International Journal of Food Microbiology 413 (2024) 110571

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Prevalence and risk factors of *Linguatula* spp. in slaughter animals in Tabriz, Iran, and methods for nymphal stage inactivation

Nasser Hajipour [a,*], Hosein Baghaefar [a], Parviz Hassanzadeh [a], Jennifer Ketzis [b,**]

[a] Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
[b] Biomedical Sciences, Ross University School of Veterinary Medicine, Basseterre, St Kitts, West Indies, Saint Kitts and Nevis

ARTICLE INFO

Keywords:
Sodium chloride
Temperature
Goats
Camels
Buffalo
Sheep
Cattle
Seasonality

ABSTRACT

Linguatula is a food born zoonotic parasite in which carnivores and herbivores serve as final and intermediate hosts, respectively. Human infection with *Linguatula* spp. occurs following consumption of raw or undercooked infected internal organs of the intermediate host and/or consumption of water and/or vegetables contaminated with eggs released from final hosts. The aim of this study was to determine prevalence and risk factors of *Linguatula* spp. in sheep, cattle, buffalo, goats and camels slaughtered at the Tabriz abattoir, Iran. In addition, effect of temperature and sodium chloride (NaCl) on survival time of *Linguatula* spp. nymphs was assessed. For this purpose, 25,520 mesenteric lymph nodes from 2552 animals and the livers and lungs from 656 animals were collected randomly and examined. To evaluate the effect of temperature and NaCl on the survival of *Linguatula* spp. nymphs in infected livers and lungs, 30 g of each liver and lung with dimensions of 2 × 3 × 4 cm, were exposed to temperatures of −20, 10, 50, 60 and 72 °C and NaCl concentrations of 5 %, 10 %, 15 % and 20 % for 3, 6, 12, 24, 48 and 72 h, in triplicate. Based on the mesenteric lymph nodes, 25.7 % (656 of 2552 animals) were infected with *Linguatula* spp. Of the 656 liver and lungs assessed, 141 (21.5 %) and 62 (9.5 %) were infected with *Linguatula* spp., respectively. The rate of infection of mesenteric lymph nodes in all animals was significant with age ($P < 0.05$), with more older animals infected. In regards to sex, except for camels, more female animals were infected than male animals ($P < 0.05$). There was a significant difference in survival of nymphs based on temperature and/or NaCl and time ($P < 0.0001$). At 72 h, all temperatures assessed except 10 °C, resulted in all nymphs being inactivated. Sodium chloride was more effective against *Linguatula* spp. nymphs in livers than in lungs with 100 % efficacy only achieved against nymphs in livers at 20 % concentration after 48 h and at 10 and 15 % concentration after 72 h. Based on these results, heating and application of common salt as a food preservative in meat products reduces the survival time of *Linguatula* spp. nymphs and their use could decrease the risk of food-born microorganisms.

1. Introduction

Belonging to the class Pentastomida, *Linguatula serrata* and its haplotypes and potentially closely related *Linguatula* species are zoonotic arthropods (Hendrix, 1998) which usually infect dogs, and occasionally, foxes, cats and other carnivores as the final host and herbivores as the intermediate host. The adult parasite can be detected in the upper respiratory system, nasal airways and frontal sinuses of the final host (Hendrix, 1998). Nasopharyngeal discharges of the final host spread the egg into the environment which are then consumed by the intermediate herbivore host. Following emergence from the eggs, the larva spread throughout the internal organs of the intermediate host reaching the mesenteric lymph nodes (MLNs), liver, lungs and spleen, where they develop into infective nymphs. The final hosts are infected following ingestion of the infested viscera of intermediate hosts (Tavassoli et al., 2018).

Human infection with *L. serrata* occurs following consumption of raw or undercooked infected liver, lungs or other infected organs of the intermediate host and can manifest as a nasopharyngeal or Halzoun disease. Within Iran, the consumption of a delicious food called Joghul and Boghul, in which the liver, lungs and intestines of primarily sheep but also other ruminants are briefly cooked, can lead to *Linguatula* infection

* Correspondence to: N. Hajipour, Shohadaye Ghavvas Blvd, Opposite to Khavaran Town, Tabriz, East Azarbaijan Province 5166616471, Iran.
** Correspondence to: J. Ketzis, PO Box 334, Basseterre, St. Kitts, West Indies, Saint Kitts and Nevis.
E-mail addresses: n.hajipour@tabrizu.ac.ir (N. Hajipour), JKetzis@RossU.edu (J. Ketzis).

https://doi.org/10.1016/j.ijfoodmicro.2024.110571
Received 12 May 2023; Received in revised form 16 November 2023; Accepted 6 January 2024
Available online 12 January 2024

if these organs are infected with the nymphal stage. In humans, the nymph and/or mature parasites migrate to the nasopharyngeal tract and frontal sinuses leading to a variety of symptoms, ranging from mild inflammation of the upper respiratory tract to temporary conductive deafness (Khalil et al., 2013). The visceral form of the infection occurs when humans consume water or vegetables contaminated with eggs and, depending on the organ system involved, a range of symptoms including abdominal pain, chronic cough, or night sweats can be observed (Mateva et al., 2013). Most research on the epidemiology of *Linguatula* spp. and sources of human infection have focused on mesenteric and/or mediastinal lymph nodes of ruminants (Hajipour and Tavassoli, 2019; Nourollahi et al., 2011, 2010; Oryan et al., 2011; Sudan et al., 2014; Tajik et al., 2008; Tavassoli et al., 2007a, 2007b). Fewer studies have been performed on liver or lung infection of various animals with *Linguatula* spp. nymphs (Haddadzadeh et al., 2010; Hami et al., 2009; Morales Muñoz et al., 2020; Oryan et al., 2011; Shakerian et al., 2008; Shekarforoush et al., 2004; Tajik and Sabet Jalali, 2010; Yakhchali et al., 2009). Although *Linguatula* is a public health issue, there is no standard and comprehensive method for diagnosing the infection in offal during abattoir inspection. Ruminant infection has no considerable clinical signs and such animals are major sources of infection for humans. Hence, it is necessary to understand the prevalence and risk factors for the parasite in ruminants and to identify efficient disinfection and inactivation methods to improve food safety and public health.

