Prevalence and Molecular Characterization of *Trichinella* Species in Wild Boar (*Sus scrofa*) Populations of Khuzestan Province, Iran

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Abstract

Trichinellosis is a zoonotic parasitic disease caused by nematodes of the genus *Trichinella*, maintained primarily through a sylvatic cycle involving wildlife reservoirs. Wild boars (*Sus scrofa*) play a key epidemiological role in sustaining transmission and represent a potential source of human infection through the consumption of undercooked meat. This study aimed to detect and molecularly characterize *Trichinella* species in wild boars from Khuzestan Province, southwestern Iran, during 2022–2023. Muscle samples from five anatomical sites (tongue, larynx, diaphragm, heart, and mediastinum) and blood samples were collected from 36 wild boars. Parasitological examination was performed using artificial enzymatic digestion, while molecular detection targeted the mitochondrial small subunit ribosomal RNA gene (rrnS) using polymerase chain reaction (PCR). Serological analysis was conducted using a commercial ELISA kit to detect anti-*Trichinella* IgG antibodies.

No larvae were detected by enzymatic digestion (0%). However, PCR analysis identified *Trichinella britovi* DNA in 9 of 36 animals (25.0%), with positive samples mainly from tongue muscle, followed by diaphragm and heart tissues. Eleven tissue samples were PCR-positive, and two animals showed multi-tissue involvement. ELISA revealed a high seropositivity rate (86.1%),

indicating widespread exposure within the wild boar population. Sequence analysis of PCR products confirmed 100% homology with *T. britovi* reference sequences in GenBank.

The discrepancy between digestion and molecular findings suggests low-intensity or unevenly distributed infections, highlighting the higher sensitivity of molecular methods for detecting minimal parasite burdens. The predominance of *T. britovi* supports the existence of an active sylvatic transmission cycle in Khuzestan. These findings emphasize the zoonotic risk associated with wild boar meat consumption and underscore the need for continued surveillance and public health awareness programs targeting hunters and high-risk populations.

Keywords: *Trichinella britovi*; wild boar; molecular detection; Khuzestan Province

Introduction

Trichinellosis, a significant zoonotic disease caused by parasitic nematodes of the genus *Trichinella*, continues to pose substantial public health risks and economic challenges worldwide (1). The disease's transmission cycle involves both domestic and wild animals, with swine and carnivores serving as primary reservoir hosts. While the sylvatic cycle of *Trichinella* species has been extensively documented across all continents except Antarctica since the 19th century (2), significant gaps remain in our understanding of its current epidemiology in many regions (3).

Global epidemiological data of *Trichinella* infection an annual incidence rate of 469.2 to 985.3 cases per billion people, with mortality rates ranging from 0.3 to 0.8 per billion annually. Although wildlife maintains the primary reservoir for *Trichinella* infections, the spillover to domestic animals presents significant challenges for disease control in endemic regions.

The pathogenesis of trichinellosis begins when larvae-infected muscle tissue from host animals such as pigs, wild boars, and rodents is consumed. The ingested larvae develop into adult worms in the small intestine and produce new larvae that migrate via the bloodstream to various tissues, predominantly targeting muscle. Their migration leads to muscle inflammation and calcified cyst formation, particularly in the abdominal wall, tongue, and larynx (4). While the disease prevalence remains low in Islamic countries due to religious dietary restrictions on pork consumption, it affects up to 12% of the human population in certain regions of Europe and South America (5).

In Iran, while human cases remain sporadic, infections have been documented in wildlife, particularly among carnivores and wild boars, across various regions including the Caspian area, Isfahan, Ardabil, Khuzestan, Khorasan Razavi, and Bandar Abbas (6). The first human case of trichinellosis was reported in 1965, suggesting the existence of a sylvatic *Trichinella* cycle within the country's wildlife (7). Subsequent investigations, particularly in the northern provinces, have corroborated these findings (8, 9). A significant milestone in Iranian trichinellosis surveillance occurred in 2008 with the first documented family outbreak, attributed to the consumption of infected wild boar meat (10).

