Original Article

Serological Detection of Trichinellosis among Suspected Wild Boar Meat Consumers in North and Northeast of Iran

Faramarz Koohsar ¹, *Saied Reza Naddaf ², Mohammad Bagher Rokni ¹, Hamed Mirjalali ³, Mehdí Mohebali ¹, Reza Shafiei ⁴, *Gholamreza Mowlavi ¹,⁵

¹. Department of Medical Parasitology & Mycology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran
². Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran
³. Foodborne and Waterborne Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
⁴. Vector-Borne Diseases Research Center, North Khorasan University of Medical Sciences, Bojnord, Iran
⁵. Center for Research of Endemic Parasites of Iran (CREPI), Tehran University of Medical Sciences, Tehran, Iran

Received 05 Nov 2020
Accepted 18 Feb 2021

Keywords:
Trichinellosis; Wild boar; Seroprevalence; Iran

*Correspondence Email: saiedrezanaddaf@gmail.com molavig@yahoo.com

Abstract

Background: Trichinellosis is a foodborne zoonosis disease worldwide. Humans acquire infection by ingesting raw or uncooked animal flesh containing viable Trichinella larvae. The most common reservoirs of this helminth are pigs and wild boars. In northern Iran, hunting and consuming wild boars meat by some communities, including ethnic Armenians, may expose them to trichinellosis. Here, we investigated anti-Trichinella IgG antibodies in high-risk individuals in northeastern Iran.

Methods: From Mar to Aug 2020, we collected 189 blood samples from individuals with a history of wild boar meat consumption and examined the sera for anti-Trichinella IgG antibodies using a commercial ELISA kit (NovaTec Immunodiagnostica GmbH, Germany). Sera from 30 individuals with no history of eating wild boar meat was used to determine the range of actual negative values and possible cross-reactivity with other similar antigens.

Results: Of the 189 participants, 5 (2.6%) had anti-Trichinella IgG antibodies (OD, 1.176 ±0.154). None of the 30 negative controls became positive (OD, 0.198 ± 0.044). The age, gender, occupation, and education showed no significant association with Trichinella seropositivity rate (P>0.05). All five seropositive cases were among 112 individuals (4.46% seropositivity) that resided in the western part of the study area, stretching from Behshar to Gorgan.

Conclusion: Eating wild boar meat might expose individuals to trichinellosis in the north and northeast of Iran. Further studies with more individuals from different parts of the country and confirmation of the ELISA by additional tests like Western blot will give a more in-depth insight into human trichinellosis epidemiology in Iran.
Introduction

Human trichinellosis is a food-borne zoonosis disease transmitted through ingesting raw or undercooked meat containing viable *Trichinella* larvae (1). This helminth infection ranks the seventh among the ten foodborne parasite infections threatening millions of people worldwide (2). *Trichinella* genus comprises nine species and three genotypes, all infecting mammals, including humans, while one species and two other species also infect birds and reptiles, respectively (3). The species exhibit a cosmopolitan distribution in all the continents but Antarctica and circulate in nature by correlated synanthropic domestic and sylvatic cycles (4). Despite being regarded as a neglected tropical disease, human trichinellosis occurs in 55 countries, specifically in developing countries (5). Over the last decade, human trichinellosis outbreaks have occurred in some countries, including China, Estonia, and the island of bally in Indonesia (1, 6, 7). The infection is considered an emerging or re-emerging zoonotic parasitic disease in several parts of the world (8). Globally, around 11 million people have trichinellosis (9), and annually, an average of 5751 new cases are diagnosed with five deaths (7, 10, 11).

*Trichinella* larvae have been reported in about 100 animal species, including humans. Pigs are the primary source of human trichinellosis (4, 12). However, the flesh of walruses, bears, badgers, horses, dogs, and wild boars, also plays a crucial role (13). This zoonotic disease is a public health concern and an economic threat to porcine husbandry and food safety (9).

*Trichinella* infection in humans exhibits two phases of intestinal and muscular involvement (4). Initial clinical symptoms that appear after ingestion of *Trichinella* larvae are abdominal pain and diarrhea. About two or three weeks post-infection, when the larvae penetrate the muscular tissues, clinical manifestations such as fever, myalgias, peri-orbital edema, allergic skin reactions, myocarditis, and encephalitis may appear (14).