The purpose of this research, therefore, was to assess the prevalence of *Linguatula* spp. nymphal stages in liver and lungs of ruminants slaughtered at the Tabriz abattoir in the northwest of Iran and identify possible factors, such as season, age, and sex, associated with the infestation. In addition, the effects of temperature and salinity on survival of *Linguatula* spp. nymphs in infected livers and lungs of ruminants were investigated.

2. Materials and methods

2.1. Area of study

This study was conducted in Tabriz in East Azerbaijan Province in northwestern Iran, a region with a population of almost 1.5 million and geographical directions of 38° 4′ North and 46° 18′ East. This city, located between Sahand and Eynali mountains, has a humid continental climate with regular seasons. The climate is temperate during the summer months and the winters are long with temperatures reaching −10 °C. Summer is categorized as the months of July, August and September, autumn as October, November and December, winter as January, February and March and spring as April, May and June. The annual average rainfall is 320 mm (Mansouri Daneshvar et al., 2019). For mean temperature and precipitation in Tabriz during the time of this study, see supplemental Table 1.

2.2. Sampling

From April 2019 to April 2020 on three to four days of each week, 25,520 MLNs from 2552 animals and 656 livers and lungs from 656 animals were collected after slaughter at the Tabriz abattoir. Animals were selected randomly during each visit and sex and age (based on dentition) of each sampled animal were recorded. After the first animals were identified and followed from preslaughter through processing, the next available animal was selected for sampling. From each animal 10 to 12 MLNs were collected randomly. The liver and lungs of animals whose lymph nodes were found to be infected with *Linguatula* spp. were selected for examination. In all work with the *Linguatula* spp. nymphs, differentiation of stage and sex was not performed.

2.3. Mesenteric lymph node examination

The samples were cut into small pieces (approximately 2 × 2 mm) and then incubated in saline (0.9 % NaCl) for 5 to 6 min at room temperature to allow nymphs to naturally emerge from the tissue. Nymphs were collected from the saline by forceps. After recovering nymphs, they were flattened, dehydrated in ascending grades of ethyl alcohol, cleared in creosote and examined under a stereomicroscope (10× and 40× magnification) (Hajipour et al., 2019).

2.4. Liver and lung examination

The liver and lung examination was performed in two steps. In the first step, the organ was sliced into 4 to 5 mm thick sections and observed carefully to find encapsulated or free nymphs. In the second step, 100 g of mixed sliced samples were digested in 200 ml of preheated digestion fluid containing 5 g pepsin and 25 ml hydrochloric acid in 1000 ml distilled water and incubated at 37 °C for 24 h. After 24 h, the contents of the container containing the digestion solution, which included the digested liver and lungs, and the larvae released from them during digestion, were examined for *Linguatula* spp. nymph under a stereomicroscope. The remaining parts of the livers and lungs of animals identified as positive were placed in a refrigerator at 4 °C (Fig. 1).

2.5. Effect of different temperatures on Linguatula nymphs in infected livers and lungs

To evaluate the effect of temperature on the survival of *Linguatula* spp. nymphs in infected livers and lungs, 30 g samples of the previously identified positive liver and lung were used. Thirty grams were selected based on the number of nymphs (approximately 50 to 60) found in the 100 g subjected to the digestion method. The 30 g sample, with dimensions of 2 × 3 × 4 cm, were placed in plastic bags and exposed to temperatures of 10, 50, 60, 72 and −20 °C for 3, 6, 12, 24, 48 and 72 h in triplicate using a water bath or freezer. Infected liver and lungs stored at 4 °C were used as controls.

2.6. Effect of different concentrations of sodium chloride on Linguatula nymphs in infected livers and lungs

To evaluate the effect of sodium chloride, 30 g samples of the previously identified positive liver and lung, with an estimated 15 to 20 nymphs, with dimensions of 2 × 3 × 4 cm, were placed in plastic bags and exposed to concentrations of 5 %, 10 %, 15 % and 20 % for 3, 6, 12, 24, 48 and 72 h in triplicate. Infected liver and lungs stored at 4 °C were used as controls.

2.7. Evaluation of survival rate of Linguatula nymphs in infected livers and lungs treated to different temperatures and sodium chloride

After the infected liver and lungs were treated at different temperatures and concentrations of sodium chloride, they were slightly crushed and immersed in pepsin digestion solution, then incubated at 37 °C for 24 h. The motility of nymphs was then assessed under a stereomicroscope as described by Mir et al. (2009) with those exhibiting movement categorized as having survived the treatment.

2.8. Statistical analysis

The resulting data were statistically analyzed by one-way analysis of variance (ANOVA) and if the difference in means was significant ($P < 0.05$), Duncan's post hoc test was used to track the difference between groups. Data analysis was done using SPSS version 21 software. The $P < 0.05$ or ($P < 0.001$) level was considered as significant.

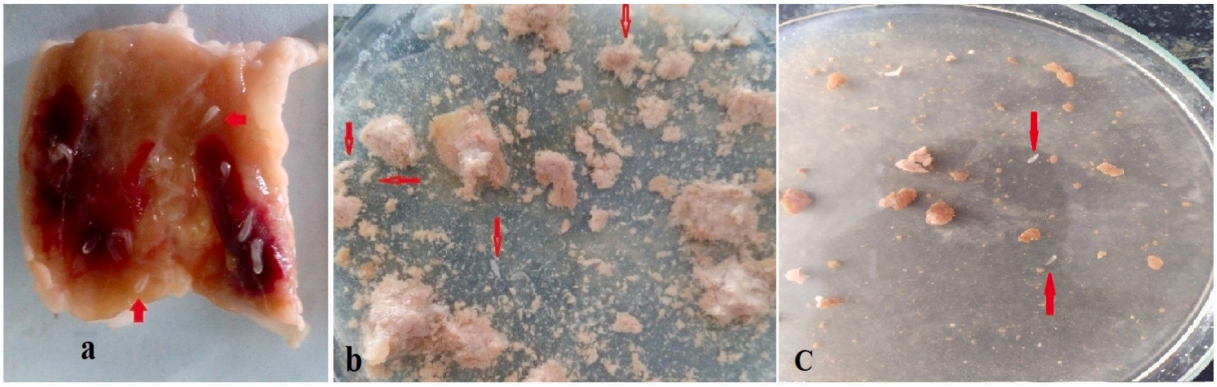

Fig. 1. *Linguatula* nymphs in mesenteric lymph nodes (a), Infected liver digested in pepsin (b) and Infected lung digested in pepsin.

