The Prevalence of *Linguatula serrata* Nymphs in Mesenteric Lymph Nodes in Cattle

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Abstract: Problem statement: The *Linguatula serrata* is one of the parasitic zoonoses. Human beings may also be infected by both the nymph stage, a condition called nasopharyngeal linguatulosis or Halzoun syndrome and the egg, a condition called visceral linguatulosis. **Approach:** The aim of the present study was to evaluate the prevalence of nymphal stages of *L. serrata* in mediastenal and mesenteric lymph nodes of cattle slaughtered in Kerman slaughterhouse, south east of Iran. For this purpose, mesenteric and mediastenal lymph nodes of 450 cattle of different sex and age were examined. **Results:** A prevalence of 16.22% was observed in mesenteric lymph nodes. Higher prevalence of infection was observed in animals aged above four years and during autumn season. The infection rate increased with age (p<0.05). Also prevalence of *L. serrata* nymphs in different seasons was significantly different (p<0.05). **Conclusion:** The high prevalence of infection observed in a ruminant is of concern owing to the zoonotic nature of the parasite and the risk of infection to humans and other animals.

Key words: *Linguatula serrata*, cattle, Kerman, Iran

INTRODUCTION

Linguatula serrata Frolich, 1789 is a complete parasite, highly specialized in endoparasitism, with the adults residing in the upper respiratory tracts of carnivore mammals, inducing parasitic rhinitis and the larvae and nymph migrating and encysting in the various visceral organs of herbivores. Omnivorous mammals, including humans, may act either as final or intermediate hosts. Therefore the presence of this infection in carnivores or herbivores is potentially zoonotic, causing visceral and nasopharyngeal linguatulosis or 'Halazoun Syndrome' in humans (Lazo et al., 1999). Linguatula serrata is tongue shaped, lightly convex dorsally and flattened ventrally. Males measure 1.8-2 cm, while females measure 8-13 cm in length. Adults inhabit the canine respiratory system as final hosts. Some of herbivores animals can act as intermediate hosts following ingestion of eggs. The eggs hatch and larvae emerge in the alimentary canal eventually migrating to various internal organs and tissues transforming into nymphs that become encapsulated (Soulsby, 1982). The eggs are expelled from the respiratory passages of the canine and, when swallowed by a suitable herbivorous animal as intermediate host, the larva reaches the mesenteric and mediastenal lymph nodes, liver and other organs, in which it develops to the infective nymphal stage after

six to nine moulting. It usually lies in a small cyst surrounded by a viscid turbid fluid. The final host becomes infected by eating the infected viscera of intermediate hosts (Soulsby, 1982). This parasite has been reported in humans in Iran (Mohammadi et al., 2008; Siavashi et al., 2002) with clinical signs of symptoms nasopharyngeal including sneezing. coughing and nasal discharge following consumption of barbecued liver (Kabab). Maleky (2001) described linguatulosis caused by L. serrata in the throat of a 28year-old woman from Tehran, Iran. The parasite in and humans causes nasopharyngeal linguatulosis (pentastomiasis) producing a condition called as halzoun or marrara syndrome (Maleky, 2001) that is often characterized by inflammation of the upper respiratory tract, swelling of the submaxillary and cervical lymph nodes and occasionally abscess formation in the eyes or ears (Khalil, 1976; Yagi et al., 1996). Sporadic incidence of halzoun in humans was also reported in Iran (Maleky, 2001). Consuming raw or under-cooked liver is not unusual in some part of Iran particularly in pregnant women. It is thought among some women, that consumption of raw or undercooked liver is helpful for growth of the fetus because of its high content of iron and vitamins. Several studies have been conducted on the prevalence rate of L. serrata in dogs (Meshgi and Asgarian, 2003), camels (Shakerian et al.,

2008; Haddadzadeh *et al.*, 2009), buffaloes (Tajik *et al.*, 2008), sheep (Shekarforoush *et al.*, 2004; Tavassoli *et al.*, 2007) and goats (Fard *et al.*, 2010; Saiyari *et al.*, 1996; Razavi *et al.*, 2004).

The aim of this study was to determine the prevalence rate of *L. serrata* nymphs in mesenteric and mediastenal lymph nodes of cattle slaughtered at Kerman slaughterhouse. Kerman is located at 30°17'13"N and 57°04'09"E southeast of Iran. The mean elevation of the city is about 1755 m above sea level. Kerman city has a moderate climate and the average annual rainfall is 135 mm. Because it is located close to the Kavir-e lut, Kerman has hot summers.

MATERIALS AND METHODS

During September 2008-September 2009, 450 cattle (198 females and 252 males) in three age groups (<2, 2-4 and >4 years old) were selected randomly at the Kerman slaughterhouse.

For this study, mediastenal and mesenteric lymph nodes were examined for nymphal stage of *L. serrata*. Samples were cut into small pieces and immersed in normal saline (0.9% NaCl) solution and left for 5-6 h to allow nymphs to come out from tissue. Recovered nymphs were flattened, dehydrated in ascending grades of ethyl alcohol and cleared in creosote before examining under a stereomicroscope. Then the negative samples were digested in 200 mL of digestion solution (5 g of pepsin, 25 mL hydrochloric acid in 1000 mL distilled water) and incubated at 37°C for 24 h Razavi *et al.*, 2004).

