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journal homepage: www.elsevier.com/locate/apjtbOriginal article <http://dx.doi.org/10.1016/j.apjtb.2015.04.006>Infestation and pathological lesions of some lymph nodes induced by *Linguatula serrata* nymphs in sheep slaughtered in Shahrekord Area (Southwest Iran)Hamidreza Azizi^{1*}, Hossein Nourani¹, Abdollah Moradi²¹Department of Pathobiology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran²School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran

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ABSTRACT

Objective: To determine the infection rates of mesenteric and mediastinal lymph nodes in relation to *Linguatula serrata* (*L. serrata*) nymphs.**Methods:** In the present study, mesenteric and mediastinal lymph nodes of 200 sheep with different sex and age that were slaughtered in a Shahrekord slaughterhouse were collected. The lymph nodes were examined macroscopically and for histopathological examination, tissue samples were taken from the gross lesions processed routinely by paraffin method and stained with hematoxylin and eosin.**Results:** Out of 200 examined sheep, the mesenteric lymph nodes in 18 sheep (9%) and the mediastinal lymph nodes of 9 sheep (4.5%) were infected by *L. serrata* nymphs. The infection rate increased with age, but no significant difference was observed between males and females or the two types of lymph nodes ($P > 0.01$). Different nonspecific gross and macroscopic lesions were seen in infected lymph nodes. *L. serrata* nymph sections were observed in some histopathological slides.**Conclusions:** It is concluded that the sheep may play an important role in linguatulosis of final hosts and human beings in this region.

1. Introduction

Parasites are organisms whose survival is dependent on the host and establishing this relationship mainly causes parasitic diseases in the hosts. *Linguatula serrata* (*L. serrata*), or worm-like and tongue-shaped invertebrates, belong to the subphylum pseudo-arthropod and the family Linguatulidae. *L. serrata* is one of the parasites prevalent worldwide in different climatic zones [1,2]. After ingestion of eggs by the intermediate host, the larvae hatch, then after passing from the intestinal wall, reach the mesenteric lymph nodes through the blood stream. The parasite attacks different organs depending on the hosts: in horses and camels they mainly attack mesenteric lymph nodes; in cows and goats, they mainly attack the hepatic lymph nodes and in sheep, they attack mediastinal lymph nodes. Parasites

enter these organs and can cause pathological lesions and signs related to the infected organ. Finally, the larvae become nymphs and remain in place for a few years until they are calcified. After the intermediate host's infected viscera is eaten by the final hosts, the life cycle of *L. serrata* will be complete [1,3,4]. Symptoms of parasites in the intermediate hosts include emaciation, paleness of mucous membranes, ascites and serous accumulation in the abdominal cavity, peritoneal inflammation and intestinal adhesion. Important symptoms caused by the disease in sheep include hyperplasia of pulmonary lymphatic tissue and pneumonia. In human beings, horses and camels, symptoms are mainly related to intestinal lymph nodes and cause them to swell. Symptoms in human beings are sneezing, swelling of the face, increased nasal discharge, eye irritation and severe itching at the site of the throat and mouth due to stimulation of the respiratory mucosa or possible allergic reaction to the parasite nymph. Treatment of the disease may be performed by surgical removal of the parasite and administration of antihistamines [2,5]. The prevalence of *L. serrata* infection in dogs was 27.83% and the infection rate for goats, buffaloes, cattle and sheep was 50.75%, 26.6%, 36.62% and 42.69%, respectively.

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The prevalence rate in all animals was significantly associated with age and sex ($P \leq 0.05$) [6]. The extent of prevalence of infection in cattle can be caused by geographical and climatic conditions and access of the final host to the infected viscera of ruminants. In Iran, the prevalence of the adult form of *L. serrata* infection in dogs, 55% in the Marand area and 76.47% in the Shiraz area, have been reported [5,7]. Chaharmahal and Bakhtiari Province, with 1 529 000 sheep is one of the major provinces in the breeding and production of sheep. Despite the high rate of adult form of infection of *L. serrata* in dogs in the Shahrekord area, in respect of the infection and lesions caused by *L. serrata*, no research has been conducted in sheep as yet. In this study, the amount of infection and pathological lesions caused by *L. serrata* nymph were studied in slaughtered sheep in the Shahrekord area.