3. Results

3.1. Prevalence, risk factors

Out of 2552 animals studied, 656 (25.7 %) were infected with *Linguatula* nymphs. The prevalence of nymphs in MLNs, livers and lungs of the different animals are summarized by sex, age and season in Tables 1–5. The results showed that the prevalence of infection with *Linguatula* nymphs in MLNs was significantly higher in goats (63 %) than in cattle (14.8 %), sheep (21.5 %), buffaloes (10.5 %) and camels (17.3 %) ($P < 0.05$). The rate of nymph infection in livers and lungs was observed as 30.2 % and 13.3 % in goats, 19.8 % and 8.7 % in sheep, 11.1 % and 11.1 % in camels, 7.1 % and 4.8 % in buffalos and 6.8 % and 1.7 % in cattle ($P > 0.05$). The results of statistical analysis showed that the rate of infection of mesenteric lymph nodes in all animals and the level of infection of liver and lungs in goats was significant with increasing age and sex, with more female than male animals infected ($P < 0.05$). The relationship between the level of infection and the seasons was not significant, except in sheep and goats. However, the rate of infection in all animals except camels was higher in the autumn than in other seasons. Although a difference was seen in the infection rate between males and females in all studied animals, it was not significant within the same age except for camels ($P > 0.05$).

3.2. Effect of temperature on Linguatula nymphs in infected livers and lungs

Approximately 15–20 nymphs were recovered from each sample, which were sufficient for the assessments and suggest similar distribution across the samples used. There was a significant difference in survival of nymphs based on temperature and time ($P < 0.001$) (Table 6). At 10 °C after 3, 6 and 12 h, survival was >90 % for nymphs in livers and lungs. At 72 h. all temperatures, except 10 °C resulted in all nymphs being inactivated. In general, 72 °C and − 20 °C, at all time points, resulted in the lowest survival of the nymphs.

3.3. Effect of sodium chloride on Linguatula nymphs in infected livers and lungs

As with the samples used for the temperature evaluation, approximately 15–20 nymphs were recovered from each sample, which were sufficient for the assessments and suggest similar distribution across the samples used. Sodium chloride was more effective against *Linguatula* spp. nymphs in livers than in lungs with 100 % efficacy only achieved against nymphs in livers at 20 % concentration after 48 h and at 10 and 15 % concentration after 72 h (Table 7). There was a clear relationship of concentration and time in survival of the nymphs in livers and lungs.

Table 1
The prevalence of *Linguatula* nymphs in liver, lungs and mesentery lymph nodes in cattle by season, age and sex from 2019 to 2020.

Organ	Season	Number animals	No. infected animals (%)	Number of infected animals (%)				
				Age groups (years)			Sex	
				<2	2–4	>4	Male	Female
MLNs	Spring	200	19 (9.5)	2 (1)	5 (2.5)	12 (6.0)	7 (3.5)	12 (6.0)
	Summer	200	29 (14.5)	6 (3)	9 (4.5)	14 (7.0)	10 (5.0)	19 (9.5)
	Autumn	200	40 (20.0)	7 (3.5)	13 (6.5)	20 (10.0)	13 (6.5)	27 (13.5)
	Winter	200	30 (15.0)	5 (2.5)	8 (4.0)	17 (8.5)	14 (7.0)	16 (8.0)
	Total	800	118 (14.8)	20 (2.5)	35 (4.4)	63 (7.9)	44 (5.5)	74 (9.3)
	P value		0.32	0.000			0.002	
Liver	Spring	19	1 (5.3)	0	0	1 (5.3)	0	1 (5.3)
	Summer	29	1 (3.4)	0	0	1 (3.4)	0	1 (3.4)
	Autumn	40	4 (10)	0	1 (2.5)	3 (7.5)	1 (2.5)	3 (7.5)
	Winter	30	2 (6.7)	0	1 (3.3)	1 (3.3)	0	2 (6.7)
	Total	118	8 (6.8)	0	2 (1.7)	6 (5.1)	1 (0.8)	7 (5.9)
	P value		0.745	0.023			0.025	
Lung	Spring	19	0	0	0	0	0	0
	Summer	29	0	0	0	0	0	0
	Autumn	40	1 (2.5)	0	0	1 (2.5)	0	1 (2.5)
	Winter	30	1 (3.3)	0	0	1 (3.3)	0	1 (3.3)
	Total	118	2 (1.7)	0	0	2 (1.7)	0	2 (1.7)
	P value		0.690	0.287			0.154	

Table 2
The prevalence of *Linguatula* nymphs in liver, lungs and mesentery lymph nodes in buffalo by season, age and sex from 2019 to 2020.

Organ	Season	Number animals	No. infected animals (%)	Number of infected animals (%)				
				Age groups (years)			Sex	
				<2	2–4	>4	Male	Female
MLNs	Spring	100	7 (7.0)	1 (1.0)	1 (1.0)	5 (5.0)	2 (2.0)	5 (5.0)
	Summer	100	8 (8.0)	1 (1.0)	3 (3.0)	4 (4.0)	2 (2.0)	6 (6.0)
	Autumn	100	17 (17.0)	2 (2.0)	4 (4.0)	11 (11.0)	5 (5.0)	12 (12.0)
	Winter	100	10 (10.0)	1 (1.0)	3 (3.0)	6 (6.0)	3 (3.0)	7 (7.0)
	Total	400	42 (10.5)	5 (1.3)	11 (2.8)	26 (6.5)	12 (3.0)	30 (7.5)
	P value		0.090	0.000			0.003	
Liver	Spring	7	0	0	0	0	0	0
	Summer	8	0	0	0	0	0	0
	Autumn	17	2 (11.8)	0	1 (5.9)	1 (5.9)	1 (5.9)	1 (5.9)
	Winter	10	1 (10.0)	0	0	1 (10.0)	0	1 (10.0)
	Total	42	3 (7.1)	0	1 (2.4)	2 (4.8)	1 (2.4)	2 (4.8)
	P value		0.610	0.327			0.549	
Lung	Spring	7	0	0	0	0	0	0
	Summer	8	0	0	0	0	0	0
	Autumn	17	1 (5.9)	0	0	1 (5.9)	0	1 (5.9)
	Winter	10	1 (10.0)	0	0	1 (10.0)	0	1 (10.0)
	Total	42	2 (4.8)	0	0	2 (4.8)	0	2 (4.8)
	P value		0.705	0.557			0.147	

Table 3
The prevalence of *Linguatula* nymphs in liver, lungs and mesentery lymph nodes in camels by season, age and sex from 2019 to 2020.