Lymph nodes from each animal were removed and bagged separately and were fixed in 10% buffered neutral formalin. The samples were processed by the standard paraffin wax technique and were cut in 5 μm thickness stained with routine Haematoxylin and Eosin (HE) method. The stained samples were examined under a light microscope.

The computer software, SPSS Version 9.0 for Windows (SPSS Inc., Chicago, IL, USA) was used for analysis. To compare relative frequency of infection between different groups of lymph nodes Chi-square tests was used. Differences were considered significant when p<0.05.

RESULTS

The prevalence rate of *L. serrata* nymphs in mediastenal and mesenteric lymph nodes of 450 cattle

slaughtered at Kerman slaughterhouse, Iran in different sex and age groups and different seasons are summarized in Table 1.

Seventy three out of 450 (16.22%) cattle were infected with nymph stages of *L. serrata*. 34 out of 252 males (13.4%) and 39 out of 198 females (19.6%) were found to be positive. Seventy three out of 450 (16.22%) cattle had nymphs in their mesenteric lymph nodes. Thirty out of 450 (6.66%) cattle had nymphs in their mediastenal lymph nodes. The results showed a higher infection rate of mesenteric lymph nodes compared with that of mediastenal mesenteric lymph nodes (p<0.05). The infection rate increased with age (p<0.05).

The mesenteric lymph nodes revealed oedema and haemorrhage, with a loss of lymphocytes. In histopathology, the nymph of *L. serrata*, with segmented body and transversely striated spines were observed within oedematous cyst-like spaces surrounded by a wall composed of fine fibrous and dense lymphocytic zones, often with several lymphoblasts. Dead larvae were observed with chronic granulomatous inflammation, characterized with accumulation of epithelioid macrophages and giant cells around the necrotic areas (Fig. 1).

Multifocal caseative necrosis with calcification was observed in some lymph nodes. In severely affected cases, such lesions were coalescent.

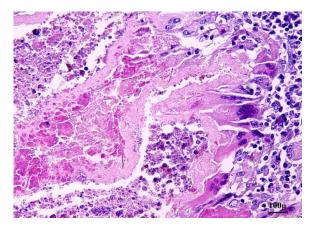


Fig. 1: Dead larvae of *L. serrata*, with chronic granulomatous inflammation (H and E)

Table 1: The prevalence of Linguatula serrata nymphs in mesenteric lymph nodes cattle

	Age (years)			Gender			Seasons		
	<2	2–4	>4	Male	Female	Spring	Summer	Autumn	Winter
No. of cattle	220	138	92	252	198	120	110	110	110
No. of infected cattle (%)	17 (7.72)	31 (22.4)	25 (27.17)	34 (13.4)	39 (19.6)	18 (15)	20 (18.1)	25 (22.7)	10 (9.09)

DISCUSSION

Several studies have been conducted in Iran to determine the prevalence of linguatulosis in ruminants. The prevalence rates of 19% in cattle of India (Ravindran *et al.*, 2008), 44% in cattle in Urmia slaughterhouse, Iran (Tajik *et al.*, 2006), 29.9% in goats in Shiraz, Iran (Razavi *et al.*, 2004), have been reported from different countries. In the present study, the prevalence rate of 16.22% was observed in cattle. High prevalence rate of infection in this area is a manifestation of climatic parameters that enhance survival of parasite eggs in vegetables, fruits and water resources and possibly, the suitable temperature and humidity play important roles in the epidemiology of this infection.

The high prevalence rate of 16.22% infection in lymph nodes of cattle should be considered as an important risk factor for humans. Larval and nymphal stages of *Linguatula serrata* have been recorded from humans in some countries such as Iran (Maleky, 2001).

The prevalence of *L. serrata* nymphs in females was significantly higher than that of males (p<0.05). The infection rate increased with age (p<0.05).

The prevalence rate of *L. serrata* in mediastenal lymph nodes (6.66%) was lower than that of mesenteric lymph nodes (16.22%). According to the life cycle of *L. serrata*, mesenteric lymph nodes, located in the way of portal circulation before the other organs so the infection rate of these are higher than other organs.

CONCLUSION

Considering results, it could be concluded that prevalence of infection in cattle is high and emphasized the need for more investigation on mesenterinc lymph nodes in cattle.

Recently, *L. serrata* found a more public health importance and new human cases is reported from Iran and should be considered as an important risk marker for human being infection.

Larval or nymphal infection is asymptomatic in herbivores. Larval and nymphal stages of *L. serrata* in humans were previously reported from Iran (Maleky, 2001). Human infection is as the result of ingestion of third stage larvae of *L. serrata* found in raw liver or lymph nodes of sheep, goats and cattle. Ingestion of *L. serrata* nymphs can cause halzoun or marrara syndrome.

Because of the veterinary and medical importance of linguatulosis, we suggest that further serious investigation be conducted in both carnivores and herbivores.

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