Prevalence of Linguatula Serrata Infection in Domestic Ruminants in West Part of Iran: Risk Factors and Public Health Implications

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Abstract
Linguatulosis is one of the neglected parasitic zoonoses. Domestic ruminants are the important source for human infection. The main goal of the present investigation was to evaluate the prevalence of nymphal stages of L. serrata in domestic ruminant in Hamedan province, western Iran. The mesenteric and mediastinal lymph node of 1080 animals were taken randomly from different slaughterhouses of Hamedan province. All lymph nodes were examined for nymphal stage of L. serrata using parasitological methods (digestion technique). The nymph of L. serrata was found in 31.4% (69/220) of goats, 15% (60/400) of sheep, and 7.4% (34/460) of cattle (P=0.457). The infection rate in beef cattle, dairy cattle and native cattle was 1.3%, 6.4% and 12.6%, respectively (P=0.981). No evidence of correlation between gender, age groups, and L. serrata infection rate in sheep and cattle (P>0.05). In goats, the infection rate in >2yr old (48%) was higher than ≤2yr old (8.6%) (P<0.0001, OR=5.6); also the infection rate was 23.7% and 35.7% in male and female goats, respectively (P=0.065). This is the first report of linguatulosis in domestic animals from western Iran. The results indicate that linguatulosis may partly be responsible for economic losses in domestic ruminant husbandry in this region. Therefore, designing control strategies for a better management in animals farm to reduce the risk of zoonotic outbreaks is highly recommended.

Introduction
Linguatulosis caused by Linguatula serrata (Pentastomida: Linguatulidae) which is entitled “tong-worm” is a cosmopolitan, zoonotic infection (Sadeghi-Dehkordi et al., 2014; Yazdani et al., 2014). The adult parasite is found in the nasal airway, frontal sinus and tympanic cavity of dogs and cats (Rezaei et al., 2011). Sheep, goats, cattle, or rodents play the role of intermediate hosts in which visceral linguatulosis has been described (Oluwasina et al., 2014). The larval stages of the parasite develop in mesenteric lymph nodes (MLNs), liver or lungs. Final hosts are infested by eating infected organs. Expelled eggs from the respiratory passages of final host (by coughing or sneezing) are ingested by herbivorous, the intermediate host (Aydenizoz et al., 2012).

On histopathological examinations, chronic parasitic granuloma is the major finding in infected MLNs and surrounded by infiltration of mononuclear fibrosis. Also, there are necrotic areas in lymphoid tissue with reduced cellularity in lymphoid follicles. The visceral form of infection usually remains asymptomatic; pharyngitis and coughing was reported in the nasopharyngeal form (Aydenizoz et al., 2012).

There is no clinical and pathological importance in domestic animals; but these animals are important for human infection and other public health implications (Ravindran et al., 2008; Nourollahi Fard et al., 2010; Yazdani et al., 2014). Humans may be infected by the ingestion of nymphs of L. serrata (an accidental final host: nasopharyngeal linguatulosis or Halzoun syndrome) or by the consumption of infective eggs (intermediate host: visceral linguatulosis). Close contact to dogs and their secretions predispose to infection with L. serrata (Nourollahi Fard et al., 2010; Oluwasina et al., 2014).

The epidemiology of L. serrata infection in man is complicated because both eggs and infective larvae can become established. Eggs hatch in the alimentary tract
and primary larvae subsequently invade the body cavity to encyst on the viscera, producing visceral linguatulosis, whereas ingested infective larvae attempt to migrate to the nasal passages, producing nasopharyngeal linguatulosis. People may suffer from irritation in their nose and throat. Deaths have been reported due to blocked air passages (Yazdani et al., 2014).

In the previous researches from Iran, the \textit{L. serrata} infection was reported in different animals, such as dogs (Rezaei et al., 2011), camels (Haddadzadeh et al., 2009), buffaloes (Alborzi et al., 2013), sheep (Nourrollahi Fard et al., 2011), goats (Rezaei et al., 2012), and cattle (Tajik et al., 2006). Also, linguatulosis in humans has recently been reported in Kerman province, Southeastern Iran (Yazdani et al., 2014).

The current investigation was aimed to determine the mesenteric and mediastinal lymph node infection with \textit{L. serrata} nymphs in cattle, sheep and goats in Hamedan province, west part of Iran.