Organ	Season	Number animals	No. infected animals (%)	Number of infected animals (%)				
				*Age groups (years)			*Sex	
				<4	4–8	>8	Male	Female
MLNs	Spring	15	1 (6.7)	0	0	1 (6.7)	0	1 (6.7)
	Summer	12	5 (41.7)	0	1 (8.3)	4 (33.3)	1 (8.3)	4 (33.3)
	Autumn	13	2 (15.4)	0	0	2 (15.4)	0	2 (15.4)
	Winter	12	1 (8.3)	0	0	1 (8.3)	0	1 (8.3)
	Total	52	9 (17.3)	0	1 (1.9)	8 (15.4)	1 (1.9)	8 (15.4)
	P value		0.076	0.004			0.37	
Liver	Spring	1	0	0	0	0	0	0
	Summer	5	1 (20.0)	0	0	1 (20.0)	1 (20.0)	0
	Autumn	2	0	0	0	0	0	0
	Winter	1	0	0	0	0	0	0
	Total	9	1 (11.1)	0	0	1 (11.1)	1 (11.1)	0
	P value		0.268	0.325			0.343	
Lung	Spring	1	0	0	0	0	0	0
	Summer	5	1 (20.0)	0	0	1 (20.0)	0	1 (20.0)
	Autumn	2	0	0	0	0	0	0
	Winter	1	0	0	0	0	0	0
	Total	9	1 (11.1)	0	0	1 (11.1)	0	1 (11.1)
	P value		0.268	0.325			0.343	

Given the small sample size for lungs and liver, statistical analysis results must be interpreted with caution.

4. Discussion

The results of the present study showed that the *Linguatula* nymph infection rate in MLNs of cattle (14.75 %), buffaloes (10.5 %), camels (17.3 %), sheep (21.5 %) and goats (63 %) from this region was higher than previously reported rates in other surveys from Iran which found 6.9–12.8 % of cattle (Hashemnia et al., 2018; Tabaripour et al., 2017), 5.1–5.7 % of buffaloes (Tajik and Sabet Jalali, 2010), 13.5–16.2 % of camels (Haddadzadeh et al., 2010; Oryan et al., 2011), 9–19.7 % (Azizi et al., 2015; Dehkordi et al., 2014; Gharekhani et al., 2017; Hashemnia et al., 2018; Kheirabadi et al., 2015) and 16.8–59.8 % of goats (Dehkordi et al., 2014; Gharekhani et al., 2017; Hajipour et al., 2019; Hashemnia et al., 2018; Nourollahi et al., 2010; Rezaei et al., 2011; Tabaripour et al., 2017) to be infected. However, the prevalence found in different animals in this study was low according to other reported results in previous studies within Iran (Alborzi et al., 2013; Bamorovat et al., 2014; Nourollahi et al., 2012, 2011; Rezaei et al., 2012, 2011; Tajik et al., 2008; Youssefi et al., 2014). The reasons for the difference in prevalence in the studies conducted in different regions are probably due to different weather conditions such as annual rainfall, humidity, and temperature; the population of dogs in the region also likely plays a role (Hajipour and Tavassoli, 2019).

The prevalence of infection with *Linguatula* nymphs was significantly higher in goats than in cattle, sheep, camels and buffaloes. The reason is not clear but it may be related to different forage habitats of goats. Also, goats are often grazed ahead of the other livestock, which could be another reason for the observed increase in the risk of infection (Hajipour and Tavassoli, 2019; Tavassoli et al., 2014). The prevalence of *Linguatula* nymphs in the MLNs of female ruminants except for camels was significantly higher than in those of males, which may be a result of the higher mean age of females at slaughter.

With the exception of livers and lymph nodes of sheep and goats in the autumn, no significant difference was observed between the infection rate in different seasons ($P > 0.05$). However, in the autumn the prevalence trended toward being higher than in other seasons which was similar to the results of previously published studies (Hajipour et al., 2019; Mirzaei et al., 2011). However, studies by Tabaripour et al. (2017) and Kheirabadi et al. (2015), who also found seasonal differences,

Table 4
The prevalence of *Linguatula* nymphs in liver, lungs and mesentery lymph nodes in sheep by season, age and sex from 2019 to 2020.

Organ	Season	Number animals	No. infected animals (%)	Number of infected animals (%)				
				Age groups (years)			Sex	
				<1	1–2	>2	Male	Female
MLNs	Spring	200	23 (11.5)	3 (1.5)	6 (3.0)	14 (7.0)	8 (4.0)	15 (7.5)
	Summer	200	25 (12.5)	3 (1.5)	7 (3.5)	15 (7.5)	10 (5.0)	15 (7.5)
	Autumn	200	79 (39.5)	8 (4.0)	16 (8.0)	55 (27.5)	25 (12.5)	54 (27.0)
	Winter	200	45 (22.5)	7 (3.5)	12 (6.0)	26 (13)	17 (8.5)	28 (14.0)
	Total	800	172 (21.5)	21 (2.6)	41 (5.1)	110 (13.8)	60 (7.5)	112 (14.0)
	P value		0.000	0.000			0.000	
Liver	Spring	23	2 (8.7)	0	1 (4.3)	1 (4.3)	1 (4.3)	1 (4.3)
	Summer	25	3 (12.0)	0	1 (4.0)	2 (8.0)	1 (4.0)	2 (8.0)
	Autumn	79	23 (29.1)	1 (1.3)	2 (2.5)	20 (25.3)	5 (6.3)	18 (22.8)
	Winter	45	6 (13.3)	0	2 (4.4)	4 (8.9)	1 (2.2)	5 (11.1)
	Total	172	34 (19.8)	1 (0.6)	6 (3.5)	27 (15.7)	8 (4.7)	26 (15.1)
	P value		0.041	0.073			0.121	
Lung	Spring	23	1 (4.3)	0	0	1 (4.3)	0	1 (4.3)
	Summer	25	1 (4.0)	0	0	1 (4.0)	0	1 (4.0)
	Autumn	79	10 (12.7)	0	1 (1.3)	9 (11.4)	2 (2.5)	8 (10.1)
	Winter	45	3 (6.7)	0	1 (2.2)	2 (4.4)	1 (2.2)	2 (4.4)
	Total	172	15 (8.7)	0	2 (1.2)	13 (7.6)	3 (1.7)	12 (7.0)
	P value		0.387	0.129			0.206	

Table 5
The prevalence of *Linguatula* nymphs in liver, lungs and mesentery lymph nodes in goats by season, age and sex from 2019 to 2020.

