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Infestation and pathological lesions of some lymph nodes induced by *Linguatula serrata* nymphs in sheep slaughtered in Shahrekord Area (Southwest 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

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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. 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.

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

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 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 linguatulusis.

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.



Figure 1. Nymph of *L. serrata*.

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.

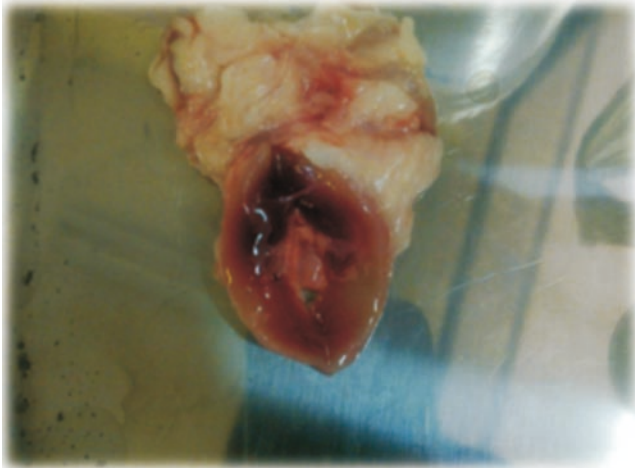


Figure 2. Congestion and hemorrhage in the lymph node infected with nymph of *L. serrata*.

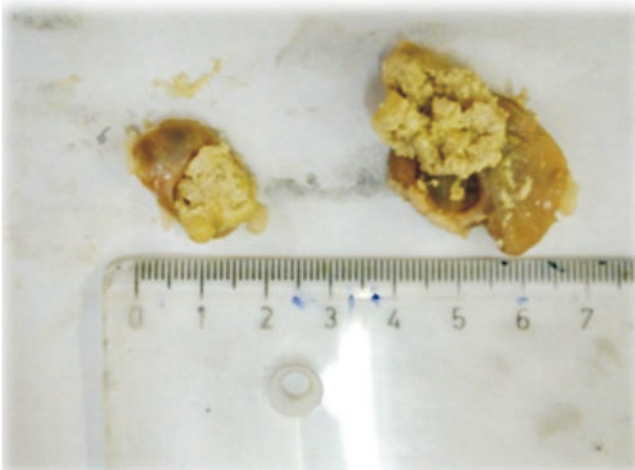


Figure 3. Focal of caseous necrosis and calcification in the lymph node infected with nymph of *L. serrata*.

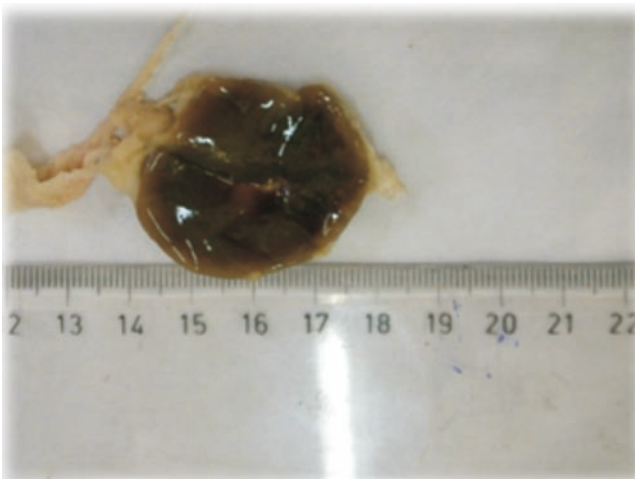


Figure 4. Swelling, softening and discoloration of lymph node infected with nymph of *L. serrata*.

Under microscopic examination of infected lymph nodes, the sections of *L. serrata* were clear in a cystic space (Figure 5). In some lymph nodes, there was mild fibrosis in the vicinity of *L. serrata* (Figure 6). Also, spinous processes can be seen on the surface in histopathological section of *L. serrata*. (Figure 7). Hyperemia, hemorrhage and severe edema (Figure 8), hemosiderin pigments (Figure 9) and drainage of this organ from lymphocytes (Figure 10) were observed in existence of *L. serrata*.

