Original Article

Seroprevalence of *Linguatula serrata* infection among sheep in Fars province, south of Iran

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Summary

Linguatula serrata is an important zoonotic parasite at a global scale. The epidemiological role of sheep in transmission of linguatulosis has recently been demonstrated, but there is still a lack of information on the subject. The aim of the present study was to evaluate the occurrence and seroprevalence of *L. serrata* infection among sheep in Fars province, south of Iran, from December 2014 to September 2015. Blood samples were collected from 180 sheep in Shiraz abattoir. The antibody detection against *L. serrata* was made by counter immunoelectrophoresis (CIEP). Specific antibodies against *L. serrata* were detected in 84 (46.66%) out of 180 ovine sera. Out of 38 males, 21 under 1 year old (55.26 %) and out of 81 males, 36 older than 1 year (44.44%) were infected with nymphs. Fifteen out of 30 females under 1 year old (50%) and 12 out of 31 females above 1 year old (38.7%) were infected with nymphs. The age and the sex of infected sheep showed no significant differences between positive and negative cases ($P \leq 0.05$). The results of this study showed the presence of *L. serrate* among sheep in Iran, which could be a public health concern. According to the relatively high prevalence of *L. serrata* infection in sheep, implementation of control measures to reduce infection in both definitive and intermediate hosts are needed.

Key words: Fars, *Linguatula serrata*, Sheep, seroprevalence, counter immune electrophoresis (CIE).

Introduction

Linguatulosis is a zoonotic disease caused by *Linguatula serrata*, a cosmopolitan parasite, from phylum Pentastomida (Gosling, 2005; Muller, 2002). The adult parasite infects the nasal sinuses and nasopharynx of canine (final host) (Muller, 2002; Bowman et al., 2004). Eggs containing fully developed larvae are discharged into the environment by nasal secretion and ingested by intermediate hosts (herbivorous animal). The larvae then migrate into the mesenteric lymph nodes (MLNs), liver and lung where they develope into infective nymphs (Tajik et al., 2008). In the final host, *L. serrata* causes catarrhal inflammation of the respiratory tract (Bowman et al., 2004). Linguatulosis in human can be caused by either the egg (visceral form) or nymph stage of the parasite (nasopharyngeal form) (Khalil et al., 1965).

Nasopharyngeal linguatulosis, known as Halzoun or Marrara syndrome in human, often occurs after consumption of the raw or undercooked viscera (liver, lung and lymph nodes) of infected animals. The syndrome has been reported in many countries including Africa, South-East Asia and the Middle East (Beaver et al.,

1984; Drabick et al., 1987; EL-Hassan et al., 1991). The prevalence of *L. serrate* has been studied in various animals in Iran, including dogs (Meshgi et al., 2003; Oryan et al., 2008; Rezaei et al., 2011), sheep (Shekarforoush et al., 2004; Nourollahifard et al., 2011), goats (Razavi et al., 2004), and cattle (Hami et al., 2009; Tajik et al., 2011). There are also some reports of human linguatulosis in Iran (Sadjjadi et al., 1998; Maleki et al., 2001; Anaraki mohammadi et al., 2008; Tabibian et al., 2008).

The epidemiological role of sheep in linguatulosis has recently been demonstrated, but there is still a lack of information on the subject. Since sheep may serve as a possible reservoir of infection for human beings, the public health implications of these results are quite significant. Some researchers described clinical signs of the syndrome including pharyngitis, salivation, dysphagia and coughing. Visceral linguatulosis generally remains asymptomatic (Khalil et al., 1965). Since clinical symptoms and macroscopic examinations are variable, the diagnosis often relies on serological tests (Mehlborn, 2008). Serological tests are a major component of the success in any surveillance system. For this purpose, the antibody against *L. serrate* infection was determined using counter immunoelectrophoresis (CIE). Considering the importance of linguatulosis for both animal and public health, the aim of the study described here was to evaluate the occurrence and seroprevalence of *L. serrate* infection among sheep in Fars province, South of Iran. This study is the first serological survey on linguatulosis in sheep from this region of the country. Since sheep may serve as a potential source of infection for human beings, the public health implications of these results are quite significant.

Materials and Methods

Sampling

A total of 180 blood samples were randomly collected from sheep in Shiraz abattoir from December 2014 to September 2015. After clotting the samples at room temperature, the sera were separated and stored at -20°C until testing for the presence of antibodies against *L. serrata*.

Preparation of somatic antigen

Nymphs of the parasite were obtained from mesenteric lymph nodes of infected sheep and used for preparation of somatic antigens by sonication (250 nymphs/10ml, PBS-RPMI1640). Freshly collected nymphs were washed 3-4 times in PBS and then sonicated by an ultrasound processor with pulse-type vibration for 2/5min and 10 pulses of 15sec under chilled condition (Charley et al., 1965). Finally, the suspension was centrifuged at 2500 x g and 4ºC for 5 min. The supernatant was collected (Alborzi et al., 2015). The protein concentration was estimated (160µg/ml), (Bradford 1978). The prepared antigen was stored in small aliquots at -80°C until used.

