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Phylogenetic, molecular, and microscopic investigation of *Linguatula serrata* infection in stray and road-killed dogs in Northwest Iran

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Abstract

Background *Linguatula serrata* is a cosmopolitan zoonotic parasite. Canids serve as definitive hosts, while herbivores acts as intermediate hosts. Adult *L. serrata* are found in the nasal and respiratory passages, whereas the immature stages are located in the mesenteric lymph nodes, liver, spleen, lungs, and, occasionally in other organs. Humans can serve as intermediate hosts, with visceral infections, or as definitive hosts with nasopharyngeal infections. This study aimed to investigate the infection rate of stray and road-killed dogs with *L. serrata* and to explore its phylogeny.

Material and methods A total of 150 stray dogs and 100 road-killed dogs were examined for *L. serrata* eggs in feces and nasal secretions using microscopy and molecular tests, and for adult *L. serrata* through necropsy.

Results The results showed that 174 of 250 (69.6%) studied dogs were positive for *L. serrata* by at least one of the examination methods. The prevalence in road-killed and stray dogs was determined to be 72% and 68%, respectively. The *L. serrata* infection rate based on fecal microscopy in the road-killed and stray dogs was 29% and 46.7%, respectively, and by fecal PCR was 59% and 48.7%, respectively. The rate of infection with *L. serrata* based on nasal secretions in road-killed and stray dogs was 14% and 26%, respectively, using microscopy, and 44% and 43.3%, respectively, using PCR. In the road-killed dogs, based on necropsy, 46% were positive. The results of this study show that PCR is more effective than microscopy for detecting *L. serrata* infections in dogs. PCR amplicon of the expected size for *Linguatula* of approximately 595 bp for 18S rRNA were generated from the *L. serrata* isolates using described specific primers.

Conclusion It can be concluded that the rate of infection in dogs and possibly other carnivores, herbivores, and man, is high in this locality; hence, strict control measures should be conducted to overcome the risk of infection with this zoonotic disease.

Keywords *Linguatula serrata*, PCR, Dogs, Feces, Nasal secretions, Necropsy

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Introduction

Belonging to the class Pentastomida, *Linguatula serrata* Fröhlich, 1789 is a zoonotic aberrant arthropod [1] that primarily infects dogs, and occasionally, foxes, cats, and other carnivores as the final hosts and herbivores such as sheep, goats, cattle, and camels as the intermediate hosts. The adult parasite can be detected in the upper respiratory system, nasal airways, and frontal sinuses of the final hosts. Nasopharyngeal discharges from the final hosts spread the eggs into the environment. Larvae emerge from the eggs, after being ingested by herbivores, and spread throughout the internal organs of the intermediate hosts reaching the mesenteric lymph nodes (MLNs), liver, lungs and, spleen, where they develop into infective nymphs [2, 3]. The final hosts are infected following ingestion of the viscera of intermediate hosts that contain infective nymphs [2]. Humans can serve as final or intermediate hosts. Consumption of raw or undercooked infected internal organs of intermediate hosts can result in nasopharyngeal disease with symptoms ranging from mild inflammation of the upper respiratory tract to temporary conductive deafness [4–6]. Consumption of *L. serrata* eggs (typically via water or vegetables) results in the visceral form in humans. Symptoms depend on the organs involved and can include abdominal pain, chronic cough, or night sweats [7]. In severe infections, secondary septicemia, pneumonia, or severe enterocolitis can occur [8, 9].

Recently, cases of linguatulosis in humans have been reported in various provinces of Iran, with an increasing incidence observed [9–15]. There are also reports indicating the endemicity of linguatulosis in goats, sheep, cattle, and camels in various regions of Iran and other countries [2, 16, 17]. Based on the above reports and given that dogs are the major source of linguatulosis of herbivorous animals and man, it is speculated that the prevalence of linguatulosis may be very high in stray dogs from different regions in Iran.

In Urmia, Iran, the prevalence has been reported as 1.9% in road-killed dogs [18] and 81.0% in stray dogs [19], with both studies using microscopic examination of the nasal cavity and brain. In other studies within Iran, mostly using necropsy, prevalence has ranged from 27.8% to 76.5% in stray dogs, showing a wide variation depending on the location [20–23]. In other parts of the world where *L. serrata* occurs, reported infection rates range from as low as 0.4% to over 67% [24–26]. Wild and stray dogs often show higher infection rates, with 67.6% of dogs in south-eastern Australia reported to be positive [27]. However, only 3.5% of stray dogs in the Rabat region, Morocco were positive for infection [28]. The infection rate in owned dogs in Thessaloniki, Greece was 0.4% [29] but was over 37.4% in Jalingo, Nigeria [30].

Given regional variations and the lack of information on the prevalence of linguatulosis in stray dogs in the southern areas of Iran, determining the frequency of infestation of this disease in stray dogs from a public health perspective is of great significance. However, it is a time-consuming, expensive, and hazardous task for research personnel due to the risk of direct contact with infected animals or their tissues, which can lead to zoonotic transmission of *L. serrata*. Adequate information on the prevalence of linguatulosis of stray dogs in this populated area is not yet available. Therefore, the present study was undertaken to examine the prevalence of this disease in stray dogs and road-killed dogs of the Tabriz area of the East Azerbaijan province in Iran using microscopy and PCR (feces and nasal secretions) and necropsy.