Khuzestan Province, located in southwestern Iran, represents a critical area for studying *Trichinella* species due to its diverse wildlife and suitable ecological conditions. Previous studies have identified *Trichinella britovi* in the region's wild boar populations, emphasizing the zoonotic potential and necessity for continued surveillance. Modern molecular techniques, particularly PCR-based methods targeting the 5S ribosomal DNA intergenic spacer region (5S ISR) and mitochondrial large subunit ribosomal DNA (mt-lsrDNA), have proven effective for species identification and phylogenetic analysis across all 12 *Trichinella* genotypes. These markers have facilitated the identification of novel species or genotypes in various hosts, including crocodiles in Zimbabwe and pumas in Argentina (11).

This study aims to employ PCR and traditional enzymatic digestion methods, to identify and characterize *Trichinella* species in wild boar populations of Khuzestan Province during 2022-2023.

Materials and Methods

Study Area and Sample Collection

This study was conducted in Khuzestan Province, southwestern Iran, following approval from the Ethics Committee of Jundishapur University of Medical Sciences, Ahvaz (IR.AJUMS.MEDICINE.REC.1401.079). Thirty-six wild boars (*Sus scrofa*) were captured from sugarcane fields in collaboration with the Veterinary Faculty and licensed hunters affiliated with the Khuzestan Veterinary Network, and were humanely euthanized by licensed hunters using a single, close-range gunshot to the vital thoracic area, in accordance with national wildlife control regulations. During necropsy, tissue samples were collected from five anatomical sites: tongue, larynx, diaphragm, heart, and mediastinum. All samples were immediately snap-frozen in liquid nitrogen (-196°C) and transported to the laboratory under controlled conditions. Samples were stored at -70°C until further analysis.

Parasitological Examination

Muscle samples (1-2 g) from each anatomical site were examined for *Trichinella* larvae using the artificial digestion method (12). Briefly, tissues were finely minced and digested in 20-30 mL of pepsin-hydrochloric acid solution (0.5% pepsin, 1.5% HCl) at 37°C for 24 hours. Post-digestion, samples were homogenized and microscopically examined for the presence of *Trichinella* larvae under light microscopy.

Molecular Analysis

DNA Extraction

Genomic DNA was extracted using a commercial kit (Sinaclon, Cat. No: EX6011) following the manufacturer's protocol with modifications (13). Briefly, 1-3 g of each sample was centrifuged (30,000 rpm, 5 min), and the resultant pellet was washed thrice with PBS buffer (1,500 × g, 3 min, 4°C). Sample lysis was performed using 400 µL lysis buffer, followed by the addition of 300 µL precipitation solution. The mixture was transferred to filter microtubes and centrifuged (12,000-13,000 rpm). After sequential washing steps with wash buffers I and II, DNA was eluted with 50 µL pre-heated (65°C) elution buffer. The final DNA preparation was obtained by centrifugation at 13,000 rpm and stored at -20°C.

PCR Amplification

PCR amplification targeted a fragment of the mitochondrial small subunit ribosomal RNA gene (rrnS) of *Trichinella* spp. PCR reactions were performed in 25 µL volumes containing 12.5 µL Master mix [Amplicon], 1 µL each of forward (5′-CATGGTTAGGTGAGATATTGCCTGC-3′) and reverse (5′-GGTCCTCCTTCCAGAAGATCTACTTTG-3′) primers (14), 5 µL DNA template, and 5.5 µL sterile deionized water. Amplification was conducted under the following conditions: initial denaturation at 94°C for 7 min; 45 cycles of denaturation (94°C, 1 min), annealing (60°C, 30 sec), and extension (72°C, 1 min); followed by a final extension at 72°C for 10 min. The PCR products were analyzed by 1.5% agarose gel electrophoresis, stained with ethidium bromide, and visualized under UV illumination.

PCR products were subjected to unidirectional sequencing (Takapoo Zist Company). The resulting sequences were compared with reference sequences deposited in the NCBI GenBank database for species identification and phylogenetic analysis.