Hunting animals for recreation and the increased traditional habits of eating undercooked and raw meat have introduced wild boar meat as the second source of human trichinellosis (15-19). The wild boar population is increasing rapidly in Iran, mainly due to the religious beliefs that ban this animal meat (19). Various molecular markers have identified *T. britovi* as the only infecting species in wild boars and other animals (13, 20-22). In Iran, some communities like Armenian minorities enjoy hunting wild boars and consuming meat. However, observations suggest that people might also practice illegal hunting and consuming wild boar meat in some regions.

Following detecting *Trichinella* infection in the wildlife, attempts were made to detect the infection in humans. In an exhaustive study from 1967 to 1970, examining 4838 intercostal and diaphragm muscle samples provided by Tehran Legal Medicine Organization revealed no *Trichinella* infection (23). Later, a few reports indicated human trichinellosis following eating wild boar meat (19, 24, 25).

In epidemiological studies, serological assays like ELISA provide reliable tools for detecting anti-*Trichinella*-specific antibodies in humans and animals (1).

Here, we used ELISA to investigate anti-*Trichinella* antibodies among people with a history of consuming wild boar meat in the north and northeast of Iran.

Materials and Methods

Study Population

The study population included individuals from different counties in Golestan Province, north of Iran, and two counties, Bojnourd and Behshar, in adjacent provinces of North
Khorasan and Mazandaran, respectively (Fig. 1). These provinces cover 48000 km² and have a mild subtropical and cold desert climate with an average annual temperature of 16-25 °C.

In these regions, wild boars as vertebrate pests destroy farmlands and crops, and hunting these animals is commonly practiced by local and outside hunters, including Armenian minorities.

**Fig. 1:** Map of Iran and the study area. The values show the number of participants in each county.

**Sample collection**

From Mar to Aug 2020, we collected 189 blood samples from high-risk people who claimed to have practiced eating wild boar meat in a face-to-face interview. The inclusion criterion was eating wild boar meat at least four times during the last year. Most participants had consumed grilled wild boar meat. A questionnaire form addressing sociodemographic variables, including education, age, occupation, and gender, was filled out for each individual. Amounts of 2 ml of the blood were obtained from the participants by venipuncture. Blood samples were transferred in a cool box to the Microbiology Laboratory of the Golestan University of Medical Science and centrifuged at 1000 g for 5 minutes. The recovered sera were stored at -20 °C until used. Sera from 30 individuals who claimed they had never consumed wild boar meat in their lifetime were used to determine the range of actual negative values.

**Ethical commitment**

The Ethics Committee of the Tehran University of Medical Science approved all procedures (No. IR.TUMS.SPH.REC.1396.3996). Written consent was obtained from all participants or their guardians before sample collection.
ELISA

Serum samples were examined for anti-Trichinella IgG antibodies using a commercial qualitative ELISA Kit (NovaTec Immunodiagnostica GmbH, Germany) in an ELISA automatic instrument Chemwell 2910 (Awareness, USA). The manufacturer-stated diagnostic sensitivity and specificity for the ELISA kit were >95% and 94.8%, respectively. Similar epidemiological studies had previously deployed the same kit successfully (26-30).

Statistical analysis

Table 1: Trichinella infection seroprevalence in people with a history of wild boar meat consumption in the Northeast of Iran