Materials and methods

Study area

With a population of over 1.5 million and geographic coordinates of 38° 4′ N and 46° 18′ E, Tabriz County, East Azerbaijan Province, in northwest Iran, was the location of the study. This city has a humid continental climate with distinct seasons, and it is situated between the Eynali and Sahand mountains. About 320 mm of rainfall is recived annually. During the warmer summer months, the climate is temperate; while the long, harsh winters bring temperatures as low as −10 °C.

Study animals

This study was conducted between March 2016 and March 2021 in Tabriz, East Azerbaijan Province, and involved 150 stray dogs and 100 road-killed dogs. All procedures were conducted in compliancewith ethical guidelines and were approved by the institutional animal care and use committee (approval number: 2312). Fecal samples from stray dogs were collected non-invasively from naturally defecated material, ensuring no harm to the animals. Road-killed dogs were obtained through local authorities or standard municipal road-cleaning operations, ensuring that no animals were euthanized specifically for research purposes. Necropsy procedures were carried out with strict adherence to ethical and biosafety standards. Using the following formula [31], the number of samples needed was determined, considering a 50–70% prevalence of *L. serrata* (derived from research in the region's herbivores), a 95% confidence interval, and a 5% accuracy.

$$N_\kappa = \frac{4(1-\kappa)}{W_\kappa^2}\left[(1-\kappa)(< spanclass =' convertEndash' >< spanclass =' convertEndash' > 1 - 2 < /span >< /span > \kappa) + \frac{\kappa(2-\kappa)}{2\pi(1-\pi)}\right]z_{1-\alpha/2}^2$$

where K is the expected kappa coefficient of 0.7, α is the error rate, d is the accuracy, π is the predicted prevalence, W_k is the kappa width, and α is the 5% accuracy,

The Bureau of Veterinary Medicine and the Tabriz Environment Protection Organization granted authorization for the samples to be collected (Letter Number 2312). The Pardis Animal Shelter provided the stray dogs, who were housed there both during and after the study. As part of the trial, none of the dogs had anesthesia or were put to sleep.

As previous studies suggest [32], dogs were grouped into four age groups based on their teeth condition: under six months, six to less than 24 months, two to four years, and more than four years.

Detection of eggs by microscopy

For fecal and nasal egg detection, all dogs (250) were used. Fecal samples from road-killed dogs were collected directly from the rectum, while naturally defecated samples were used for stray dogs. Feces were examined using direct smear and flotation, using a saturated sugar solution, described by [33], with some modifications. Initially, 2 g of feces were mixed with 10 ml water, sieved (a 100 µm mesh), and centrifuged at 1800 g for 5 min. After pouring off the supernatant, the sediment was mixed with the saturated sugar solution and centrifuged with a coverslip on the tube at 1000 g for 5 min. The coverslip was subsequently examined under magnifications of 40× and 100× to detect *L. serrata* eggs. A portion of the sediment was stored at −20 °C for DNA extraction and PCR analysis.

To evaluate the presence of *L. serrata* eggs in nasal secretions, 2 ml of PBS (phosphate-buffered saline, pH = 7) was injected into the sinuses, and then 1–2 drops of the secretions were placed on a slide, covered with lamel and examined at a 40× magnification. Additionally, a sample of the secretions was stored at −20 °C for DNA extraction. Morphological identification of the eggs was performed following the criteria outlined by Soulsby (1982).

Detection of *L. serrata* adult by necropsy

The heads of the 100 road-killed dogs were removed from the bodies and transferred to the necropsy laboratory. After collection of the nasal secretions, the skin was separated from the underlying bones, and the skull was sagittally cleaved on either side of the midline using a motorized thin-bladed saw to access both the frontal sinuses and nasal cavities. The nasopharynx area, nasal turbinates, sinuses, eustachian tubes, trachea, and brain were visually examined for the presence of *L. serrata* (Fig. 1) with any parasites seen carefully removed. Each section of the skull was submerged in warm physiological saline overnight. The next day, the head was discarded, the supernatant fluid decanted, and the sediment examined for parasites. The number of *L. serrata* in each infected dog was recorded. The collected adult parasites were preserved in 10% buffered formalin for species identification or stored at −20 °C for DNA extraction and PCR analysis. For morphological identification, the parasites were washed under running water, mounted on slides, cleared with lactophenol, and stained using azocarmine. The length and width of the parasites were measured, and the morphology and sex were studied using a light microscope.