Serological Analysis

Blood samples were collected from wild boars immediately after euthanasia by cardiac puncture using sterile syringes and transferred into plain vacuum tubes without anticoagulant. The samples were allowed to clot at room temperature and then centrifuged at 3,000 rpm for 10 min to separate the serum. The obtained sera were aliquoted and stored at −20°C until serological analysis.

Trichinella spiralis-specific IgG antibodies were detected in wild boar serum samples using a commercial ELISA kit (Cortez ELISA IgG Kit, Cat No. 8207-35) according to the manufacturer's instructions. Absorbance measurements were performed using a Stat Fax 4200 ELISA reader.

Results

Enzymatic Digestion Findings

No *Trichinella* larvae were detected through the enzymatic digestion method in any of the 36 wild boar tissue samples examined (0%). All sediment samples obtained after artificial digestion were carefully examined under light microscopy at ×40 and ×100 magnification by two independent observers. Despite thorough microscopic evaluation, no Trichinella larvae were observed in any of the examined preparations.

Molecular Detection

PCR analysis identified *T. britovi* in 9 out of 36 wild boars (25.0%). While the total number of positive PCR samples was 11, two animals showed co-infection in multiple tissues, accounting for the difference between sample positivity and animal positivity rates. The anatomical distribution of positive samples was: tongue muscle (8 samples), diaphragm (2 samples), and heart muscle (1 sample). Among the nine infected animals, seven had single-tissue infections, while two demonstrated multi-tissue involvement with positive results in both tongue and diaphragm muscles. All positive samples displayed characteristic amplification bands at 195 bp (Figure 1; Table 1).

Serological Analysis

ELISA testing revealed *Trichinella* antibodies in 31 of the 36 wild boars (86.1%). The high seropositivity rate suggests extensive exposure to *Trichinella* within the sampled population, with significantly higher detection rates compared to molecular methods.

Sequence Analysis

Sequencing was performed on nine PCR-positive samples, with seven yielding interpretable results. All successfully sequenced samples showed 100% homology with *Trichinella britovi* reference sequences in the GenBank database. Species identification was performed by comparing the obtained rrnS sequences with reference sequences available in the NCBI GenBank database using the BLASTn algorithm. Sequences showing ≥99–100% identity and query coverage with reference *T. britovi* sequences were assigned to this species. No significant similarity was observed with other *Trichinella* species, thereby confirming the species-level identification. Two samples produced illegible sequencing data and were excluded from phylogenetic analysis.

Table 1. Detection of *Trichinella britovi* in Wild Boar Samples Using Different Diagnostic Methods.

Diagnostic Method	Sample Type	Results		Prevalence %
		Total (n)	Positive (n)	
Enzymatic Digestion	All tissues	108	0	0
PCR	Tongue muscle	36	8	22.2
	Diaphragm	36	2	5.6
	Heart muscle	36	1	2.8
ELISA	Blood	36	31	86.1
DNA Sequencing	PCR positive samples	9	7	77.8

