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■ Infection and pathological lesions of lymph nodes induced by *Linguatula serrata* nymphs in one-humped camels in Iran

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ABSTRACT. *Linguatula serrata* (Pentastomida: Linguatulidae) known tong-worm is a cosmopolitan, zoonotic, and obligate endoparasite. The parasite lives in the nasopharyngeal region of the final hosts, which primarily include dogs and other carnivores. Various herbivores, including, camels serve as the best intermediate hosts for nymph stages. In present study the mesenteric lymph nodes of 101camels were examined for infection to *L. serrata* macroscopically and histopathologically. The infected and normal lymph nodes were processed for histopathology. The results indicated that out of 101 sampled 33 (32.67%) were infected. Macroscopic examination revealed that the infected lymph nodes are swollen and dark, with rubbery consistency, some with subcapsular hemorrhage on cutting. Extensive hemorrhage occurred in various parts of infected lymph nodes. A section of *L. serrata* parasite was observed near one of the hemorrhage centers. Neutrophil count was very high in these centers and giant cells were present around the parasite, indicating granulomatous reaction. Our findings confirmed that different regions of Iran is an endemic for *L. serrata* infections. Because *L. serrata* is a zoonotic parasite, preventive measures should be adopted to disrupt the parasite's life cycle and minimize the risk of infection in both humans and other animals.

Keywords: *Linguatula serrata*, infection, pathology, one-humped camel, Iran

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INTRODUCTION

The phylum Pentastomida consists of approximately 100 species of endoparasites that primarily infect the respiratory tract of vertebrates. These parasites are important in veterinary and human medicine. They are classified into two families, Linguatulidae and Procephalidae, which include the zoonotic parasitic species of the genera *Linguatula* and *Armillifer* (Dawn and Lee, 1981). *Linguatula serrata* known as tongue-worm is a cosmopolitan, zoonotic, and obligate endoparasite. The parasite lives in the nasopharyngeal region of the final hosts, which primarily include dogs, foxes, felines, and other carnivores. Various herbivores, including cattle, camels, buffaloes, sheep, and goats, serve as the best intermediate hosts for nymph stages. Eggs produced by the female parasite exit the primary host through its nasopharyngeal secretions. They are then swallowed by grazing ruminants and hatch into larvae in their small intestine. These larvae must travel to the intermediate host's mesenteric lymph nodes (MLNs), liver, and lungs for further development into infective nymphs (Tajik et al., 2008). The final hosts are infected by the ingestion of viscera of infected herbivores. Similarly, the consumption of contaminated food is the primary source of infection with *L. serrata* in humans. (Siavashi et al., 2002). The parasite causes a syndrome, known as halzoun or Marrara, in humans (Schlossberg, 2004). 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). Extensive research has also evaluated the prevalence of *L. serrata* infection in dogs (Oryan et al., 2008; Rezaei et al., 2011), camels (Haddadzadeh et al., 2009; Radfar et al., 2010; Tajik et al., 2007), buffaloes (Tajik et al., 2008), sheep (Fard et al., 2011; Tavassoli et al., 2007b), cattle (Tajik et al., 2006; Youssefi and Moalem, 2010), and goats (Fard et al., 2010b; Tavassoli et al., 2007a) in different regions of Iran. The current investigation was aimed to determine the infection rate of MLNs and pathological changes induced by *L. serrata* nymphs in camels in different parts of Iran.

MATERIALS AND METHODS

Sampling

A total of 101 camels were randomly selected from a slaughterhouse in Kahrizak (Southwest of Tehran, Iran) during November 2012 to June 2013. The camels had been brought for slaughter from different parts of the country.

Parasitology

The presence or absence of *L. serrata* nymphs in the MLNs of animals was examined; each individual lymph node was cut longitudinally, placed in petri dishes containing normal saline and examined under a dissecting microscope for *L. serrata* nymphs.

Figure 1. Macroscopic presentation of mesenteric lymph nodes infested with nymphs of *Linguatula serrata* after fixation in 10% formalin

- A- Presence of *Linguatula serrata* in a section of the node
- B- Extensive hemorrhage in the node
- C- Cystic spaces around the parasite



(Tavassoli et al., 2010a, Ghorashi et al., 2016). The total number of nymphs per lymph node was recorded as an indicator of infection severity.