DNA extraction from feces, nasal secretions, and adult parasites

DNA was extracted from feces containing egg deposits, nasal secretions, and adult parasites using a DNA

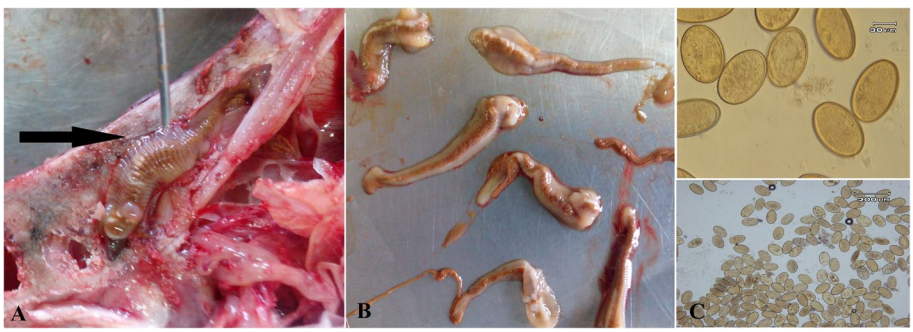

Fig. 1 Adult form of *L. serrata* (female) harvested from infected dog by necroscopy (**A** and **B**); Eggs of *L. serrata* harvested from infected dog by microscopic method (**C**)

isolation kit (MBST, Iran) according to the manufacturer's protocol. In brief, individual parasites, 50 ml of feces containing egg deposits, or nasal secretions were lysed in 180 µl lysis buffer and 20 µl proteinase K (10 mg/ml) for 20 min to 2 h at 55 °C. Subsequently, 360 µl binding buffer was added, and the mixture was incubated for 10 min at 70 °C. Next, 270 µl ethanol (96–100%) was added to the solution, vortexed, and transferred to the MBST column. The column was centrifuged at 8000 × g, washed twice with 500 µl washing buffer, and then centrifuged at 12,000 × g. DNA was eluted from the column using 100 µl elution buffer. The extracted DNA was visualized on 0.8% agarose gel stained with ethidium bromide and observed under a UV transilluminator. The DNA concentration was quantified by measuring absorbance at 260 nm (A260). DNA was either used immediately in PCR or stored at −20 °C for future use.

Polymerase chain reaction (PCR), sequencing and phylogenetic analyses

To investigate *Linguatula serrata*, specific primers targeting the 18S small subunit ribosomal RNA (18S rRNA) gene were employed. The primers, 18S-F (5'-CCATGGTTGTCACGGGTGACG-3') and 18S-R (5'-CTTGCGACGATCCAAGAATTT-3'), were designed based on complete 18S rRNA sequences available in GenBank (accession no. JX088397).

PCR amplification was performed in 25 µL reaction volumes using an I-Cycler thermal cycler (Bio-Rad). The reaction mixture consisted of: 1 µL of genomic DNA (5 ng), 25 µM of each primer, 1.5 mM $MgCl^2$, 1250 µM of each dNTP, 1 × GoTaq® Green Flexi Reaction Buffer, and 1 U of GoTaq DNA polymerase (Promega, USA). The thermal cycling protocol included an initial denaturation at 94 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 10 s. A final extension step at 72 °C for 1 min completed the protocol.

The amplified PCR products were resolved by 2% agarose gel electrophoresis, stained with DNA-safe stain (Yekta Tajhiz Azma, Iran; Cat no. YT0001), and visualized using a Gel Documentation System (Axygen®, Germany). PCR amplicons were purified with the QIAquick® PCR Purification Kit (QIAGEN) according to the manufacturer's instructions. The purified PCR products were submitted to Bioneer Co. (Korea) for sequencing. Sequence analysis and phylogenetic studies were conducted using MEGA software (version 11.0.13). The neighbor-joining method was employed to construct phylogenetic trees, with bootstrap analysis performed using 1,000 replicates to ensure robustness.

L. serrata sequences were aligned with reference strains retrieved from GenBank to confirm identity and infer evolutionary relationships. The final sequence data were submitted to the NCBI GenBank database under the accession number OR125935.1.

Calculation of the agreement of two tests

The following formula was used to calculate the agreement between the two tests [31, 34, 35]. If the Kappa value was less than 0.2, 0.2–0.4, 0.4–0.6, 0.61–0.8, and more than 0.8, the agreement between the two tests is considered weak, moderate, or almost good, good and very good, respectively [36, 37].

$$p_{Exp} = \frac{(n_{00} + n_{01})(n_{00} + n_{10})}{n^2} + \frac{(n_{11} + n_{10})(n_{11} + n_{01})}{n^2}; p_{Agree} = \frac{n_{00} + n_{11}}{n}$$

$$\kappa = \frac{p_{Agree} - p_{Exp}}{1 - p_{Exp}}$$

where P_{Exp}: Chance agreement; P_{Exp}: True agreement, n_{00}: The number of animals that were not infected in both tests; n_{11}: Number of animals infected in both tests; n_{10} and n_{01}: The number of animals that were infected by one test and uninfected by another test and K: agreement of two tests or Kappa.

Statistical analysis

Associations between host factors (age and sex) and prevalence of infection were evaluated using the chi-square test with SPSS software version 21. Differences were considered significant at a P-value < 0.05.