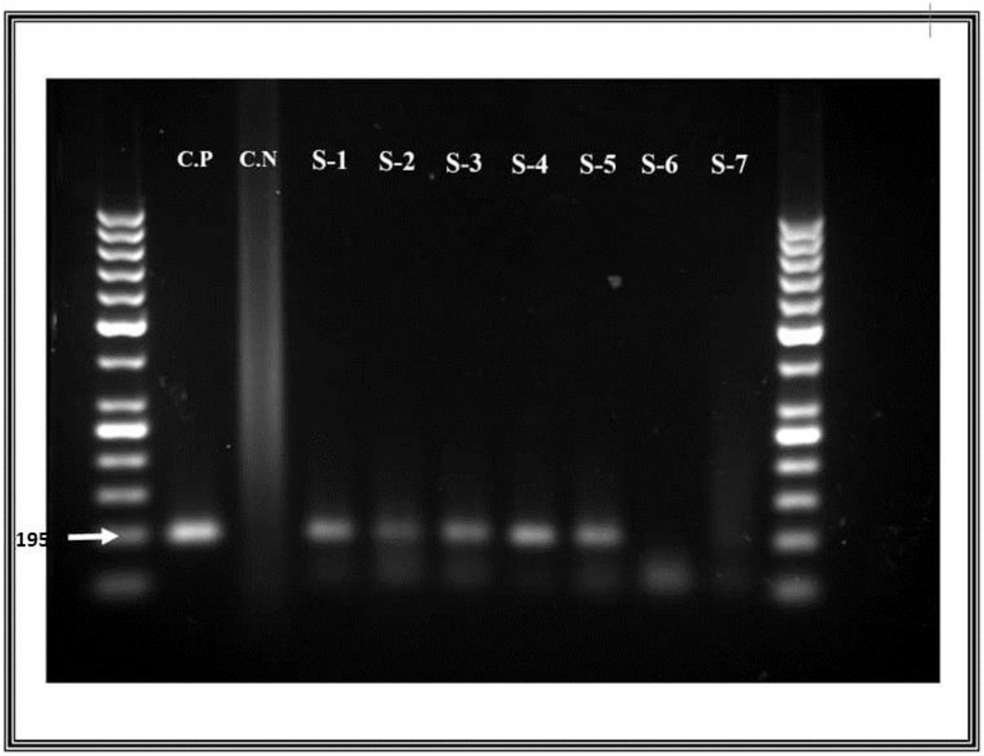

Figure 1. PCR Analysis of *Trichinella* Species from Boar Using a 100 bp Ladder. C.P: Positive Control, C.N: Negative Control, S1-S5: Positive Samples, S6-S7: Negative Samples.

Discussion

The results showed that no *Trichinella* larvae were detected in any of the 36 samples using the pepsin enzymatic digestion method, while the molecular method detected 11 positive samples, confirmed by a 195 bp band on agarose gel electrophoresis. The higher sensitivity of the molecular method compared to enzymatic digestion has been reported in previous studies, confirming its efficacy in detecting low-level infections (15). The absence of detectable larvae by enzymatic digestion despite positive PCR and serological findings may be explained by several factors. First, low-intensity infections with larval burdens below the detection limit of the digestion method may have occurred, as PCR is known to be more sensitive for detecting minimal amounts of parasite DNA. Second, *Trichinella* larvae are unevenly distributed within muscle tissues, and focal localization may result in false-negative digestion outcomes when only limited tissue portions are examined. Third, the presence of degraded or non-viable larvae, particularly in chronic infections, may prevent larval recovery while still allowing molecular detection. In addition, serological positivity reflects previous exposure and may persist even when larval loads have declined. Finally, infections with *T. britovi* are often characterized by lower muscle larval densities compared to *T. spiralis*, which may further reduce the likelihood of larval detection by conventional methods.

The discrepancy between ELISA and molecular findings observed in this study is expected and reflects the inherent differences between serological and molecular diagnostic approaches. Commercial ELISA assays are based on *Trichinella spiralis* excretory–secretory antigens, which are highly conserved among encapsulated *Trichinella* species and therefore detect genus-specific antibodies with known cross-reactivity. In contrast, PCR targets species-specific genetic markers, allowing precise identification of the circulating species. Accordingly, ELISA positivity indicates exposure to *Trichinella* spp., whereas molecular analysis confirms *T. britovi* as the infecting species in wild boars from Khuzestan Province.

These findings highlight the importance of monitoring *Trichinella* infections in local wildlife to ensure public health and safety. In line with the findings of this study, previous research has also demonstrated the presence of *Trichinella britovi* in wildlife. For instance, Mirjalali and colleagues (2014) identified the etiological agent of *Trichinella* species in Khuzestan Province, southwestern Iran, using molecular analyses. They collected muscle samples from 32 roadkill animals, including 14 dogs and 18 golden jackals (*Canis aureus*), and found larvae in two jackals, which were later confirmed to be *Trichinella britovi* through molecular analysis. This study reinforced that *T. britovi* is a dominant species in Iranian wildlife, particularly in Khuzestan (16).