Histopathology

After confirmation of infection, ten infected lymph nodes and two healthy specimens from animals were isolated for histopathological analysis. The MLNs transferred to 10% buffer formal saline and processed for histopathology. Paraffin blocks were made; 4-5 micron sections were cut and stained with hematoxylin and eosin. They were examined under light microscope and observations were recorded.

Data analysis

The Chi-Square test (SPSS version 17.0) was used to compare the relative frequency of infection lymph nodes based on color and consistency.

RESULTS

Of the 101 sampled camels, 33 (32.67%) camels showed at least one parasite in the lymph nodes. Totally, 463 lymph node samples, of which 113 (24.4%) samples were, infected with *L. serrata* nymphs. The mean number of parasitic nymphs in MLN was 1.13, with a range of 1-28 nymphs, and a total of 1878 parasites were isolated.

Macroscopic findings:

Macroscopic examination revealed that the infected MLNs are swollen and dark, with rubbery consistency, some with subcapsular haemorrhage on cutting. Infection severity and number of parasites in black and hemorrhagic lymph nodes were significantly higher than those in normal color lymph nodes ($p < 0.05$). When the severely infected nodes were initially isolated, pus discharge along with parasitic nymphs was observed; nymphs 'structures were severely damaged. In some nodes, hemorrhage with abscess and cystic spaces formation as well as calcification was present (Figure 1).

Microscopic findings:

Extensive hemorrhage occurred in various parts of infected MLNs. A section of *L. serrata* parasite was observed near one of the hemorrhage centers. There were small accumulations of pus and abscess formations in multiple areas. Bacterial colonies were also seen in pus and abscess formation locations. Neutrophil count was very high in these centers and giant cells were present around the parasite, indicating granulomatous reaction (Figure 2 and 3), (Table 1).

Infected lymph nodes showed follicular hyperplasia with active germinal centers containing numerous mitotic figures (Figure 4).

In addition, the average number of lymph node

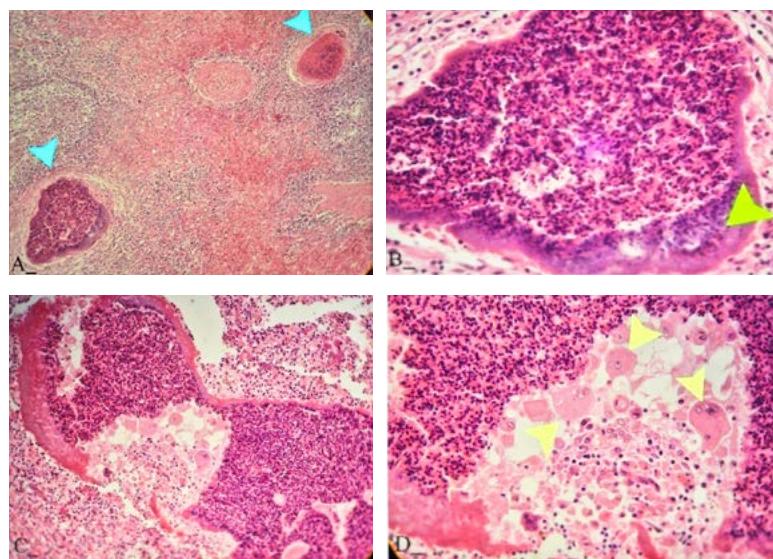


Figure 2. A microabscess in the node and the hemorrhage around it (100 \times , H&E staining)

B microabscess in the node and purple-colored colonies of bacteria around it (400 \times , H&E staining)

C-An abscess and granulomatous formations and bacterial colonies around it (200 \times , H&E staining)

D-Presence of giant cells in granulomatous formations (400 \times , H&E staining)