Organ	Season	Number animals	No. infected animals (%)	Number of infected animals (%)				
				Age groups (years)			Sex	
				<1	1–2	>2	Male	Female
MLNs	Spring	125	43 (34.4)	5 (4.0)	12 (9.6)	26 (20.8)	15 (12.0)	28 (22.4)
	Summer	125	57 (45.6)	12 (9.6)	15 (12.0)	30 (24.0)	23 (18.4)	34 (27.2)
	Autumn	125	120 (96.0)	23 (18.4)	35 (28.0)	62 (49.6)	25 (20.0)	95 (76.0)
	Winter	125	95 (76.0)	12 (9.6)	24 (19.2)	59 (47.2)	32 (25.6)	63 (50.4)
	Total	500	315 (63.0)	52 (10.4)	86 (17.2)	177 (35.4)	95 (19.0)	220 (44.0)
	P value		0.000	0.000			0.002	
Liver	Spring	43	5 (11.6)	1 (2.3)	1 (2.3)	3 (7.0)	2 (4.7)	3 (7.0)
	Summer	57	8 (14.0)	1 (1.8)	2 (3.5)	5 (8.8)	3 (5.3)	5 (8.8)
	Autumn	120	61 (50.8)	5 (4.2)	18 (15.0)	38 (31.7)	22 (18.3)	39 (32.5)
	Winter	95	21 (22.1)	5 (5.3)	6 (6.3)	10 (10.5)	9 (9.5)	12 (12.6)
	Total	315	95 (30.2)	12 (3.8)	27 (8.6)	56 (17.8)	36 (11.4)	59 (18.7)
	P value		0.000	0.000			0.002	
Lung	Spring	43	3 (6.9)	0	2 (4.7)	1 (2.3)	2 (4.7)	1 (2.3)
	Summer	57	5 (8.7)	0	1 (1.8)	3 (5.3)	0	5 (8.8)
	Autumn	120	23 (19.2)	0	8 (6.7)	15 (12.5)	6 (5.0)	17 (14.2)
	Winter	95	11 (11.5)	0	4 (4.2)	7 (7.4)	5 (5.3)	6 (6.3)
	Total	315	42 (13.3)	0	15 (4.8)	26 (8.3)	13 (4.1)	29 (9.2)
	P value		0.061	0.000			0.037	

showed higher prevalence in summer and spring, respectively. This seasonal difference is likely due to grazing and exposure periods. If intermediate hosts grazing in spring swallow the infectious eggs, *Linguatula* nymphs will be seen in the autumn, since the time from ingestion of eggs to formation of the nymphal stage requires 6 months. In the location of the study reported here, suitable weather conditions for egg development occur in the late spring, coinciding with the time of grazing sheep, resulting in the autumn nymphs in MLNs (Hajipour et al., 2019).

Studies on means of inactivating *Linguatula* nymphs are needed to identify procedures that could be implemented post-slaughter to decrease human exposure and infection. However, few studies have been performed on inactivation methods of *Linguatula* nymphs in vitro. Heat and sodium chloride are two effective methods for inactivating *Linguatula* nymphs, although the best temperature and concentration combined with exposure times needs to be determined. Heat results in the denaturation and coagulation of cellular proteins leading to the death of the nymphs while exposure to sodium chloride removes water from the body of the parasites resulting in plasmolysis and subsequent death (Jay et al., 2008). Studies by Basti et al. (2011) and Hajimohammadi et al. (2012) showed much lower survival rates of the nymphs at similar temperatures and much quicker death with, for example, only 8 % survival after 0.5 h at 60 °C. However, in these previous studies, the nymphs were isolated while in the study presented here in, the nymphs were within the liver or lungs.

The survival time of the nymphs stored at the high temperatures (50 °C, 60 °C and 72 °C) were short and all the isolated nymphs were found dead after maximum 1.5 h. The other ones stored at freezing temperature (−18 °C) were more resistant and died after 3 h (Basti et al., 2011).

Hajimohammadi et al. (2012) showed that the survival time of the nymphs stored in 10 % NaCl solution was shorter compared to the present study and all of them were dead after 3 h. But the other ones maintained in 2 % NaCl solution were significantly more resistant ($p < 0.05$) and survived for 2 days. All the nymphs pertaining to each 60 °C and 72 °C treatments were found dead after first 5-min storage interval; the nymphs stored at 50 °C died totally after 20 min.

The time required for heat to penetrate tissues varies which is reflected in the higher survival rate of *Linguatula* nymphs in the lungs compared to the liver at the same temperatures and treated time. In addition, some protease enzymes can help to increase the survival time

Table 6
Percent survival of *Linguatula* nymphs in infected livers and lung at different temperatures and exposure times (Mean ± SE).

Organ	Temp. (°C)	Exposure time (hours)					
		3	6	12	24	48	72
Liver	10	100^{c}	100^{d}	93.3 ± 6.6^{b}	90.3 ± 5.7^{b}	79.3 ± 10.4^{b}	56.2 ± 28.2^{b}
	50	100^{c}	28.1 ± 7.7^{b}	10.8 ± 5.8^{a}	9.7 ± 5.0^{a}	5.5 ± 5.5^{a}	0.0^{a}
	60	57.9 ± 14.4^{b}	45.3 ± 5.3^{c}	4.7 ± 4.7^{a}	4.7 ± 4.7^{a}	0.0^{a}	0.0^{a}
	72	24.6 ± 16.9^{a}	20.1 ± 1.1^{b}	3.3 ± 3.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}
	−20	14.0 ± 1.6^{a}	3.3 ± 3.3^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}
Lung	10	100^{c}	100^{c}	100^{b}	87.7 ± 6.1^{b}	74.6 ± 12.9^{b}	66.4 ± 18.9^{b}
	50	100^{c}	44.0 ± 9.7^{b}	18.0 ± 11.7^{a}	15.0 ± 8.2^{a}	6.6 ± 6.6^{a}	0.0^{a}
	60	62.6 ± 6.4^{b}	36.1 ± 7.3^{ab}	6.6 ± 6.6^{a}	6.6 ± 6.6^{a}	0.0^{a}	0.0^{a}
	72	27.7 ± 114.6^{a}	22.2 ± 14.6^{ab}	4.1 ± 4.1^{a}	0.0^{a}	0.0^{a}	0.0^{a}
	−20	13.3 ± 13.3^{a}	6.6 ± 6.6^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}

Lowercase letters (a-d) in each column indicate statistical significance.