Borji et al. conducted a study in Mashhad, northeastern Iran, to detect *Trichinella* species in local carnivores. They collected muscle tissues from 120 stray dogs, 26 wild boars, 25 rodents, two foxes, and two hyenas. Using artificial digestion and compression techniques, they detected *Trichinella* larvae in three stray dogs and identified them as *T. britovi* via multiplex PCR. This is the first report of the presence of *T. britovi* in stray dogs in Iran (17). These studies underscore the importance of continued monitoring and research into *Trichinella* infections in local wildlife to ensure public health and safety. In 2021, Koohsar et al. conducted a study to investigate the presence of anti-*Trichinella* IgG antibodies among individuals at high risk of exposure in northeastern Iran. The researchers collected blood samples from 189 individuals with a history of consuming wild boar meat and identified 5 (2.6%) positive cases, whereas none of the 30 control participants tested positive. No significant correlation was found between seropositivity and demographic factors. All positive cases were located in the western part of the study area, suggesting a potential risk of trichinellosis from consuming wild boar meat in northern and northeastern Iran (9).

Studies from different regions indicate that wild boars play a key role in maintaining the sylvatic cycle of *Trichinella* spp., with marked variation in prevalence, species composition, and infection intensity depending on geographical and ecological conditions. Surveys conducted in Europe and South America have reported low to moderate prevalence in wild boars, most commonly involving *T. spiralis* and *T. britovi*, alongside occasional detection of *T. pseudospiralis* and mixed infections (18-20). These findings underscore the importance of molecular tools for detecting low-intensity infections that may be missed by conventional digestion methods, as demonstrated by PCR-based approaches with higher sensitivity (15). In agreement with these reports, the present study identified *T. britovi* DNA in wild boars from Khuzestan Province despite the absence of detectable larvae by enzymatic digestion, suggesting low parasite burdens and reinforcing the value of molecular diagnostics for surveillance. The predominance of *T. britovi* in our samples is consistent with previous studies from Iran and other parts of Europe and the Middle East, where this species is considered the dominant agent circulating in sylvatic cycles. Collectively, these data support the existence of an active sylvatic transmission cycle in the study area and highlight the continued risk of zoonotic transmission through the consumption of undercooked wild boar meat.

One limitation of the present study is the inability to perform phylogenetic analysis of the obtained isolates. Although sequence analysis and species identification were conducted using BLAST comparison, the original sequence files were not retained, which precluded the construction of a phylogenetic tree. Future studies should ensure long-term preservation of sequencing data to enable comprehensive phylogenetic and evolutionary analyses.

Conclusion

The present study reveals a relatively high prevalence of *Trichinella britovi* in wild boars in Khuzestan Province, as identified through molecular techniques and sequencing. Given the potential for this parasite to cause disease in humans, it is essential to prioritize preventive measures. Direct measures targeting wild boars are impractical and contradict ethical animal treatment principles. Therefore, health authorities in Khuzestan Province must focus on implementing health programs, managing wild boar populations, and public awareness campaigns, particularly targeting hunters. These initiatives can significantly reduce the prevalence of this infection. Furthermore, public education on the risks of consuming undercooked or raw wild boar meat and promoting safe hunting and meat-handling practices are crucial steps in mitigating the risk of *Trichinella* transmission to humans.

