rates in the mentioned animals were 26.30%, 18.32%, 14.30%, and 13.19%, respectively. No significant statistical relations were found between infection rate and species of animals. This probably indicates a regional difference in the distribution of the parasite, i.e. the high prevalence rate of infection in this area suggests its favorable climatic conditions for the survival of parasite eggs in vegetables, fruits, and water resources. Suitable temperature and humidity might thus play an important role in the epidemiology of this infection.

The high prevalence of infection in the MLNs of ruminants should be considered as an important risk factor for human infection (Maleky, 2001). Discrepancies in the rates of linguatolosis might be attributed to differences of diagnostic methods used such as digestion and/or histopathology techniques, study design, climatic variations of studied area, frequency of dogs inside and suburb of the farms and farm management as well.

In our work, the infection rate in goats was higher than other animals ($P<0.0001$). This is may be due to goats' breeding system (which increased their chance of contact with dogs), higher sensitivity, and feeding style. Moreover, goats grazing ahead of the flocks might have increased their risk of infection

(Sadeghi-Dehkordi et al., 2014). In Iran, most of sheep and goats farms are traditional; in this system animals are kept together and fed on the pastures. The animals have close contact with stray dogs. In our finding, the infection rate in animals increased with age ($P>0.05$); this is parallel to Azizi et al. (2015) report. In Kheirabadi et al. (2015) study, the infection rate in sheep with >3 years old was more than other age groups ($P<0.05$). Since *L. serrata* nymphs require five-six months for develop, the lower prevalence of infection in younger animals seems logical.

Moreover, although a significant difference in the infection rate was observed between males and females in cattle and buffaloes, no such significant differences were observed within age groups. Similar findings were reported by other researchers in Iran and Turkey (Tajik et al., 2006; Nourollahifard et al., 2010b; Nourollahifard et al., 2011; Aydenizöz et al., 2012; Alborzi et al., 2013; Nematollahi et al., 2015). Nourollahifard et al. (2010) and Sadeghi-Dehkordi et al. (2014) reported the prevalence of *L. serrata* nymphs to be significantly higher in females than in males. In similar studies in sheep and goats, there was found no significant differences between infection rate and gender (Kheirabadi et al., 2014; Azizi et al., 2015; Kheirabadi et al., 2015). They attributed this finding to the longer lifespan of female animals before being slaughtered.

Sporadic reports of human linguatolosis have been published in Iran and other countries (Maleky, 2001; Ravindran et al., 2008; Oluwasina et al., 2014;

Yazdani et al., 2014). Geographical limitations, close contact between dogs and sheep, and regular migration of nomads and their livestock and dogs (fed on uncooked offal of the ruminants) to the study area might have all contributed to the high infection rate in the area. In fact, such factors facilitate constant contact between the final host and sheep, goats, cattle, and buffaloes.

CONCLUSIONS

This is the first report of comprehensive investigation in animals' linguatolosis in Northwest of Iran. Our findings confirmed that the Meshkin Shahr is an endemic region for *L. serrata* infections. Since *L. ser-*

rata is a zoonotic parasite, preventive measures should be adopted to break the parasite's cycle and minimize the risk of infection in humans and animals.

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CONFLICT OF INTEREST STATEMENT

We declare that there is no conflict of interests.

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