Table 7
Percent survival of *Linguatula* nymphs in infected livers and lung at different concentrations of sodium chloride and exposure times (Mean ± SE).

Organ	Con. (%)	Exposure time (hours)					
		3	6	12	24	48	72
Liver	5	100^{c}	81.2 ± 2.4^{d}	61.2 ± 1.2^{c}	59.4 ± 2.0^{c}	45.3 ± 5.4^{b}	27.8 ± 2.8^{b}
	10	73.6 ± 6.1^{b}	54.2 ± 5.9^{c}	44.0 ± 9.7^{c}	14.5 ± 1.2^{b}	5.6 ± 5.6^{a}	0.0^{a}
	15	34.0 ± 20.2^{a}	38.4 ± 3.2^{b}	24.8 ± 1.5^{b}	4.2 ± 4.2^{a}	2.8 ± 2.8^{a}	0.0^{a}
	20	13.1 ± 7.2^{a}	3.3 ± 3.3^{a}	3.7 ± 3.7^{a}	2.8 ± 2.8^{a}	0.0^{a}	0.0^{a}
Lung	5	100^{c}	100^{c}	93.3 ± 6.7^{c}	77.8 ± 22.2^{ab}	58.3 ± 8.3^{b}	55.6 ± 29.4^{c}
	10	86.7 ± 13.3^{bc}	83.3 ± 9.6^{bc}	80.0 ± 11.5^{c}	52.2 ± 7.8^{c}	17.8 ± 9.7^{a}	11.1 ± 11.1
	15	58.3 ± 12.7^{ab}	54.9 ± 11.3^{ab}	50.0 ± .0^{b}	45.4 ± 7.8^{c}	16.7 ± 9.6^{a}	8.3 ± 8.3^{b}
	20	37.2 ± 14.8^{a}	30.6 ± 19.4^{a}	15.0 ± 7.6^{a}	13.3 ± 13.3^{b}	6.7 ± 6.7^{a}	6.7 ± 6.7^{b}

Lowercase letters (a-d) in each column indicate statistical significance.

of parasites such as *Clonorchis sinensis* metacercariae during long-time refrigerated storage (Li et al., 2006). Protease enzymes probably play a role in the long survival time of *Linguatula* nymphs stored at refrigeration temperature, but the detailed mechanism is not clear (Alcala-Canto et al., 2007). In a study conducted by Mir et al. (2009), it was shown that *Linguatula* nymphs can survive in PBS at room temperature and −4 °C for 4 days (Mir et al., 2009). Negrea et al. (2009) studied the effect of temperature on *Linguatula* nymphs in vitro and showed that the nymphs were destroyed after 24 h at −18 °C. At 4 °C on the first day, 50 % and 10 % on the second day were alive, and on the third day, all of them were dead, and those kept at the laboratory temperature (15–25 °C) were alive on the fifth day of treatment (Negrea et al., 2009).

The results of our studies showed that the survival rate of nymphs in liver in the concentration of 5 % and 10 % of sodium chloride after 3 h, was 100 % and 73.6 %, respectively, which was not in line with the results obtained from the studies of Hajimohammadi et al. (2017). They reported 6.6 % and 0 % survival rate of *Linguatula* nymphs in 5 % and 10 % sodium chloride concentrations after 3 h of treatment, respectively. However, with the increase in sodium chloride concentration and the duration of treatment, the survival rate of the parasite decreases, which is consistent with other studies (Hajimohammadi et al., 2017).

A limitation of the study presented herein, due to funding limitations, is a lack of morphological descriptions combined with molecular analysis to confirm the *Linguatula* species. Based on previous publications, it is most likely *Linguatula serrata* (Yektaseresht et al., 2023; Shamsi et al., 2020: Bamorovat et al., 2021; Ghorashi et al., 2016). However, mixed infections or different infections among the hosts cannot be ruled out.

5. Conclusion

In conclusion, the high prevalence of infestation observed in ruminants, especially goats, is critical due to the zoonotic nature of *Linguatula*. Considering the results, it can be concluded that the prevalence of *Linguatula* in animals in Tabriz is high which could play an important role in the epidemiology of linguatulosis in people. Further studies are needed for subtyping, speciating and determining existence of mixed infestations to expand the epidemiological understanding of the parasite. The risk of linguatulosis in people in the region could potentially be decreased through heating or the application of common salt as a food preservative, since these decrease motility of the nymphs. In regions where raw or undercooked organs may be eaten and *Linguatula* prevalence is high the people should be warned of the risk of linguatulosis and post-slaughter disinfecting methods implemented.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2024.110571.

Declaration of competing interest

The authors declare that there are no conflicts of interest relevant to this study.

Data availability

Data will be made available on request.

Acknowledgement

The part of project was funded by the University of Tabriz, Iran. The resultant part of this article is adopted from the M.Sc. thesis of Mr. Hosein Baghaefar (Code: 43.407470.2). We are grateful to staff of the Department of Food Hygiene and Aquatic, Faculty of Veterinary Medicine, University of Tabriz.

References

Alborzi, A., Molayan, P.H., Akbari, M., 2013. Prevalence of *Linguatula serrata* nymphs in mesenteric lymph nodes of cattle and buffaloes slaughtered in Ahvaz Abattoir, Iran. Iran. J. Parasitol. 8, 327–332.

Alcala-Canto, Y., Alberti-Navarro, A., Ibarra-Velarde, F., 2007. Serine protease activity demonstrated in the larval stage of the pentastomid *Linguatula serrata*. Parasitol. Res. 100, 1011–1014. https://doi.org/10.1007/s00436-006-0374-x.

Azizi, H., Nourani, H., Moradi, A., 2015. Infestation and pathological lesions of some lymph nodes induced by *Linguatula serrata* nymphs in sheep slaughtered in Shahrekord area (southwest Iran). Asian Pac. J. Trop. Biomed. 5, 574–578.

