

## Prevalence of *Linguatula serrata* infection among dogs (definitive host) and domestic ruminants (intermediate host) in the North West of Iran

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**ABSTRACT:** *Linguatula serrata* is a zoonotic parasite causing visceral and nasopharyngeal linguatulososis in humans. Dog and other canines are the main definitive hosts while most herbivores, including ruminants serve as intermediate hosts for linguatulososis. Human rarely become infected as both final and intermediate hosts. This survey aimed to assess the *L. serrata* infection rate of dogs and domestic ruminants in North West of Iran. The upper respiratory tract of 97 dogs including 45 females and 52 males and the mesenteric lymph nodes (MLNs) of 396 goats (203 females and 193 males), 406 buffaloes (166 females and 240 males), 421 cattle (209 females and 212 males) and 438 sheep (223 females and 215 males) were examined for *L. serrata*. Animals were categorized into four age groups, including under six months, six to 24 months, two to four years and more than four years. Results showed that 27.83% of dogs were infected with *L. serrata*. 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 ( $P \leq 0.05$ ). In ruminants, the highest infection rate was found in goats ( $P \leq 0.05$ ). The results from this study indicate a high rate of infection in dogs and domestic ruminants, suggesting a potential high risk of zoonotic infection in man in the investigated area.

**Keywords:** *Linguatula serrata*; dog; domestic ruminants; Iran

The phylum Pentastomida contains a highly specialized taxon with more than 120 species which infect the respiratory tracts of reptiles and other vertebrates (Riley, 1994; Riley and Henderson, 1999; Mehlborn, 2008). *Linguatula serrata*, a zoonotic and obligate endoparasite, is a member of phylum Pentastomida (Gosling, 2005; Fard et al., 2010b). The adult form of this parasite is found in the nasal airway, frontal sinus and tympanic cavity of dogs and cats (Muller, 2002; Bowman et al., 2004; Alcalá-Canto et al., 2007).

Discharged eggs from the definitive host's nasopharyngeal secretion are ingested by the grazing herbivores where they hatch into larvae. The larvae then migrate into the mesenteric lymph nodes (MLNs), liver and lung, where they develop into infective nymphs (Tajik et al., 2008). In the final host, *L. serrata* causes catarrhal inflammation of

the respiratory tract (Bowman et al., 2004). The pathogenesis of linguatulososis is not clear, but it has been shown that proteolytic enzymes may have a role in it (Alcalá-Canto et al., 2007). Clinical signs associated with infections in intermediate hosts have not yet been described carefully (Mehlborn, 2008). Linguatulososis in man can be caused by either the egg (visceral form) or nymph stage of the parasite (nasopharyngeal form) (Khalil and Schacher, 1965; Tavassoli et al., 2007a). Nasopharyngeal linguatulososis, known as Halzoun or Marrara syndrome, often occurs after consumption of the raw or undercooked viscera (liver, lung and lymph nodes) of infected animals (Beaver et al., 1984; Drabick, 1987; El-Hassan et al., 1991). On rare occasions, human become infected as definitive hosts (Symmers and Valteris, 1950; Mehlborn, 2008; Oryan et al., 2008). Linguatulososis in humans has been reported from

Africa, Southeast Asia, America and the Middle East (Gardiner et al., 1984; Barid et al., 1988; El-Hassan et al., 1991; Yagi et al., 1996; Lazo et al., 1999; Acha and Szyfres, 2003). Several reports have documented nasopharyngeal linguatulosis in Iran following consumption of liver kebab (barbecue) (Hodjati and Naghili, 1987; Fata et al., 1994; Sadjjadi et al., 1998). A 28-year-old woman affected with linguatulosis and with respiratory symptoms, including sneezing and nasal discharge, representing the clinical signs of Marrara was described in Iran (Maleky, 2001). Human nasopharyngeal linguatulosis was also reported from the Van Province of Turkey close to the North Western region of Iran (Yilmaz et al., 2011). Several other studies have assessed the prevalence of *L. serrata* in dogs (Khalil, 1970; Akyol et al., 1995; Oryan et al., 1997; Meshgi and Asgarian, 2003; Aldemir, 2004; Oryan et al., 2008), one-humped camels (Mobedi et al., 1988; Oryan et al., 1993; Tajik et al., 2007; Haddadzadeh et al., 2010; Radfar et al., 2010), two-humped camels (Haddadzadeh et al., 2009), buffaloes (Sisakumar et al., 2005; Tajik et al., 2008; Tajik and Jalali, 2010), sheep (Esmail-Nia et al., 2000; Shekarforoush et al., 2004; Tavassoli et al., 2007a; Ravindran et al., 2008; Gul et al., 2009; Fard et al., 2011), cattle (Tajik et al., 2006; Hami et al., 2009; Fard et al., 2010b; Youssef and Hadzede-Moalem, 2010), goats (Saiyari et al., 1996; Razavi et al., 2004; Tavassoli et al., 2007b; Mir et al., 2009; Fard et al., 2010a) and small ruminants (Vatsya et al., 2011). In addition, adult and nymph stages of the parasite have been isolated from red foxes (Gicik et al., 2009).