2. Materials and methods

In the winter of 2012 and spring of 2013, during a visit to a Shahrekord slaughterhouse, 200 sheep were studied randomly. Mediastinal and mesenteric lymph nodes were collected from slaughtered sheep and put into plastic cans with the number of samples written on them; the samples were placed on ice and transferred to the laboratory (Department of Parasitology) [8]. Samples collected from the slaughterhouse were transferred to the laboratory of parasitology within 3–4 h, and were studied macroscopically. Multiple sections were created with the lymph nodes immersed in normal saline (0.9%) for 5–6 h until the nymphs exited from the tissues. Then, to preserve the parasites, the tissues were placed in alcohol glycerol. Preparing a transparent slide took an hour with lactophenol. *L. serrata* nymphs were examined for size and morphology under stereomicroscope. Then lesions were sampled for histopathological investigation and put in 10% buffered formalin to stabilize. The formalin solution was replaced after 24 h. This helps the formalin to better penetrate into the lymph node. After fixation, a cross-section was prepared from each of the samples pathology slides. Samples were placed in an auto technique device. In the device, dewatering process, transparency and paraffin embedding were carried out, and then during the molding, samples were cut with a thickness of 5 μm , and stained with hematoxylin and eosin (H&E).

3. Results

Since the results of the present study were obtained from several stages, each stage will be described separately. Results were analyzed by One-way ANOVA test. From a total of 200 carcasses, *L. serrata* nymph was observed in 28 (14%). Infection rate of mesenteric lymph nodes was higher than mediastinal lymph nodes. Infection of *L. serrata* nymph in mesenteric lymph nodes in eighteen (9.0%) sheep and in mediastinal lymph nodes in nine (4.5%) sheep, and in both types of lymph nodes in one sheep (0.5%) were observed. The results can be seen in Table 1.

However, no significant relationship between the infection rate and the type of lymph was found ($P > 0.01$). *L. serrata* infection rates in females (15.27%) and males (13.28%) were similar to each other, and there is no significant difference between sex and infection rate ($P > 0.01$). Sheep under consideration in the present study were classified into four groups: less than 1 year old, 1–2 years old, 2–3 years old and more than 3

Table 1

Infection rate of nymph of the *L. serrata* in the mesenteric and mediastinal lymph nodes in 200 samples.

Type of lymph node	Infection (%)	Number of infected samples	Number of samples
Mesenteric	9.0	18	200
Mediastinal	4.5	9	200
Mesenteric and mediastinal	0.5	1	200
Total	14.0	28	200

years old. Although infection from the parasite was not seen in the group under 1 year old, the infection rate rose with increasing age, from 11.9% at age 1–2 years old to 22.03% at more than 3 years old. The results of this section are summarized in Table 2.

Table 2

Relationship between age and linguatulosis.

Age	Infection (%)	Number of infected samples	Number of samples
<1 year	0.00	0	47
1–2 years	11.90	5	42
2–3 years	19.23	10	52
>3 years	22.03	13	59
Total	14.00	28	200

There are significant differences between infection rates and age ($P < 0.01$). The appearance of the parasite was cone-shaped, in other words, the cranial part was wider than the caudal part (Figure 1). The dorsal part was convex and the ventral part was flat. The entire body surface of the nymph was formed with edge or articulation, and there was a row of spinous processes in the caudal border of these articulations.

Lesions were observed by macroscopic examination of mesenteric and mediastinal lymph nodes which were infected with *L. serrata* nymph, including hyperemia, hemorrhage (Figure 2), focal caseous necrosis and calcification (Figure 3), and severe edema, swelling and softening of the lymph nodes, and color changed to dark green (Figure 4). These lesions were more severe in the mesenteric lymph nodes.



Figure 1. Nymph of *L. serrata*.

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