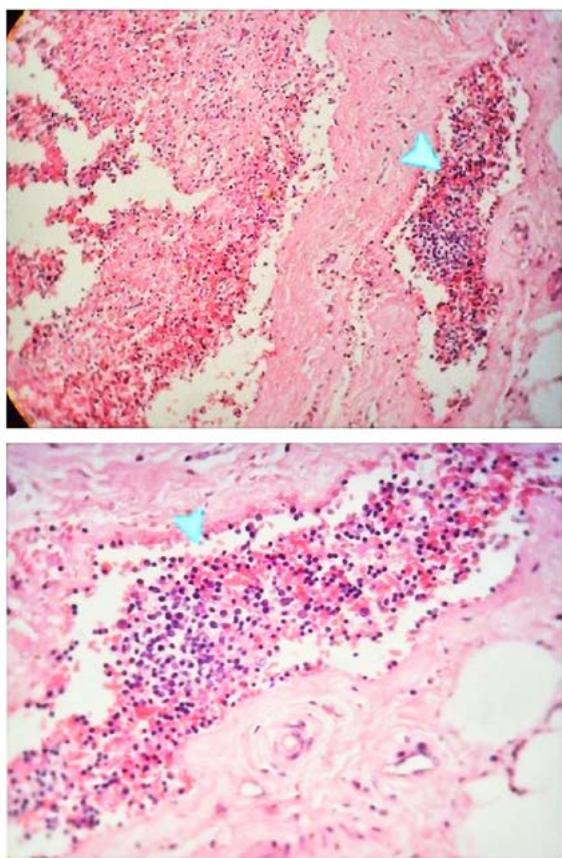


Figure 3. A cross section of a vein around the infected lymph nodes. Note the abundance of inflammatory cells compared to red blood cells (40 \times and 400 \times , H&E staining)

follicles from healthy camels at random microscopic levels ranged from 13 to 22, whereas the average number of follicles in the infected nodes ranged from 62 to 77.

DISCUSSION

Linguatulosis is an important disease concerning both veterinary and public health medicine in the world, including Iran. As an intermediate host, one- or two-humped camel, similar to other ruminants, may play a vital role in the life cycle of *L. serrata*. As the camels are mostly kept freely in the pastures, they are highly susceptible to persistent infection and its dissemination (Tajik et al., 2007). Sporadic reports of human linguatulosis have been published in Iran and other countries (Maleky, 2001; Oluwasina et al., 2014; Ravindran et al., 2008; Yazdani et al.,

2014). Human infection is caused by the ingestion of the immature stage 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 that is 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 (Yazdani et al., 2014). Previous studies in Iran have reported the prevalence rates of *L. serrata* infection as 27.8–76.5% in dogs (Meshgi and Asgarian, 2003; Rezaei et al., 2011), 19–68% in goats (Dehkordi et al., 2014; Rezaei et al., 2012; Youssefi et al., 2012), 10.2–52.5% in sheep (Dehkordi et al., 2014; Fard et al., 2011; Tavassoli et al., 2007b; Youssefi et al., 2012), and 14.8–69.1% in cattle (Alborzi et al., 2013; Fard et al., 2010a; Nematollahi et al., 2015; Rezaei et al., 2011; Tajik et al., 2006; Youssefi et al., 2012; Youssefi and Moalem, 2010). In India, infection rates have been reported to be 21% in goats and 19% in cattle (Ravindran et al., 2008). In Turkey, the infection rate was reported to be 5.4% in sheep (Aydenizoz et al., 2012). In general, MLNs are one of the first body parts to be infected with *L. serrata*. They also have a higher risk of infection compared to visceral organs (Dehkordi et al., 2014; Rezaei et al., 2011). In this study, 32.67% of the camels had *L. serrata* nymphs in their MLNs. Wahba et al. (1997) reported that *L. serrata* nymphs were found in the lymph nodes of three camels (Wahba et al., 1997). In another study, 12.5% of camels in Shiraz were infected with nymphal stage of *L. serrata* (Oryan et al., 1993). The occurrence of *L. serrata* nymphs in the left lobe of the lung of a two-humped male camel was previously reported in Tabriz, Iran (Haddadzadeh et al., 2009). In another study with slaughtered camels, Tajik et al. (2007) showed that MLNs, lungs, and the liver were infected with *L. serrata* nymphs in 75%, 29.7%, and 30.4% of animals, respectively. Shakerian et al. (2008) reported that MLNs (21%) and liver (4.5%) were infected with the infective stage of these parasites in Najaf Abad, Iran.