This study was aimed at determining the prevalence of *L. serrata* infection in the upper respiratory tract of dogs and the nymphal stage of the parasite in MLNs of domestic ruminants, including goats, buffaloes, cattle and sheep slaughtered in North Western Iran. Also, the correlation between infection in final and intermediate hosts of different ages and sexes was investigated.

## MATERIAL AND METHODS

To determine the prevalence of *L. serrata* infection in various residential areas of the North West of Iran, 97 stray dogs, including 45 females and 52 males were examined from August 2009 to September 2010. After determining sex, dogs were divided into four age groups (under six months, six

to 24 months, two to four years and more than four years) as described previously (Getty, 1975). Then, dogs were euthanized using Pentobarbital (20% solution) 150 mg/kg and according to a research proposal approved by institutional animal ethics committee. The bodies were transferred to an autopsy room and after dissecting the skin from the underlying bones, the skull was cleaved using a thin bladder saw. Then, the nasopharynx area, nasal turbinate's, paranasal sinuses, Eustachian tubes, trachea, and brain were examined for the adult form of *L. serrata*. The parasites were collected in PBS and transferred to the parasitology laboratory at the Veterinary Faculty of Urmia University for further studies.

The MLNs from 396 goats (203 females and 193 males), 406 buffaloes (166 females and 240 males), 438 sheep (223 females and 215 males) and 421 cattle (209 females and 212 males) were examined for *L. serrata* nymphs between March 2009 and October 2010. After determining sex, each ruminant species was divided into four age groups (under six months, six to 24 months, two to four years and more than four years) using the eruption of permanent incisor teeth criterion as described elsewhere (Curasson, 1947). At least three MLNs from each animal were collected in PBS and transferred to the parasitology laboratory. Lymph nodes were cut longitudinally under a dissecting microscope and were examined for *L. serrata* nymphs. The number of collected nymphs was recorded.

## Statistical analysis

Significance of differences of relative frequency of infection in different age groups and sexes were statically analysed using Chi Square and Backward elimination tests (SPSS version 17.0). Data with *P*-values equal to or less than 0.05 were considered significant.

## RESULTS

*L. serrata* nymphs were found in the MLNs of 201 goats (50.75%), 108 buffaloes (26.6%), 187 sheep (42.69%) and 150 cattle (36.6%). For goats, the number of nymphs collected from each infected lymph node varied from 1 to 325. The ranges for buffaloes, sheep and cattle were 1–23, 1–49 and 1–28, respectively.

Table 1. Prevalence rate of *L. serrata* (adult form) in different sexes and age groups of dogs

| Age group         | Total number | Number        |             | Positive number |      |
|-------------------|--------------|---------------|-------------|-----------------|------|
|                   |              | female animal | male animal | female          | male |
| Under 6 months    | 15           | 9             | 6           | 0               | 0    |
| 6–24 months       | 23           | 9             | 14          | 2               | 4    |
| 2–4 years         | 19           | 10            | 9           | 3               | 3    |
| More than 4 years | 40           | 17            | 23          | 6               | 9    |

Twenty seven (27.83%) dogs were infected with the adult form of *L. serrata*. In total, 103 *L. serrata* were collected from infected dogs. The number of parasites recovered from each dog ranged from one to 13 with an average of 3.81, of which 75 (72.8%) were from females and the remainder from males. In 16 dogs (59.25%) the parasite was found in the left

side of skull, compared to eight (29.63%) in the right side and three (11.11%) in both sides of the skull.

Tables 1 and 2 show the prevalence rates of infection in different age groups and sexes in each animal species. The correlation between different animal species based on age and sex are listed in Table 3.

Table 2. Prevalence rate of *L. serrata* (nymphal stage) in different sexes and age groups of goats, buffaloes, cattle and sheep

| Age group         | Total number | Number        |             | Positive number |      |
|-------------------|--------------|---------------|-------------|-----------------|------|
|                   |              | female animal | male animal | female          | male |
| Goats             |              |               |             |                 |      |
| Under 6 months    | 43           | 11            | 32          | 0               | 2    |
| 6–24 months       | 93           | 37            | 56          | 13              | 21   |
| 2–4 years         | 119          | 69            | 50          | 40              | 35   |
| More than 4 years | 141          | 86            | 55          | 51              | 39   |
| Buffaloes         |              |               |             |                 |      |
| Under 6 months    | 65           | 22            | 43          | 1               | 0    |
| 6–24 months       | 112          | 37            | 75          | 10              | 19   |
| 2–4 years         | 118          | 47            | 61          | 17              | 20   |
| More than 4 years | 111          | 60            | 51          | 23              | 19   |
| Cattle            |              |               |             |                 |      |
| Under 6 months    | 78           | 23            | 55          | 0               | 1    |
| 6–24 months       | 121          | 32            | 89          | 10              | 25   |
| 2–4 years         | 103          | 56            | 47          | 31              | 24   |
| More than 4 years | 119          | 98            | 21          | 48              | 11   |
| Sheep             |              |               |             |                 |      |
| Under 6 months    | 87           | 36            | 51          | 1               | 2    |
| 6–24 months       | 142          | 64            | 78          | 28              | 35   |
| 2–4 years         | 117          | 66            | 51          | 39              | 28   |
| More than 4 years | 92           | 57            | 35          | 32              | 22   |