Results

Prevalence of *L. serrata* in road-killed and stray dogs

Of the 250 dogs studied, 174 (69.6%) tested positive for *L. serrata* based on at least one of the detection method. The prevalence rates in road-killed and stray dogs were 72.0% and 68.0%, respectively (Table 1). In road-killed dogs, the highest infection rate was observed with fecal PCR (59.0%), while the lowest was detected using nasal secretion microscopy (14.0%). Similarly, in the stray dogs, the highest infection rate was found with fecal PCR (48.7%), and the lowest with nasal secretion microscopy (26.0%). Infection rates in both road-killed and stray dogs, as determined by all diagnostic methods except for nasal secretion microscopy in road-killed dogs showed a statistically significant correlation with age and sex, with infection rates increasing with age and being higher in males than in females.

Table 1 Rate of infection with *L. serrata* in road-killed and stray dogs based on age and sex using different diagnostic methods

	Method	Age (number positive; %)				Sex (number positive; %)			Total (*n* = 100)
		< 6 month (*n* = 20)	6 to > 24 month (*n* = 35)	2 to 4 years (*n* = 45)	*P*-value	Male (*n* = 64)	Female (*n* = 34)	*P*-value	
Road killed dogs (*n* = 100)	Micro fecal	2; 10.0	9; 25.7	18; 40	0.042	24; 37.5	5; 13.9	0.010	29; 29.0
	Micro NS	1; 5.0	3; 8.6	10; 22.2	0.094	11; 17.2	3; 8.3	0.179	14; 14.0
	Necropsy	3; 15.0	25; 71.4	18; 40.0	0.000	35; 54.7	11; 30.6	0.017	46; 46.0
	PCR fecal	8; 40.0	19; 54.3	32; 71.1	0.049	43; 67.2	16; 44.4	0.023	59; 59.0
	PCR NS	3; 15.0	17; 48.6	24; 53.3	0.013	34; 53.1	10; 27.8	0.012	44; 44.0
	Total	7; 35.0	28; 80.0	37; 82.2		50; 78.1	22; 64.7		72; 72.0
	Method	**< 6 month (*n* = 39)**	**6 to > 24 month (*n* = 50)**	**2–4 years (*n* = 61)**	***P*-value**	**Male (*n* = 93)**	**Female (*n* = 57)**	***P*-value**	**Total (*n* = 150)**
Stray dogs (*n* = 150)	Micro fecal	14; 35.9	30; 60.0	26; 42.6	0.055	53; 57.0	17; 29.8	0.001	70; 46.7
	Micro NS	3; 7.7	19; 30.0	17; 27.9	0.005	30; 32.3	9; 15.8	0.019	39; 26.0
	PCR fecal	10; 25.6	25; 50.0	38; 62.3	0.002	59; 63.4	14; 24.6	0.000	73; 48.7
	PCR NS	6; 15.4	17; 34.0	42; 68.9	0.000	47; 50.5	18; 31.6	0.017	65; 43.3
	Total	21; 53.8	32; 64.0	49; 80.3		73; 78.4	29; 50.8		102; 68.0

Micro fecal Microscopy of feces, *Micro NS* Microscopy of nasal secretion, *PCR NS* PCR of nasal secretion

Parasite distribution and necropsy findings

The number of parasites recovered from each road-killed dog ranged from 1 to 4, with an average of 2 *L. serrata* per infected dog. In 43 of the 150 road-killed dogs, the parasite was found on the left side of the skull, compared to 20 on the right side and 19 with parasites on both sides of the skull. No parasites were detected in the eustachian tubes, maxillary sinuses, and brain cavities.

Results of diagnostic method comparison

Good agreement was observed between the PCR of nasal secretions and necropsy with a kappa value of 0.6. In contrast, the kappa value in the two tests microscopic examination of nasal secretions versus necropsy and microscopic examination of feces versus necropsy were −0.09. and −0.16, respectively (Table 2).

Findings of molecular identification and phylogenetic analysis

PCR amplicon of the expected size for *Linguatula* was approximately 595 bp for 18S rRNA gene. One positive sample was confirmed by sequencing. Phylogenetic analysis based on the 18S rRNA nucleotide sequence revealed no significant difference between Iranian isolates (Fig. 2). Tabriz-HG (accession number: OR125935.1) exhibited the highest nucleotide sequence similarity to IR-18 s-Cattle-Kerman (accession number: KU234183.1) and IR-4-Sheep-Tabriz (accession number: KF830130.1) from Iran (Tables 3 and 4).