| Variables          | n (%) | Negative n (%) | Positive n (%) |
|--------------------|-------|----------------|----------------|
| Age (yr)           |       |                |                |
| 20 - 30            | 36 (19.05) | 35 (18.52) | 1 (0.53) |
| 30 - 40            | 39 (20.63) | 39 (20.63) | 0 |
| 40 - 50            | 52 (27.51) | 51 (26.68) | 1 (0.53) |
| ≥ 50               | 62 (32.80) | 59 (31.22) | 3 (1.59) |
| Gender             |       |                |                |
| Female             | 40 (21.16) | 39 (20.63) | 1 (0.53) |
| Male               | 149 (78.83) | 145 (76.22) | 4 (2.12) |
| Educational level  |       |                |                |
| Associate          | 17 (8.99) | 17 (8.99) | 0 |
| BS                 | 17 (8.99) | 17 (8.99) | 0 |
| Diploma            | 45 (23.80) | 42 (22.22) | 3 (1.59) |
| High school        | 53 (28.04) | 51 (26.98) | 2 (1.06) |
| Illiterate         | 35 (18.52) | 35 (18.52) | 0 |
| Primary school     | 22 (11.64) | 22 (11.64) | 0 |
| Occupation         |       |                |                |
| Cowhand            | 10 (5.29) | 10 (5.29) | 0 |
| Employee           | 36 (19.05) | 36 (19.05) | 0 |
| Farmer             | 55 (29.10) | 53 (28.04) | 2 (1.06) |
| Housekeeper        | 29 (15.34) | 28 (14.81) | 1 (0.53) |
| Freelancer         | 59 (31.21) | 57 (30.16) | 2 (1.06) |
| Total results      | 189 (100) | 184 (97.36) | 5 (2.64) |

Data analysis was performed using descriptive and quantitative statistical methods; the latter was done by employing a chi-square test. Statistical significance was considered at the P<0.05 level. SPSS software ver.22 (Chicago, IL, USA) was used for analysis purposes.

Results

Participants included 149 men (78.8%) and 40 women (21.1%) and aged 20 to 63 yr (mean age, 42.17 ± 11.35 yr (Table 1).
Out of 189 individuals, five (2.6%) had anti-Trichinella antibodies (OD, 1.176 ± 0.154), and of these, four (80%) were male, and one (20%) was female. Most seropositive cases (1.59%) were among individuals ≥50 yr old, but no significant difference was between these individuals and those under 50 yr old (P>0.05).

The mean optical density (ODs) for negative control sera was lower than the kit cut-off OD (0.33 vs. 0.53), which validates the negative control results. Our results showed a significant difference in infection rate between the counties in the eastern and western parts of the study area (P=0.003) (Table 2).

**Discussion**

Human trichinellosis has significantly decreased during the last 50 years due to pig breeding in highly contained farms and continuous surveillance programs. Post-slaughter meat inspection and continuous education of consumers in countries where meat inspection is not mandatory have also contributed as crucial disease control strategies (1, 13). Alongside the decline in domestic animal infection, i.e., horses and pigs, attention was diverted to human Trichinella outbreaks caused by wild prey meats (13, 17, 31).

Serological diagnosis of trichinellosis can shed the limelight on the disease epidemiology, where the only possible way to acquire the infection is via wild animal meat consumption. Most serological assays rely on tracing immunoglobulin G (IgG) in the blood that is detectable 15-60 d post-infection (32) and remains in the patient's blood for more than 30 years (33). Our ELISA detected anti-Trichinella IgG antibodies in 5 (2.6%) of the 189 participants, close to the 2.2% rate previously obtained by another commercial kit (ELISA IgG Kit, IBL, Hamburg, Germany) in Mazandaran, the province adjacent to our study area (19).

In similar studies in other countries, the same ELISA has successfully detected anti-Trichinella IgG antibodies in humans. In a retrospective study in southern and eastern Serbia, among 136 individuals with a trichinellosis history, 27 (19.9%) had anti-Trichinella antibodies (29). Moreover, out of 999 individuals representing Estonia's general population, 3.1% showed exposure to Trichinella infection.

### Table 2: ELISA results showing the Trichinella seropositivity in counties of north and northeast Iran

| Study zone                | Negative (%) | Positive (%) | P-value |
|---------------------------|--------------|--------------|---------|
| Behshahr (Western Area)   | 15 (7.94)    | 1 (0.53)     |         |
| Galugah (Western Area)    | 13 (6.88)    | 1 (0.53)     |         |
| Bandar-Gaz (Western Area) | 24 (12.70)   | 1 (0.53)     |         |
| Kordkuy (Western Area)    | 20 (10.58)   | 1 (0.53)     |         |
| Gorgan (Western Area)     | 40 (21.16)   | 1 (0.53)     |         |
| Ali Abad (Eastern Area)   | 13 (6.88)    | 0            |         |
| Azad Shahr (Eastern Area) | 21 (11.11)   | 0            |         |
| Gonbad (Eastern Area)     | 14 (7.41)    | 0            |         |
| Bojnord (Eastern Area)    | 24 (12.70)   | 0            |         |
| Western Area              | 112 (95.42)  | 5 (4.3)      | 0.003*  |
| Eastern Area              | 72 (38.09)   | 0 (0)        |         |
| Total results             | 184 (97.4)   | 5 (2.6)      |         |