Bamorovat, M., Zarandi, M.B., Mostafavi, M., Kheirandish, R., Sharifi, I., Radfar, M.H., 2014. The prevalence of *Linguatula serrata* nymphs in mesenteric and mediastinal lymph nodes in one-humped camels (*Camelus dromedarius*) slaughtered in Rafsanjan slaughterhouse, Iran. J. Parasit. Dis. 38, 374–377. https://doi.org/10.1007/s12639-013-0258-9.

Bamorovat, M., Sharifi, I., Oliaee, R.T., Aflatoonian, M.R., Nejad Almani, P.G., Derakhshani, A., Nasibi, S., Khedri, J., Khirandish, R., Mohammadi, M.A., 2021. Linguatulosis in small ruminants in southeastern Iran: epidemiological, histopathological and phylogenetic findings and its public health importance. Microb. Pathog. 152, 104600 https://doi.org/10.1016/j.micpath.2020.104600.

Basti, A.A., Haddadzadeh, H., Tajik, H., Hajimohammadi, B., Shirali, S., Hemati, M., Ahmadiara, E., 2011. Effect of different temperature conditions on survival time of *Linguatula serrata* nymphs. HVM Bioflux 3, 76–82.

Dehkordi, Z.S., Pajohi-Alamoti, M.R., Azami, S., Bahonar, A.R., 2014. Prevalence of *Linguatula serrata* in lymph nodes of small ruminants: case from Iran. Comp. Clin. Path. 23, 785–788.

Gharekhani, J., Esmaeilnejad, B., Brahmat, R., Sohrabei, A., 2017. Prevalence of *Linguatula serrata* infection in domestic ruminants in west part of Iran: risk factors and public health implications. J. Fac. Vet. Med. Istanbul Univ. 1, 28–31.

Ghorashi, S.A., Tavassoli, M., Peters, A., Shamsi, S., Hajipour, N., 2016. Phylogenetic relationships among *Linguatula serrata* isolates from Iran based on 18S rRNA and mitochondrial cox1 gene sequences. Acta Parasitol. 61 (1), 195–200.

Haddadzadeh, H.R., Athari, S.S., Abedini, R., Khazraii nia, S., Khazraii nia, P., Nabian, S., Haji-Mohamadi, B., 2010. One-humped camel (*Camelus dromedarius*) infestation with *Linguatula serrata* in Tabriz, Iran. J. Arthropod. Borne. Dis. 4, 54–59.

Hajimohammadi, B., Basti, A., Shirali, S., 2012. Impact of sodium chloride and heat on survival time of *Linguatula serrata* nymphs in vitro: an experimental study. J. Heal. Res. 1, 54–61.

Hajimohammadi, B., Eslami, G., Khalatbari-limaki, S., Ehrampoush, M.H., Oryan, A., Zandi, H., Dehghan, H.R., 2017. The role of *Linguatula serrata* nymph in transmission of enteric bacterial pathogens to internal organs in sheep. J. Parasit. Dis. 41, 754–760.

Hajipour, N., Tavassoli, M., 2019. Prevalence and associated risk factors of *Linguatula serrata* infection in definitive and intermediate hosts in Iran and other countries: a systematic review. Vet. Parasitol. Reg. Stud. Rep. 16, 100288.

Hajipour, N., Ketzis, J., Esmaeilnejad, B., 2019. Pathological characteristics of *Linguatula serrata* (aberrant arthropod) infestation in sheep and factors associated with prevalence in Iran. Prev. Vet. Med. 172, 104781 https://doi.org/10.1016/j.prevetmed.2019.104781.

Hami, M., Naddaf, S.R., Mobedi, I., Zare-Bidaki, M., Athari, S.S., Hajimohammadi, B., Anaraki-Mohammadi, G., 2009. Prevalence of *Linguatula serrata* infection in domestic bovids slaughtered in Tabriz Abattoir, Iran. Iran. J. Parasitol. 4, 25–31.

Hashemnia, M., Rezaei, F., Sayadpour, M., Shahbazi, Y., 2018. Prevalence of *Linguatula serrata* nymphs and pathological lesions of infected mesenteric lymph nodes among ruminants in Kermanshah, western Iran. Bulg. J. Vet. Med. 21, 94–102.

Hendrix, C.M., 1998. Diagnostic Veterinary Parasitology. Mosby St, Louis, Mo, USA.

Jay, J.M., Loessner, M.J., Golden, D.A., 2008. Modern Food Microbiology. Springer Science & Business Media.

Khalil, G., Haddad, C., Otrock, Z.K., Jaber, F., Farra, A., 2013. Halzoun, an allergic pharyngitis syndrome in Lebanon: the trematode *Dicrocoelium dendriticum* as an additional cause. Acta Trop. 125, 115–118. https://doi.org/10.1016/j.actatropica.2012.09.013.

Kheirabadi, K.P., Fallah, A.A., Azizi, H., Samani, A.D., Dehkordi, S.D., 2015. Prevalence of *Linguatula serrata* nymphs in slaughtered sheeps in Isfahan province, southwest of Iran. J. Parasit. Dis. 39, 518–521. https://doi.org/10.1007/s12639-013-0388-0.

Li, S., Kang, H.W., Choi, M.H., Hong, S.T., 2006. Long-term storage of Clonorchis sinensis metacercariae in vitro. Parasitol. Res. 100, 25–29. https://doi.org/10.1007/s00436-006-0242-8.

Mansouri Daneshvar, M.R., Ebrahimi, M., Nejadsoleymani, H., 2019. An overview of climate change in Iran: facts and statistics. Environ. Syst. Res. 8, 1–10. https://doi.org/10.1186/s40068-019-0135-3.

Mateva, S.A., Nikolova, M.R., Karaivanov, M.P., Marinova, P.E., 2013. Rare case of human visceral linguatuliasis in Bulgaria diagnosed on biopsy specimen. J. Biomed. Clin. Res. 6, 131–134.

Mir, M.S., Darzi, M.M., Hussain, I., Wani, S.A., 2009. Concurrent occurrence of visceral linguatulosis and paratuberculosis in alpine cross goats (*Capra hircus*). J. Vet. Arh. 79, 301–314.

Mirzaei, M., Asgarinezad, H., Rezaeisaghinsara, H., 2011. A survey of *Linguatula serrata* infection in sheep in Tabriz abattoir, East Azarbaijan Provice. J. Vet. Med. Lab. 3, 69–75.