Table 2 The results of Kappa in two tests

Road killed dogs	Number of infected and/or uninfected dogs				Kappa
	n_1n_1	n_0n_0	n_1n_0	n_0n_1	
Microscopy fecal versus PCR fecal	29	41	0	30	0.44
Microscopy nasal secretion versus PCR nasal	14	61	0	25	0.41
Microscopy fecal versus Autopsy	9	34	20	37	−0.16
Microscopy nasal secretion versus Autopsy	2	42	12	40	−0.09
PCR nasal secretion versus Autopsy	38	48	6	8	0.6*
PCR fecal versus Autopsy	34	29	25	12	0.27
Stray dogs					
Microscopy fecal versus PCR fecal	50	57	20	23	0.30
Microscopy nasal secretion versus PCR nasal	35	81	4	30	0.52

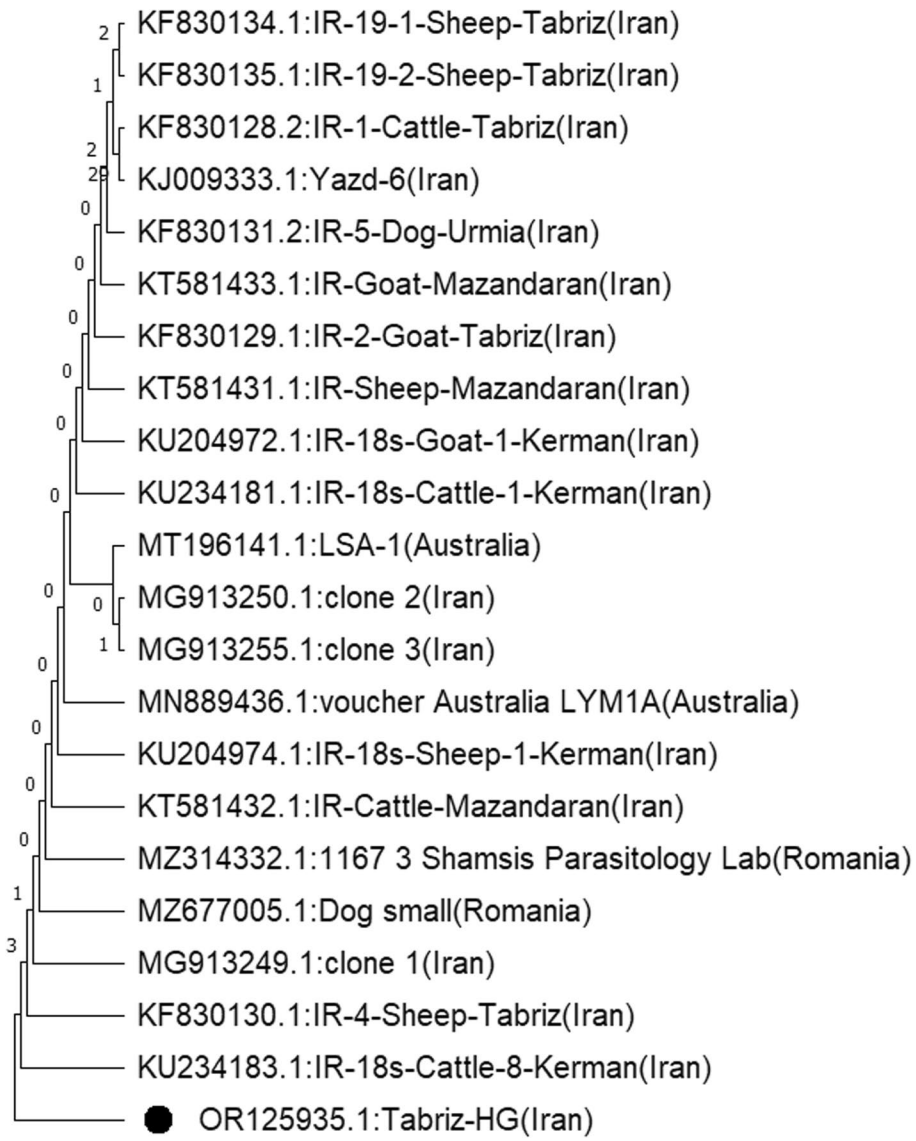

Fig. 2 Phylogenetic relationship of *L. serrata* isolates based on partial sequence of 18S rRNA gene. The numbers at nodes represent bootstrap values based on 500 replicates

Table 3 Data for some *Linguatula serrata* isolates used in this study

Isolate (strain)	Country	GenBank (accession number)
IR-18s-Cattle-Kerman	Iran	KU234183.1
Yazd-6	Iran	KJ009333.1
IR-4-Sheep-Tabriz	Iran	KF830130.1
Clone_1	Iran	MG913249.1
voucher_Australia_LYM1A	Australia	MN889436.1
LSA-1	Australia	MT196141.1
Dog_small	Romania	MZ677005.1
1167_3_Shamsis_Parasitology_Lab	Romania	MZ314332.1

Discussion

The parasitic disease linguatulosis, which is brought on by *L. serrata*, affects both animal and human populations' public health. Although most cases of the infection are subclinical, reports of severe rhinitis, bloody nasal discharge, and sporadic coughing and sneezing have been made [33]. Depending on whether the animal species is the definitive or intermediate host, different diagnostic techniques are used to identify *L. serrata* infections. The majority of studies that determine the infection rate in definitive hosts, dogs, use necropsy or identify eggs in feces or nasal swabs [21]. Our results indicate that PCR may be a more successful technique for identifying

Table 4 Percent identity of partial nucleotide sequences of the 18S rRNA gene between 22 *L. serrata* isolates