### Materials and Methods

#### Study Area
Hamedan is a mountainous province with mild climate that is located in west part of Iran (19,546 km$^2$: 34° 49’ 11” N, 48° 40’ 15” E). The mean annual rainfall and temperature is 317.7 mm and 11.3°C, respectively. The economy of this region is mainly based on agriculture and farm animal industry, including sheep, goats, and cattle.

#### Sampling
A cross-sectional study was conducted in 2013. The mesenteric and mediastinal lymph node of 400 sheep, 220 goats and 460 cattle (152 beef cattle, 110 dairy cattle and 198 native cattle) were sampled randomly from different slaughterhouses of Hamedan province (Table 1, 2). The animals were categorized into two age groups (≤2 and >2 years old in sheep and goats, ≤4 and >4 years old in cattle).

### Table 1. Prevalence of \textit{Linguatula serrata} infection in sheep and goats in different gender and age groups.

<table>
<thead>
<tr>
<th>Animals</th>
<th>Gender</th>
<th>Age groups</th>
<th>Total</th>
<th>CI 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>≤2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Sheep</td>
<td>No. of sample</td>
<td>216</td>
<td>184</td>
<td>(\chi^2=1.023)</td>
</tr>
<tr>
<td></td>
<td>No. of positive</td>
<td>36</td>
<td>24</td>
<td>(P=0.311)</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(16.7)</td>
<td>(13)</td>
<td>(Df=1)</td>
</tr>
<tr>
<td>Goat</td>
<td>No. of sample</td>
<td>80</td>
<td>140</td>
<td>(\chi^2=3.385)</td>
</tr>
<tr>
<td></td>
<td>No. of positive</td>
<td>19</td>
<td>50</td>
<td>(P=0.065)</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(23.7)</td>
<td>(35.7)</td>
<td>(Df=1)</td>
</tr>
</tbody>
</table>

\(\chi^2=\text{Chi-square test}; \ OR=\text{Odds ratios}; \ CI=\text{confidence interval}; \ Df=\text{degree of freedom}; \ *\text{Statistical analysis.}\)

### Table 2. Prevalence of \textit{Linguatula serrata} infection in cattle in different gender and age groups.

<table>
<thead>
<tr>
<th>Type of animals</th>
<th>Gender</th>
<th>Age groups</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>≤4</td>
</tr>
<tr>
<td>Beef cattle</td>
<td>No. of sample</td>
<td>152</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>No. of positive</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(1.3)</td>
<td>(0)</td>
</tr>
<tr>
<td>Dairy cattle</td>
<td>No. of sample</td>
<td>0</td>
<td>110</td>
</tr>
<tr>
<td></td>
<td>No. of positive</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(0)</td>
<td>(6.4)</td>
</tr>
<tr>
<td>Native cattle</td>
<td>No. of sample</td>
<td>43</td>
<td>155</td>
</tr>
<tr>
<td></td>
<td>No. of positive</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(25.6)</td>
<td>(9)</td>
</tr>
<tr>
<td>Total</td>
<td>No. of sample</td>
<td>195</td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>No. of positive (%)</td>
<td>13</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>(%)</td>
<td>(6.7)</td>
<td>(7.9)</td>
</tr>
</tbody>
</table>

\(\chi^2=\text{Chi-square test}; \ OR=\text{Odds ratios}; \ CI=\text{confidence interval}; \ Df=\text{degree of freedom}; \ *\text{Statistical analysis.}\)
Parasitology

The mesenteric and mediastinal lymph node of animals were examined for the presence of *L. serrata* nymphal stage. Firstly, the adipose tissue around the lymph nodes was removed. The lymph nodes were then cut longitudinally into small pieces and immersed in tepid normal saline (0.9% NaCl) solution and left for 5-6 h to allow nymphs to come out from the tissues. Recovered nymphs were flattened, dehydrated in ascending grades of ethyl alcohol and cleared in creosote before examining under a stereomicroscope. The negative samples were digested in 200 ml of digestion solution (5 g of pepsin, 25 ml HCl in 1000 ml distilled water) and incubated at 37°C for 24 h. The digestion solution (5 g of pepsin, 25 ml HCl in 1000 ml distilled water) and incubated at 37°C for 24 h. The samples were then examined for *L. serrata* nymphal stage again as described previously (Nourollahi Fard et al., 2010; Ravindran et al., 2008; Rezaei et al., 2011; Sadeghi-Dehkordi et al., 2014). In our study, MLNs was evaluated because they are one of the most target organs of infection with this parasite. Also, the possibility of infection in MLNs is higher than other visceral organs (Rezaei et al., 2011; Sadeghi-Dehkordi et al., 2014). In Iran, *L. serrata* infection rate was reported 19-68% in goats, 10.2-52.5% in sheep and 14.8-69.1% in cattle (Alborzi et al., 2013; Nematollahi et al., 2015; Nourollahi Fard et al., 2010; Nourollahi Fard et al., 2011; Rezaei et al., 2011, Rezaei et al., 2012; Sadeghi-Dehkordi et al., 2014; Tajik et al., 2006; Tavassoli et al., 2007; Youssefi and Hadizadehmoalem, 2010; Youssefi et al., 2012).