Serological screening

Counter immunoelectrophoresis (CIEP) was performed to detect the antibody against *Linguatula serrata*. Wells 5 mm in diameter were cut 1 cm apart in 1% agarose in barbiturate buffer (pH 9/5) gel in parallel rows on the right and the left side of the slide. Ten microliters of

antiserum and the same volume of the antigen (sample) were applied into the wells on the anode and the cathode sides, respectively. Protein migrated under 6 mA through the agarose gel for 2 h in an electrophoresis chamber containing barbital buffer at pH 9/5. The slide was connected to the electrolyte with a filter paper bridge. The current was then switched off and the slide was examined for precipitin lines. Development of precipitation band was considered positive in comparison with the positive control (serum containing of *L. serrata* antibody) in which there was no reaction between antigen and negative serum (negative control, serum without *L. serrata* antibody) (Alborzi et al., 2015; Arafa et al., 1999) (Figure1). The optimum test conditions were standardized with the help of positive and negative sera samples.

Statistical analysis

Data were analyzed using SPSS (version 16; SPSS). For all analyses, a *p*-value less than 0.05 was considered as significant.

Results

The results of serological examination confirmed the presence of antibodies against *L. serrata*. The prevalence of *L. serrata* antibodies in examined sheep from southwest of Iran was high. Out of the 180 sera samples screened with CIEP method, 84 (46.66%) had antibodies against *L. serrata.* Twenty one out of 38 males under 1 year old (55.26 %) and 36 out of 81 males older than 1 year (44.44%) were infected with nymphs. Fifteen out of 30 females under 1 year old (50%) and 12 out of 31 females above 1 year old (38.7%) were infected with nymphs (Table 1). There were no significant differences between the prevalence of positive cases regarding age and sex ($p \leq 0.05$) (Table 1).

Table 1. Seroprevalence of *Linguatula serrata* infection in blood sera of male and female sheep in Fars province, south of Iran**.**

Gender	(age)	Seropositive	Negative
Male	(<1Year)	21 (55.26%)	17 (44.73%)
	(>1Year)	36 (44.44%)	45 (55.55%)
Female	(<1Year)	15 (50%)	15 (50%)
	(>1Year)	12 (38.7%)	19 (61.29%)
Total		84 (46.66%)	96 (53.33%)

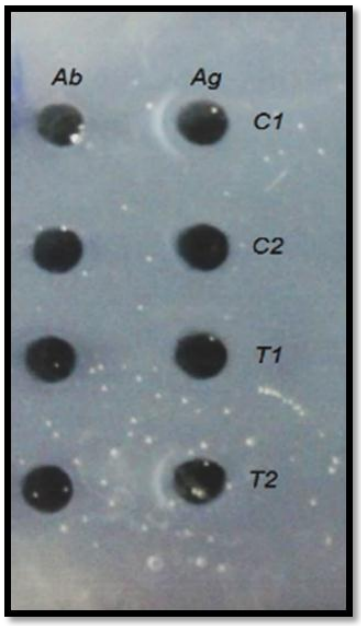

Fig. 1. Counter immune electerophoresis of sheep serum for *L. serrata* antibody in 1% agar gel: C1. Positive Control, C2. Negative Control, T1. Test serum sample (Negative), T2. Test serum sample (Positive).

Discussion

Results of the present study provided an evidence of exposure to *L. serrata* of sheep in South Iran. Based on the literature, no other serological studies have been conducted on linguatulosis in sheep in Iran. Canine are definitive hosts and the

herbivores such as sheep, goat, cattle and camel act as intermediate host in life cycle of *L. serrata*. Almost all intermediate hosts do not show any clinical signs, and the diagnosis is usually performed in postmortem examination or in the slaughterhouse (Tappe and Buttner, 2009). Reliable serological assay, which enables the detection of subclinical infection, has paramount importance (Almazan et al., 2001). In this study, the detection of antibodies against *L. serrata* among sheep was made by CIE using somatic antigens isolated from nymphs. The results showed that 84 out of 180 (46.66%) sheep was exposed to *L. serrate*. The prevalence of *L. serrata* infection in different animal species was studied based on abattoir survey in Iran by many researchers. The prevalence rates in sheep were reported to be 11.5% and 3% in the mesenteric lymph nodes and liver of sheep, respectively in Shiraz, Fars province (Shekarforoush et al., 2004). The present seroprevalence in sheep was higher than that reported in Shiraz, Fars province. In goat, the prevalence rates were reported to be 29.9% and 6.4% in MLNs and liver, respectively in Shiraz. This rate in cattle was 44% and 0.25% in Uremia and Tabriz, respectively (Razavi et al., 2004; Tabibian et al., 2012). The prevalence of adult stage has also been reported from North West (27.83%) and central part of Iran (62.2%), and from stray dogs in Shiraz, south of Iran (76.5%) (Meshgi et al., 2003; Oryan et al., 2008; Rezaei et al., 2011). It seems that the parasite is common among herbivores and canine in Iran. The Halzoun syndrome was reported from Tabriz, Fars, Tehran, and Isfahan (Montazeri et al., 1997; Sadjjadi et al., 1998; Maleki et al., 2001; Anaraki Mohammadi et al., 2008; Tabibian et al., 2008). All of these reports revealed a high rate of infection in both definit and intermediate hosts in different parts of Iran. The parasite has been reported from human beings in different areas of the world such as Morocco and Turkey (Lecorroller and Pierre, 1952; Yilmaz et al., 2011). In the present study, the presence of *L. serrata* infection in sheep with a high prevalence rate of 46.66% was evident. In conclusion, the presence of *L. serrata* antibodies in sheep sera is a public health concern, since linguatulosis is a zoonotic disease and sheep can be hazardous for public health. Linguatulosis should be considered as a public health issue for both humans and animals in Iran. In areas like Iran, where human cases of linguatulosis are not common or remain unreported, the public health implications of linguatulosis seroprevalence in sheep are quite significant. This suggests that a control program should be urgently adopted for epidemiological surveillance, and the serological test may be an important component of any control program.

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