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	OR125935.1	100																					
2	MT196141.1	99.87	100																				
3	MN889436.1	99.87	100	100																			
4	KU234181.1	99.87	100	100	100																		
5	KU204974.1	99.87	100	100	100	100																	
6	KU204972.1	99.87	100	100	100	100	100																
7	KT581432.1	99.87	100	100	100	100	100	100															
8	KT581431.1	99.87	100	100	100	100	100	100	100														
9	MZ314332.1	99.87	100	100	100	100	100	100	100	100													
10	KF830134.1	99.87	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	100												
11	KF830129.1	99.87	100	100	100	100	100	100	100	100	99.99	100											
12	MZ677005.1	99.87	100	100	100	100	100	100	100	100	99.99	100	100										
13	KT581433.1	99.87	100	100	100	100	100	100	100	100	99.99	100	100	100									
14	KU234183.1	99.87	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	100								
15	KF830131.2	99.87	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	100							
16	KF830135.1	99.87	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	100	99.99	99.99	100						
17	KF830128.2	99.87	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	100					
18	MG913250.1	99.87	100	100	100	100	100	100	100	100	99.99	100	100	100	99.99	99.99	99.99	99.99	100				
19	KF830130.1	99.87	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	99.99	100			
20	MG913249.1	99.87	100	100	100	100	100	100	100	100	99.99	100	100	100	99.99	99.99	99.99	99.99	100	99.99	100		
21	MG913255.1	99.87	100	100	100	100	100	100	100	100	99.99	100	100	100	99.99	99.99	99.99	99.99	100	99.99	100	100	
22	KJ009333.1	99.86	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	99.99	100	100	100

infection in living dogs, especially when applied to nasal secretions. By using microscopy for direct egg screening, the risk of exposure to humans may be underestimated due to the underestimation of dog prevalence.

According to the data that is being given today, stray dogs have a relatively high infection rate. The close contact of dogs and domestic animals is the main cause of the high occurrence of linguatulosis in dogs. The main source of infection for domestic dogs is the offal of ruminant domestics; in addition, eating raw sheep, goats, and other herbivorous animal viscera can cause a high rate of pentastomosis in stray dogs. The parasite spreads more easily to humans and other animals because there are a lot of stray dogs that live as scavengers and have access to offal from slaughterhouses or the carcasses of infected herbivores. The practice of feeding dogs the raw viscera of home-slaught animals and the high frequency of domestic or occupational interaction between dogs and livestock provide ideal conditions for the continuation of the parasite life cycle [18, 22]. Additionally, the care of domestic animals may encourage the pollution of feed and water meant for animals with *L. serrata* eggs by dog feces.

Our research revealed significant variations in infection rates among dogs of varying ages and sexes. The infection rate was modest in puppies under six months old, but it rose as they aged. The highest occurrence rate was reported in canines older than four years. These findings contradict those of another study, which found no significant variation in the rate of infection among stray dogs of different sexes and ages in Shiraz, Iran [22]. The absence of linguatulosis in newborn puppies has been linked to their long maturation time (> 6 months) [38] and feeding on mother's milk, which eliminates the need to give them infected ruminant offal [23].

Female dogs exhibited a lower infection rate in the study presented here than male dogs, which was similar to the study conducted by [23]. However, there was no significant difference between the sexes among the age groups. As a result, the difference could be attributed to the inclusion of more male canines in the study.

In the present study, we molecularly evaluated samples to confirm the presence of *L. serrata*, and a phylogenetic tree was constructed to investigate the relationship between isolates of Iranian origin. Our isolate, Tabriz-HG (accession number: OR125935.1), was found to be 99.86% to 99.87% identical to other isolates from Iran, including those from Yazd (KJ009333.1), IR-4-Sheep-Tabriz (KF830130.1), and clone-1 (MG913249.1).

Additionally, the Tabriz-HG isolate exhibited high nucleotide similarity (99.87%) not only to Iranian isolates but also to isolates from other countries, such as Australian isolates (MN889436.1 and MT196141.1) and Romanian isolates (MZ677005.1 and MZ314332.1) [39, 40]. These isolates have been derived from different animal species, such as cattle, sheep, and other ruminants, further supporting the hypothesis of genetic continuity and limited genetic diversity of *L. serrata* across different host species. This suggests that *L. serrata* may exhibit relatively stable genetic characteristics regardless of the host species or geographic location.

In our study, the genetic differences observed between isolates within Iran and those from other countries are minimal, which could be attributed to the high degree of host specificity of the parasite and its relatively low mutation rate over geographic distances. The minimal genetic variation in these isolates may also reflect shared environmental and ecological factors that influence the parasite's transmission dynamics across different regions.

These results align with previous studies on *L. serrata* from other countries, which have found similar high nucleotide identities across isolates from various animal species. For instance, studies on *L. serrata* isolates from ruminants in Europe, Asia, and Australia have shown identical or near-identical nucleotide sequences for certain genes, suggesting that the parasite's genetic makeup remains largely conserved, regardless of host species or geographic location [41, 42].

Based on the analysis of these sequences, the genetic differences among *L. serrata* isolates, both within Iran and internationally, appear to be relatively minor, indicating a degree of genetic stability and a lack of significant genetic differentiation among isolates across different regions and host species.