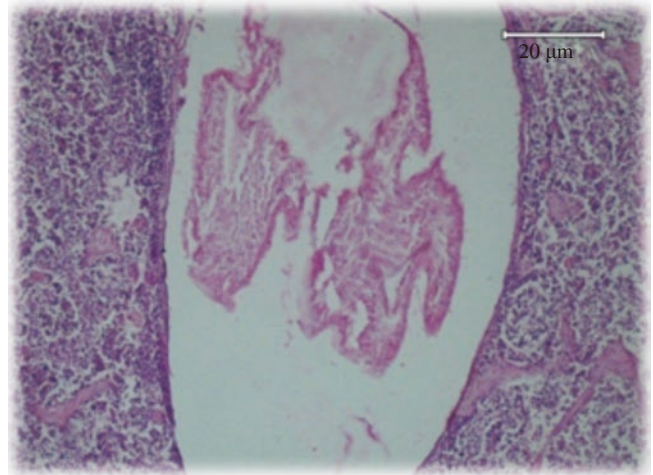


Figure 5. Section of nymph of the *L. serrata* in a cystic space in the lymph node (H&E, × 40).

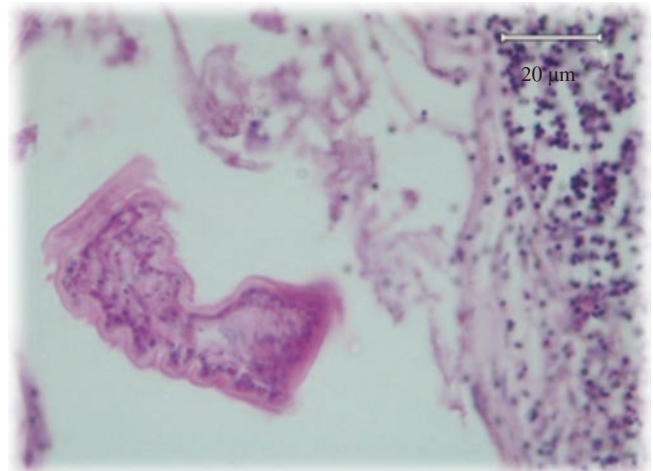


Figure 6. Mild fibrosis next to nymph of the *L. serrata* (H&E, × 40).

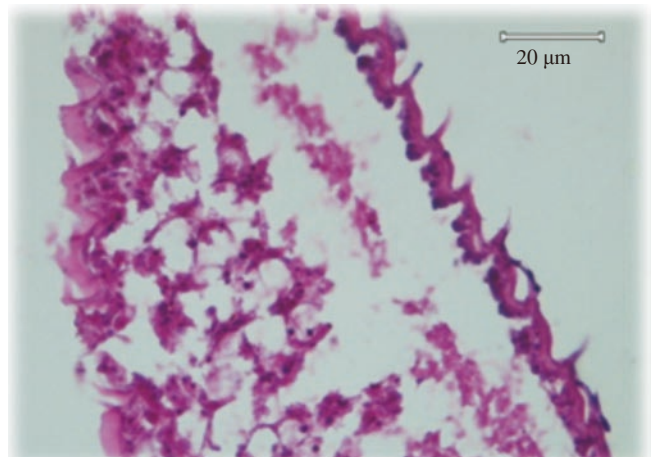


Figure 7. Microscopic section of the nymph of *L. serrata* spine like appendices on its surface (H&E, × 40).