The prevalence of infection in camels may be influenced by several factors such as the geographic and climatic variations that affect the survival of the parasite eggs. The prevalence rate (32.67%) of infection in the lymph nodes should be considered as a risk factor for infection in humans.

Data regarding the pathology of lymph nodes

Figure 4. A- Follicular hyperplasia in nodes infected with the parasitic nymphs and their expansion to medulla of the node (40 \times , H&E staining)

B- Normal node, the number, size, and distribution of its follicles (100 \times , H&E staining)

C- Abundant mitotic cells in follicle centers of lymph nodes (400 \times , H&E staining)

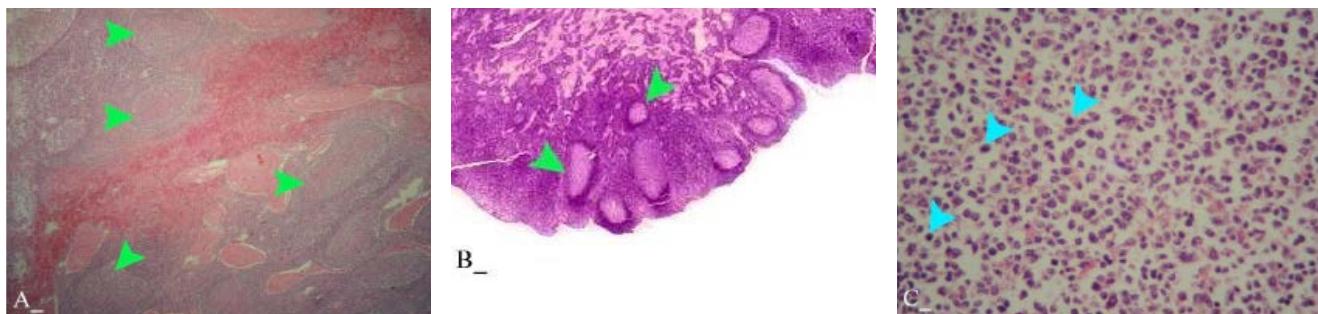


Table 1. Mean number and percentage of counted immune cells in mesenteric lymph nodes of camel in 14 random microscopic fields of destroyed space around parasites

		Neutrophil	Plasma cell	Macrophage	Lymphocyte
Camel	Mean (number \pm SD)	18 \pm 2.7	28 \pm 3.4	23 \pm 3.2	102 \pm 31.1
	Mean (percent \pm SD)	10.65 \pm 2.9	16.36 \pm 6.4	13.48 \pm 5.7	59.49 \pm 13.2

infected with *L. Serrata* nymph in camel are limited. In the present study, examination of MLNs exhibited hemorrhage, severe edema, swelling and black discoloration. Additionally, some of them showed subcapsular hemorrhage compared to healthy lymph nodes; Infection severity and the number of parasites in black and hemorrhagic lymph nodes were significantly higher than those in normal looking nodes ($p < 0.05$).

In this study, microscopic lesions of the lymph nodes infected with *L. Serrata* showed areas of pus accumulation and multiple small abscesses formations with extensive hemorrhage. Foreign body type granulomatous reaction was also observed. Follicular hyperplasia was also evident. Previous studies on the macroscopic appearance of nodes infected with parasitic nymphs have also reported enlarged lymph nodes with loose consistency and black discoloration as well as abscess formation, hemorrhagic and necrosis(Micăluș et al., 2008; Sivakumar et al., 2005).

CONCLUSION

These findings are consistent with results of the present study. Based on our knowledge of zoonotic parasites, geographical limitations, close contact between dogs and camel, and regular migration of nomads and their livestock and dogs (fed on uncooked offal of the ruminants) might have contributed to the high infection rate in this area. In fact, such factors facilitate constant contact between the final host and camel. Our findings confirmed that in different regions of Iran is an endemic for *L. serrata* infections. Since *L. serrata* is a zoonotic parasite, preventive measures should be adopted to disrupt the parasite's life cycle and minimize the risk of infection in both humans and other animals.

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CONFLICT OF INTEREST STATEMENT

We declare that there is no conflict of interests.

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