Conclusion

The high prevalence of *L. serrata* found in dogs in this study indicates that the human population of Tabriz is at a potentially high risk of *L. serrata* exposure. Although linguatulosis is difficult to diagnose in dogs, with PCR being the preferred method based on the study results presented herein, it should not be ignored. A strict control program conducted by the city authorities and education of the population on the epidemiology of the parasite could reduce the infection risk. Any measures taken in this direction may also help to decrease other zoonoses in which dogs play a role. Regular molecular monitoring of parasitic isolates circulating within a region should be done to continuously have accurate and updated information on the status of pathogens. Examining more samples collected from different hosts and regions will help to better understand the possible distribution of isolates and their evolution.

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Authors' contributions
N. H. Conceptualization, Supervision, Methodology, Project administration, Investigation, Writing- Original Draft preparation, Visualization; J. K. Conceptualization, Methodology, Formal analysis, Writing - Review & Editing and M. G.. Methodology.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. As fecal samples were collected from the rectum of the animals and stray animals were used, approval by the ethical committee of the Animal Welfare Committee Ferdowsi University of Tabriz was required as well as permission from the Tabriz Environment Protection Organization and Bureau of Veterinary Medicine (Letter Number 2312). Informed consent was obtained from the animal shelter from where the stray dogs were sourced.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Hendrix CM. Diagnostic veterinary parasitology. 2nd edition. USA: Mosby St. Louis, Mo; 1998. p. 220-238.
2. Hajipour N, Tavassoli M. 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 Reports. 2019;16: 100288.
3. Tavassoli M, Tamaddonfard E, Mirshekar F, Hajipour N, Erfanparast A. A behavioral evaluation of the effects of ingestion of *Linguatula serrata* nymphs in rats. Vet Parasitol. 2018:254:78–81.
4. Kheirabadi KP, Fallah AA, Azizi H, Samani AD, Dehkordi SD. Prevalence of *Linguatula serrata* nymphs in slaughtered sheeps in Isfahan province, southwest of Iran. J Parasit Dis. 2015;39:518–21.
5. Khalil G, Haddad C, Otrock ZK, Jaber F, Farra A. Halzoun, an allergic pharyngitis syndrome in Lebanon: the trematode *Dicrocoelium dendriticum* as an additional cause. Acta Trop. 2013;125:115–8.
6. Sarmadian H, Nasiri Z, Saeedinia S, Moradi Y, Eshrati B, Ghasemikhah R, et al. Clinical manifestation and epidemiological findings of human *Linguatula serrata* infection in Iran: systematic review. J Parasit Dis. 2021;45:50–8.
7. Mateva SA, Nikolova MR, Karaivanov MP, Marinova PE. Rare case of human visceral linguatuliasis in Bulgaria diagnosed on biopsy specimen. J Biomed Clin Res. 2013;6:131–4.
8. Tappe D, Buttner DW. Diagnosis of human visceral pentastomiasis. PLoS Negl Trop Dis. 2009;3: e320.
9. Yazdani R, Sharifi I, Bamorovat M, Mohammadi MA. Human linguatulosis caused by *Linguatula serrata* in the city of Kerman, south-eastern Iran-Case report. Iran J Parasitol. 2014;9:282.
10. Anaraki Mohammadi G, Mobedi I, Ariaiepour M, Pourmohammadi Z, Zare BM. A Case report of nasopharyngeal linguatuliasis in Tehran, Iran and characterization of the isolated *Linguatula serrata*. Iran J Parasitol. 2008;3:53–5.
11. Hamid T, Hossein YD, Bahadoran-Bagh-Badorani Mehran FS, Masood EH. A case report of Linguatula serrata infestation from rural area of Isfahan city, Iran. Adv Biomed Res. 2012:1-3.
12. Janbakhsh A, Hamzavi Y, Babaei P. The first case of human infestation with *Linguatula serrata* in Kermanshah province. J Kermanshah Univ Med Sci (J Kermanshah Univ Med Sci). 2015;19:58–61.
13. Montazeri A, Gamali R, Kazemi A. A case report of Halzon syndrome. Tehran Univ Med J. 1997;55:20–4.
14. Sadjjadi SM, Ardehali SM, Shojaei A. A case report of *Linguatula serrata* in human pharynx from Shiraz, southern Iran. Med J Islam Repub Iran. 1998;12:193–4.
15. Siavoshi MR, Asmar M, Vatankhah A. Nasopharyngeal pentastomiasis (Halzoun): report of 3 cases. Iran J Med Sci. 2002;27:191–2.
16. Islam R, Anisuzzaman, Hossain MS, Alam Z, Islam A, Khan AHNA, et al. Linguatula serrata, a food-borne zoonotic parasite, in livestock in Bangladesh: Some pathologic and epidemiologic aspects. Vet Parasitol Reg Stud Rep. 2018:13:135–40.
17. Shamsi S, Halajian A, Barton DP, Zhu X, Smit WJ, Roux F, et al. Occurrence and characterisation of tongue worms in South Africa. Int J Parasitol Parasites Wildl. 2020;11:268–81.
18. Hajipour N, Tavassoli M, Eslamlo AA, Javadi SH. Investigation of histopathological changes caused by adult stage of *Linguatula serrata* in dog. Comp Clin Path. 2018;27:717–20.
19. Rasoli S, Amniattalab A, Sadagian M, Haji KB, Azizpour SA, Jafari K. A servey on the prevalence rate of *Linguatula serrata* in stary dogs of the city of Urimia. Vet J Islam Azad Univ Tabriz. 2010;4:765–71.
20. Bahrami AM, Yousofzadeh S, Kermanjani A. Study of *Linguatula serrata* infection rate among shepherd and stray dogs in ILAM (Western IRAN). J ilam Univ Med Sci. 2011;19:60–5.
21. Meshgi B, Asgarian O. Prevalence of *Linguatula serrata* infestation in stray dogs of Shahrekord. Iran J Vet Med B Infect Dis Vet Public Health. 2003;50:466–7.
22. Oryan A, Sadjjadi SM, Mehrabani D, Rezaei M. The status of *Linguatula serrata* infection of stray dogs in Shiraz. Iran Comp Clin Path. 2008;17:55–60.
23. Rezaei F, Tavassoli M, Mahmoudian A. Prevalence of *Linguatula serrata* infection among dogs and domestic ruminants in North West of Iran. Vet Med (Praha). 2011;56:561–7.
24. Aldemir OS. The distribution of *Linguatula serrata* in stray dogs in the Erzurum Province. Turkish J Parasitol. 2004;28:42–4.
25. Khalil GM. Incidence of *Linguatula serrata* infection in Cairo mongrel dogs. J Parasitol. 1970;56:485.
26. Soriano SV, Pierangeli NB, Roccia I, Bergagna HFJ, Lazzarini LE, Celescinco A, et al. A wide diversity of zoonotic intestinal parasites infects urban and rural dogs in Neuquén, Patagonia. Argentina Vet Parasitol. 2010;167:81–5.
27. Shamsi S, McSpadden K, Baker S, Jenkins DJ. Occurrence of tongue worm, *Linguatula* cf. *serrata* (Pentastomida: Linguatulidae) in wild canids and livestock in south-eastern Australia. Int J Parasitol Parasites Wildl. 2017;6:271–7.
28. Pandey VS, Dakkak A, Elmamoune M. Parasites of stray dogs in the Rabat region. Morocco Ann Trop Med Parasitol. 1987;81:53–5.
29. Haralabidis ST, Papazachariadou MG, Koutinas AF, Rallis TS. A survey on the prevalence of gastrointestinal parasites of dogs in the area of Thessaloniki. Greece J Helminthol. 1988;62:45–9.
30. Oluwasina OS, ThankGod OE, Augustine OO, Gimba FI. *Linguatula serrata* (Porocephalida: Linguatulidae) infection among client-owned dogs in Jalingo. North Eastern Nigeria: Prevalence and public health implications. J Parasitol Res; 2014. p. 2014.
31. Motamed N, Zamani F. Sample size in medical research: a practical approach. 1st edition. Tehran: Aser Roshanbini; 2016. p. 120-150.
32. Getty R. Sisson and Grossman's the Anatomy of the Domestic Animals. 5th. Philadelphia: Saunders; 1975. p. 180-200.