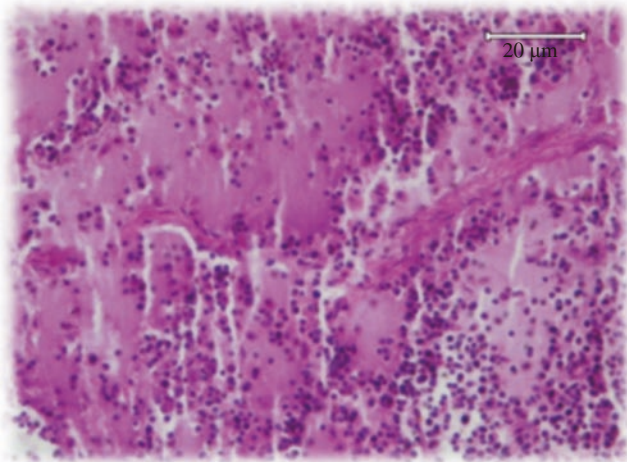


Figure 8. Severe edema of the lymph node infected with nymph of *L. serrata* (H&E, × 40).

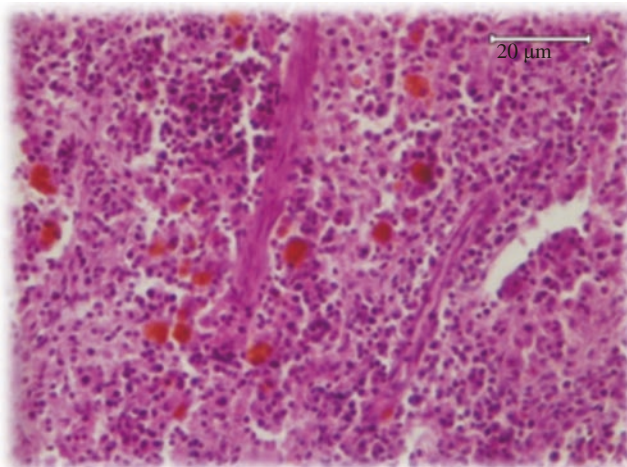


Figure 9. Hemosiderin pigment in the lymph node infected with nymph of *L. serrata* (H&E, × 40).

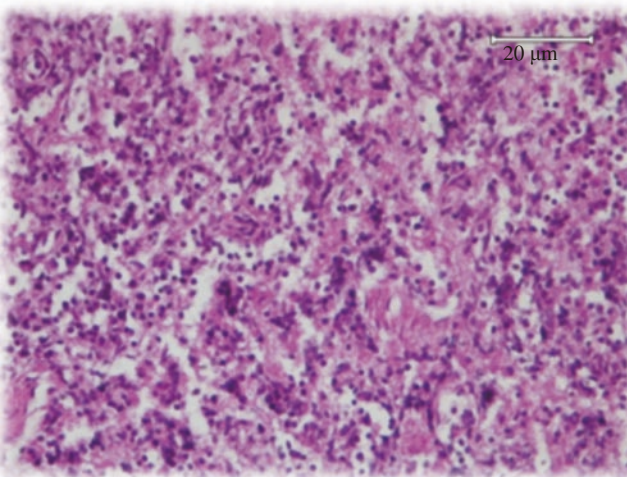


Figure 10. Lymphocyte depletion of the lymph node infected with nymph of *L. serrata* (H&E, × 40).

4. Discussion

Linguatulosis is a common disease with worldwide distribution,

prevalent mainly in tropical, subtropical and humid climates. The adult form of the parasite can be seen in canine nose and airways as final hosts and the immature form can be seen in the mesenteric lymph nodes of herbivores as intermediate hosts. Although in the infected intermediate host no clinical signs were seen, in human beings as accidental hosts, infection can cause severe lesions and symptoms[3]. This study aimed to investigate the amount of infection and pathological lesions caused by *L. serrata* nymph in slaughtered sheep in the Shahrekord Area. In this study, during December 2012 and May 2013, from 200 slaughtered sheep, mesenteric and mediastinal lymph nodes were randomly investigated. From a total of 200 carcasses, *L. serrata* nymph was observed in 28 (14%) carcasses. Infection rate of mesenteric lymph nodes was higher than mediastinal lymph nodes. Prevalence rates of 25.5%[4] and 16.1%[2] have been reported from different regions of Iran. Several studies have been conducted on the prevalence of *L. serrata*. The prevalence rates of 49.1% in goats in Kerman[9], 16.1% in sheep in Kerman[2], 14.8% in cattle in Babol[10], 16.22% in cattle in Kerman[11], 25.5% in sheep in Urmia[4] and 16.1% in sheep in Kerman[2] have been reported from different regions of Iran.