The infection rate has been reported as 21% and 19% in goats and cattle, respectively in India (Ravindran et al., 2008), and 5.4% in sheep in Turkey (Aydenizoz et al., 2012). However, in our study the infection rates were 31.4% in goats, 15% in sheep and 7.4% in cattle; no significant differences were found between infection rate and species of animals. The infection rate in native cattle (12.6%) was higher than dairy cattle (6.4%) and beef cattle (1.3%). This might be due to the differences in breeding system of animals that increased the chance of contact with dogs. Sensitivity of some species and goats’ feeding style might have affected on the rate of the infection. On the other hand, goats grazing ahead of the flocks might be another reason to have increased risk of infection (Sadeghi-Dehkordi et al., 2014). The high prevalence of linguatulosis in dogs and ruminants may play an important role in epidemiology of human and animals linguatulosis (Meshgi and Asgarian, 2003).

In the present study, there was no significant relationship between gender and infection rate in animals. Nourollahi Fard et al. (2010) and Sadeghi-Dehkordi et al. (2014) reported that the prevalence of *L. serrata* nymphs in females was significantly higher than that of males (P<0.05). It is probably due to the fact that those animals live longer before being slaughtered. Also, the most of management system is traditional in sheep and goats in Iran. Moreover, there are many reports that did not show significant correlation between infection rate and gender, parallel to our finding (Nourollahi Fard et al., 2010, 2011).

In current work, no significant differences was found between infection rate and age groups in sheep and cattle (P>0.05, Table 1, 2). But, the infection rate in goats of > 2yr old (48%) was significantly higher than 2yr old (8.6%) (P<0.0001, OR=5.6, Table 1); this rate was 23.7% in male and 35.7% in female goats (P=0.065).

Discussion

Linguatulosis is a widespread parasitic zoonosis. The prevalence of linguatulosis in dogs has been reported between 27.8 and 76.5% in different regions of Iran (Meshgi and Asgarian, 2003; Rezaei et al., 2011). Some studies were performed on the prevalence of linguatulosis in domestic ruminants in Iran and other regions of world such as liver, lung and spleen (Nourollahi Fard et al., 2011; Rezaei et al., 2011; Sadeghi-Dehkordi et al., 2014). In our study, MLNs was evaluated because they are one of the most target organs of infection with this parasite. Also, the possibility of infection in MLNs is higher than other
sampling, climatic variations in studied regions, management and hygienic measures of farms (intensive/extensive or stray dogs’ management).

Human linguatulosis with sporadically distribution have been recorded in Africa, Europe, USA and Iran (Oluwasina et al., 2014; Ravindran et al., 2008; Yazdani et al., 2014). Some locals also believe that eating the raw or undercooked offals, especially liver of farm animals (cattle, goats or sheep), is a useful means to promote the fetus growth during pregnancy because of its high content of iron and vitamins. In the Middle East, Halzoun also occurs after religious feasts in which uncooked sheep or goats may be served (Oluwasina et al., 2014; Sadeghi-Dehkordi et al., 2014).

Physicians should be aware and consider L. serrata infection in patients with complaints of upper respiratory tract symptoms, especially in endemic regions, where humans consume raw or uncooked liver or when they are in close contact with domestic and home-reared animals.

In conclusion, this is the first report of linguatulosis in domestic animals from western Iran. The results indicate that linguatulosis may partly be responsible for economic losses in domestic ruminant husbandry in this region. Therefore, designing control strategies for a better management in animal farms to reduce the risk of zoonotic outbreaks is highly recommended.

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