References

1. Pozio E, Gomez Morales MÁ. Trichinella and Trichinellosis: from wildlife to the human beings. Zoonoses: Infections Affecting Humans and Animals: Springer; 2023. p. 529-44.
2. Pozio E. World distribution of Trichinella spp. infections in animals and humans. Veterinary parasitology. 2007;149(1-2):3-21.
3. Eslahi AV, KarimiPourSaryazdi A, Olfatifar M, de Carvalho LMM, Foroutan M, Karim MR, et al. Global prevalence of Trichinella in pigs: A systematic review and meta-analysis. Veterinary Medicine and Science. 2022;8(6):2466-81.
4. Zarlenga DS, Hoberg EP, Thompson P, Rosenthal B. Trichinella: Becoming a parasite. Veterinary Parasitology. 2025;333:110220.
5. Gallardo M, Mateos L, Artieda J, Wesslen L, Ruiz C, García M, et al. Outbreak of trichinellosis in Spain and Sweden due to consumption of wild boar meat contaminated with Trichinella britovi. Euro Surveillance: Bulletin Europeen sur les Maladies Transmissibles= European Communicable Disease Bulletin. 2007;12(3):E070315. 1-E. 1.
6. Borhani M, Fathi S, Harandi MF, Simsek S, Ahmed H, Wu X, et al. Trichinella infections in animals and humans of Iran and Turkey. Frontiers in Medicine. 2023;10:1088507.
7. Moein M. First report of human trichinosis in Iran. J Med the Univ Med School. 1966;5:259-67.
8. Rostami A, Khazan H, Kia EB, Bandehpour M, Mowlavi G, Kazemi B, et al. Molecular identification of Trichinella spp. in wild boar, and serological survey of high-risk populations in Iran. Food Control. 2018;90:40-7.
9. Koohsar F, Naddaf SR, Rokni MB, Mirjalali H, Mohebali M, Shafiei R, et al. Serological detection of trichinellosis among suspected wild boar meat consumers in North and Northeast of Iran. Iranian Journal of Parasitology. 2021;16(2):253.
10. Kia E, Meamar A, Zahabiun F, Soodbaksh A, Kordbacheh P. An outbreak of human trichinellosis due to consumption of boar meat infected with Trichinella sp. Iran J Infect Dis Trop Med. 2008;41:35-8.
11. De Bruyne A, Yera H, Le Guerhier F, Boireau P, Dupouy-Camet J. Simple species identification of Trichinella isolates by amplification and sequencing of the 5S ribosomal DNA intergenic spacer region. Veterinary parasitology. 2005;132(1-2):57-61.
12. Pozio E, Foggin C, Marucci G, La Rosa G, Sacchi L, Corona S, et al. Trichinella zimbabwensis n. sp.(Nematoda), a new non-encapsulated species from crocodiles (Crocodylus niloticus) in Zimbabwe also infecting mammals. International Journal for Parasitology. 2002;32(14):1787-99.
13. Song YY, Zhang XZ, Wang BN, Weng MM, Zhang ZY, Guo X, et al. Molecular characterization of a novel serine proteinase from Trichinella spiralis and its participation in larval invasion of gut epithelium. PLoS Neglected Tropical Diseases. 2023;17(9):e0011629.
14. Cuttell L, Corley SW, Gray CP, Vanderlinde PB, Jackson LA, Traub RJ. Real-time PCR as a surveillance tool for the detection of Trichinella infection in muscle samples from wildlife. Veterinary Parasitology. 2012;188(3-4):285-93.
15. Cuttell L, Corley SW, Gray CP, Vanderlinde PB, Jackson LA, Traub RJ. Real-time PCR as a surveillance tool for the detection of Trichinella infection in muscle samples from wildlife. Veterinary Parasitology. 2012;188(3):285-93.

16. Mirjalali H, Rezaei S, Pozio E, Naddaf SR, Salahi-Moghaddam A, Kia EB, et al. Trichinella britovi in the jackal Canis aureus from south-west Iran. Journal of Helminthology. 2014;88(4):385-8.
17. Borji H, Sadeghi H, Razmi G, Pozio E, La Rosa G. Trichinella infection in wildlife of northeast of iran. Iran J Parasitol. 2012;7(4):57-61.
18. Hidalgo A, Villanueva J, Becerra V, Soriano C, Melo A, Fonseca-Salamanca F. Trichinella spiralis Infecting Wild Boars in Southern Chile: Evidence of an Underrated Risk. Vector-Borne and Zoonotic Diseases. 2019;19(8):625-9.
19. Balić D, Marucci G, Agičić M, Benić M, Krovina Z, Miškić T, et al. Trichinella spp. in wild boar (Sus scrofa) populations in Croatia during an eight-year study (2010–2017). One Health. 2020;11:100172.
20. Kärssin A, Häkkinen L, Vilem A, Jokelainen P, Lassen B. Trichinella spp. in Wild Boars (Sus scrofa), Brown Bears (Ursus arctos), Eurasian Lynxes (Lynx lynx) and Badgers (Meles meles) in Estonia, 2007–2014. Animals. 2021;11(1):183.