Morales Muñoz, P., Carrillo Parraguez, M., González Marambio, M., Carvallo Chaigneau, F., 2020. Histopathological lesions compatible with nymphs of *Linguatula serrata* in bovine liver. Austral J. Vet. Sci. 52, 19–23.

Negrea, O., Liviu, O., Miclaus, V., Miresan, V., Răducu, C., 2009. Epizootological aspects regarding in vitro resistance of *Linguatula serrata* larva stages. Med. Vet. 52, 691–693.

Nourollahi, F.S.R., Kheirandish, R., Norouzi, A.E., Fathi, S., 2010. The prevalence of *Linguatula serrata* nymphs in goats slaughtered in Kerman slaughterhouse, Kerman, Iran. Vet. Parasitol. 171, 176–178. https://doi.org/10.1016/j.vetpar.2010.03.010.

Nourollahi, F.S.R., Kheirandish, R., Norouzi, A.E., Fathi, S., 2011. Mesenteric and mediastinal lymph node infection with *Linguatula serrata* nymphs in sheep slaughtered in Kerman slaughterhouse, Southeast Iran. Trop. Anim. Health Prod. 43, 1–3.

Nourollahi, F.S.R., Ghalekhani, N., Kheirandish, R., Fathi, S., E., N.A., 2012. The prevalence of *Linguatula serrata* nymphs in camels slaughtered in Mashhad slaughterhouse, Northeast, Iran. Asian Pac. J. Trop. Biomed. 2, 885–888. https://doi.org/10.1016/S2221-1691(12)60247-0.

Oryan, A., Khordadmehr, M., Ranjbar, V.R., 2011. Prevalence, biology, pathology, and public health importance of linguatulosis of camel in Iran. Tropl. Anim. Health Prod. 43, 1225–1231. https://doi.org/10.1007/s11250-011-9830-4.

Rezaei, F., Tavassoli, M., Mahmoudian, A., 2011. Prevalence of *Linguatula serrata* infection among dogs and domestic ruminants in North West of Iran. Vet. Med. (Praha). 56, 561–567.

Rezaei, F., Tavassoli, M., Javdani, M., 2012. Prevalence and morphological characterizations of *Linguatula serrata* nymphs in camels in Isfahan Province, Iran. Vet. Res. Forum 3, 61–65.

Shakerian, A., Shekarforoush, S.S., Ghafari Rad, H., 2008. Prevalence of *Linguatula serrata* nymphs in one-humped camel (*Camelus dromedarius*) in Najaf-Abad, Iran. Res. Vet. Sci. 84, 243–245. https://doi.org/10.1016/j.rvsc.2007.04.015.

Shamsi, S., Barton, D.P., Zhu, X., Jenkins, D.J., 2020. Characterisation of the tongue worm, *Linguatula serrata* (Pentastomida: Linguatulidae), in Australia. Int. J. Parasitol. Parasites Wildl. 11, 149–157. https://doi.org/10.1016/j.ijppaw.2020.01.010.

Shekarforoush, S.S., Razavi, S.M., Izadi, M., 2004. Prevalence of *Linguatula serrata* nymphs in sheep in Shiraz, Iran. Small Rumin. Res. 52, 99–101. https://doi.org/10.1016/S0921-4488(03)00224-4.

Sudan, V., Jaiswal, A.K., Shanker, D., 2014. Infection rates of *Linguatula serrata* nymphs in mesenteric lymph nodes from water buffaloes in North India. Vet. Parasitol. 205, 408–411. https://doi.org/10.1016/j.vetpar.2014.07.025.

Tabaripour, Rabeeh, Fakhar, M., Alizadeh, A., Youssefi, M.R., Tabaripour, Reza, Teshnizi, S.H., Sharif, M., 2017. Prevalence and histopathological characteristics of *Linguatula serrata* infection among slaughtered ruminants in Mazandaran Province, northern Iran. Comp. Clin. Path. 26, 1259–1265.

Tajik, H., Sabet Jalali, F.S., 2010. *Linguatula serrata* prevalence and morphometrical features: an abattoir survey on water buffaloes in Iran. Ital. J. Anim. Sci. 9, 348–351. https://doi.org/10.4081/ijas.2010.e65.

Tajik, H., Tavassoli, M., Javadi, S., Baghebani, H., 2008. The prevalence rate of *Linguatula serrata* nymphs in Iranian River Buffaloes. Asian J. Anim. Vet. Adv. 3, 174–178.

Tavassoli, M., Tajic, H., Dalir-Naghadeh, B., Hariri, F., 2007a. Prevalence of *Linguatula serrata* nymphs and gross changes of infected mesenteric lymph nodes in sheep in Urmia, Iran. Small Rumin. Res. 72, 73–76. https://doi.org/10.1016/j.smallrumres.2006.08.013.

Tavassoli, M., Tajik, H., Dalir Naghadeh, B., Lotfi, H., 2007b. Study of *Linguatula serrata* infestation in mesenteric lymph nodes of goats in slaughterhouse of Urmia, Iran. Sci. Iran. Vet. J. 3, 84–90.

Tavassoli, M., Gorashi, S.A., Shamsi, S., Hajipour, N., 2014. Molecular differences between *Linguatula serrata* isolated from differernt farm animals of Iran. In: The 22nd Iranian Congress on Infectious Diseases and Tropical Medicine. Iran.

Tavassoli, M., Tamaddonfard, E., Mirshekar, F., Hajipour, N., Erfanparast, A., 2018. A behavioral evaluation of the effects of ingestion of *Linguatula serrata* nymphs in rats. Vet. Parasitol. 254, 78–81.

Yakhchali, M., Athari, S.H., Hajimohammadi, B., Raeisi, M., 2009. Prevalence of *Linguatula serrata* in the ruminants slaughtered in Urmia slaughterhouse, Iran. J. Vet. Res. 64, 329–332.

Yektaseresht, A., Razavi, S.M., Sebdani, M.M., Ahmadi, A., 2023. Molecular characterization and phylogenetic analysis of *Linguatula serrata* isolated from camels, sheep and goats in Iran. J. Parasit. Dis. 47, 410–415. https://doi.org/10.1007/s12639-023-01587-6.

Youssefi, M., Tabaripour, R., Gerami, A., Omrani, V., 2014. Electrophoretic pattern of *Linguatula serrata* larva isolated goat mesenteric lymph node. J. Parasit. Dis. 40, 292–294. https://doi.org/10.1007/s12639-014-0497-4.