Table 3. Infection rate of *L. serrata* in animals of different ages and male and female animals

| Group             | Stray dogs (%) | Goats (%) | Buffaloes (%) | Cattle (%) | Sheep (%) |
|-------------------|----------------|-----------|---------------|------------|-----------|
| <b>Age</b>        |                |           |               |            |           |
| Under 6 months    | 0              | 4.7       | 1.5           | 1.3        | 3.4       |
| 6-24 months       | 26.1           | 36.6      | 25.9          | 28.9       | 44.4      |
| 2-4 years         | 31.6           | 63        | 31.4          | 53.4       | 58.3      |
| More than 4 years | 37.5           | 63.8      | 36.9          | 49.6       | 58.7      |
| <b>Sex</b>        |                |           |               |            |           |
| Male              | 30.8           | 50.3      | 24.2          | 28.8       | 40.5      |
| Female            | 24.4           | 51.2      | 30.7          | 42.6       | 44.8      |

## DISCUSSION

Previously, the prevalence of linguatulosis in dogs from Iran was shown to be 76.2% in Shiraz (Oryan et al., 2008), 65.5% in Shahre-Kord (Meshgi and Asgarian, 2003), and 76.47% in the Fars province (Oryan et al., 1997). In Turkey 20% of dogs were found to be infected with *L. serrata* (Akyol et al., 1995). In the current study, about one-third of dogs were found to be infected with *L. serrata* and the close contact between dogs and livestock explains the greater rates of infection in intermediate hosts (ruminants). Feeding dogs with infected viscera was described to increase the incidence of linguatulosis in dogs (Soulsby, 1982).

Significant differences were found between the rates of infection in dogs of different ages and sexes (Table 1). In puppies under six months, no parasites were found, but with increasing age, the incidence of infection increased significantly and the highest prevalence rate was found in dogs of more than four years of age. These findings contradict the results from another study (Oryan et al., 2008), that indicated no significant difference in the rate of infection among stray dogs with different sexes and ages in Shiraz, Iran. It has been postulated that the absence of linguatulosis in young puppies is associated with their long maturation period (> six months) (Tavassoli et al., 2001) and feeding on mother's milk, which obviates the need to feed them with the infected offal of ruminants (Oryan et al., 2008).

Female dogs showed a lower infection rate in this study. However, no significant difference was found within the age groups between both sexes, which may be linked to the random use of more male animals.

In previous studies the prevalence of *L. serrata* nymphs has been investigated in different visceral organs and lymph nodes, but in the current study, only MLNs from a broad range of animals were examined at the same time, providing a better understanding of the infection status in the investigated area. Independent studies in Urmia, North West of Iran, showed the infection rates of MLN to be 68% in goats (Tavassoli et al., 2007b), 44% in cattle (Tajik et al., 2006), 52.5% in sheep (Tavassoli et al., 2007a) and 18.75% in buffaloes (Tajik et al., 2008). However, in our study the infection rates of different ruminants were determined together and were 50.75%, 26.6%, 36.2% and 42.9%, respectively.

The high prevalence of *L. serrata* in dogs in the North West of Iran can be a major source of infection in intermediate hosts, especially for intermediate ruminants. Similar to what was shown in dogs the results showed that the infection rate was significantly higher in older ruminants, which is related to the long period of time required for nymphs to develop (Razavi et al., 2004; Mehlborn, 2008; Fard et al., 2010b, 2011).

The prevalence of *L. serrata* nymphs in the MLNs of female ruminants was significantly higher than in those of males, which may be a result of the higher mean age of females used in this study.

The prevalence of infection with *L. serrata* nymphs was higher in goats and sheep than in cattle and buffaloes, which may be linked to the close contact between dogs and small ruminants. The high rate of infection of MLNs found in this study was consistent with the results from a previous study (Tavassoli et al., 2007a).

The high rate of infection in dogs in this area indicates a greater risk of infection for humans. The consumption of raw liver by pregnant women

and other people as a rich source of iron and other nutrients may also increase the risk of infection (Sadjjadi et al., 1998; Oryan et al., 2008).

The high prevalence of linguatulosis in dogs and other animals found in this study highlights the need of improving preventative measures to reduce the rate of infection, which may pose a hazard to human health.

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