In all reports related to *L. serrata* infection in domestic ruminants, the infection rate of liver, lung and spleen lymph nodes was higher than other organs. This is due to penetration of the parasite nymph through the gut into the mesenteric lymph nodes at first, and then spreading to other visceral organs. In the study which was carried out by our laboratory, the infection rate of mesenteric lymph nodes and the mediastinal lymph nodes was 15.1% and 7.3%, respectively. The results showed infection rate of mesenteric lymph nodes higher than mediastinal mesenteric lymph nodes ($P < 0.05$)[12]. The infection rates of mediastinal and hepatic lymph nodes in the study of Yakhchali *et al.* have been reported as 65.79% and 32.28%[13]. With the results of studies on *L. serrata* infection rate in sheep in the different regions of Iran, it is concluded that the infection rate in sheep was very different in these studies[2,10]. The mesenteric lymph nodes, livers, and lungs of 75 goats and 225 sheep (19 females and 206 males) were examined for *L. serrata* nymphs by Dehkordi *et al.*[1]. The results showed that 30.6% of goats and 10.2% of sheep were infected with *L. serrata* nymphs[1]. The study of Aydenizoz *et al.* showed that 14 sheep (5.4%) were infested on gross examination and nymphal stages of *L. serrata* were detected in 97 of 1 729 mesenteric lymph nodes (5.2%)[14]. Gül *et al.* in the Van province of Turkey, in a study of 347 sheep mesenteric lymph nodes and 224 sheep mediastinal lymph nodes, observed *L. serrata* nymph in mesenteric lymph nodes at a rate of 5.19%, but in the mediastinal lymph node there was no *L. serrata* nymph[15]. Mir *et al.* studied the simultaneous incidence

of linguatulosis and Johne's disease in goats and suggested that linguatulosis may be predisposing Johne's disease[16]. Nourollahi Fard *et al.* studied *L. serrata* infestation rate in mesenteric and mediastinal lymph nodes of 407 slaughtered goats in Kerman city[9]. The results showed that 49.1% of goats were infected with the parasite. Prevalence rates of the parasite in the fall were 76.4%, spring 50.0%, summer 38.6%, and winter 31.8%. No significant difference in prevalence rates between males and females was observed[9]. In this study, there was significant association between age and infection rate that, with increasing age, infection rate was also increased. In other words, there was direct association between age and rate of infection ($P < 0.01$). This finding was the same with the results of other researchers like Nourollahi Fard *et al.* and Youssefi *et al.*[2,10]. There are no studies about pathology of the infected lymph nodes to *L. serrata* nymph in sheep, and in the present study macroscopic lesion in mesenteric and mediastinal lymph nodes include hyperemia, hemorrhage, severe edema, swelling and softening of the lymph nodes, color change (dark green), focal caseous necrosis and calcification, in which the severity of the lesions was greater in mesenteric lymph nodes. In this study, microscopic lesions of lymph nodes due to *L. serrata* include observing the sections of *L. serrata* in a cystic space, hyperemia, hemorrhage and severe edema, hemosiderin pigment, lymphocytic necrosis, draining the lymph nodes from lymphocytes, mild fibrosis in the vicinity of *L. serrata* and extensive necrosis focals. Given our knowledge of zoonotic parasites, it is important to carry out further investigations about human infections, and it is also necessary to pay special attention to zoonotic parasitic diseases between dogs and humans. According to the results of this study, with a prevalence rate of 14% in slaughtered sheep, it is concluded that sheep can play an important role in *L. serrata* infection of final host.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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