33. Soulsby EJL. Helminths, arthropods and protozoa of domesticated animals. Philadelphia: Lea and Febiger; 1982.
34. Cohen J. A co-efficient of agreement for norminal scales. Educ Psychol Meas. 1960;20:111–6.
35. Whitley E, Ball J. Statistics review 4: sample size calculations. Crit care. 2002;6:1–7.
36. Landis JR, Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33:159–74.
37. Kundel HL, Polansky M. Measurement of observer agreement. Radiology. 2003;228:303–8.
38. Tavassoli M, Javadi Sh, Hadian M. Experimental infection and study of life cycle of *Linguatula serrata* in dogs. J Vet Res. 2001;56:1–3.
39. Berberich M, Grochow T, Roßner N, Schmäschke R, Rentería-Solís Z. *Linguatula serrata* in an imported dog in Germany: Single-case or emerging disease? Vet Parasitol Reg Stud Reports. 2022;30: 100717.
40. Shamsi S, Barton DP, Zhu X, Jenkins DJ. Characterisation of the tongue worm, *Linguatula serrata* (Pentastomida: Linguatulidae), in Australia. Int J Parasitol Parasites Wildl. 2020;11:149–57.
41. Ghorashi SA, Tavassoli M, Peters A, Shamsi S, Hajipour N. Phylogenetic relationships among *Linguatula serrata* isolates from Iran based on 18S rRNA and mitochondrial cox1 gene sequences. Acta Parasitol. 2016;61:195–200.
42. Sudan V, Shanker D, Jaiswal AK. First report of molecular characterization and sequence phylogenetic analysis of *Linguatula serrata* from India. Acta Parasitol. 2018;63:781–3.

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