*P<0.05 is considered a significant result
and Western blot confirmed the infection in 2.7% of ELISA positive cases (26). The sero-
positivity in our study was less than rates in
China (3.1%-5.3%) (34-36), Argentina (4.5%) (37), Turkey (4%) (38), Indonesia (19.8%) (39),
Estonia (3.3%) (26), Papua New Guinea (10%) (40), Laos (19.1%) (41), and East
Greenland (3.1%) (42), but higher than rates in
Mexican suburban population (1.9%) (43),
and Greenland children (1.09%) (44).

In our investigation, the risk factors, like age,
gender, job, and educational level, were not
significantly associated with Trichinella sero-
positivity. However, seropositive rates were
higher among farmers (3.6%, 2/55) and self-
employed people (3.4%, 2/59), which might
be because these individuals are at higher risk
of exposure via eating wild boar meat, similar
to previous reports (19, 26, 40, 43).

In our research, the seropositivity rate was
higher in males (2.9%, 4/149) and increased
with age, showing the highest rate in ≥50
(4.8%, 3/62). Nevertheless, these associations
were not statistically significant. Similar studies
in Iran (19), Papua New Guinea (40), and
Laos (41) have reported higher seropositivity
rates in men than women for trichinellosis.
Our results also showed a higher seropositivity
rate in western counties than in eastern
counties (P≤0.05). All five seropositive cases
were among 112 individuals (4.46% seroposi-
tivity) that resided in the western part of the
study area, stretching from Behshar to Gorgan,
Iran. Among the limitations in our study was
that some individuals with other social prob-
lems, e.g., drug-addictions feared disclosing
their identity and were hesitant to give blood
samples.

Conclusion

Eating wild boar meat intentionally or un-
knowingly is a possible source of trichinellosis
in the Northeast of Iran. Further studies with
more individuals from different parts of the
country and confirmation of the ELISA by
additional tests like Western blot will give a
more in-depth insight into human trichinello-
sis epidemiology in Iran.

Acknowledgements

This survey was part of the Ph.D. thesis pro-
ject financially supported by the Tehran Univer-
sity of Medical Sciences (TUMS). We thank
Dr. Nasser Behnampoor and Dr. Mostafa
Ghorbani for analyzing the data, Dr. Hamed
Kalani for help with sample collection, and
the Department of Laboratory Sciences, Para-
medical School, Golestan University of Med-
ical Sciences.

Conflict of interest

The authors declare that there is no conflict
of interests.

References

1. Yang Y, Cai YN, Tong MW, et al. Serological
tools for detection of Trichinella infection in
animals and humans. One Health. 2016;2:25-
30.
2. World Health Organization (2015). WHO
estimates of the global burden of foodborne
diseases: Foodborne disease burden epidemiolo-
gy reference group 2007-2015. World Health
Organization. https://apps.who.int/iris/handle/10665/199350.
3. Tasić NM, Ignjatović A. Serological diagnosis of
trichinellosis in patients in southern and eastern
Serbia in the period from 2007 to 2018.
Veterinarski Glasnik. 2019;73:157-167.
4. Gottstein B, Pozio E, Nöckler K. Epidemiology,
diagnosis, treatment, and control of trichinellosis.
Clin Microbiol Rev. 2009;22:127-145.
5. Sun G-G, Wang Z-Q, Liu C-Y, et al. Early
serodiagnosis of trichinellosis by ELISA using
excretory–secretory antigens of Trichinella spiralis
adult worms. Parasit Vectors. 2015;8:484.
6. Liu M, Boireau P. Trichinellosis in China:
Epidemiology and control. Trends Parasitol.
2002;18(12):553-556.
7. Pozio E. World distribution of *Trichinella* spp. Infections in animals and humans. Vet Parasitol. 2007;149(1-2):3-21.

8. Bai X, Hu X, Liu X, et al. Current research of trichinellosis in China. Front Microbiol. 2017;8:1472.

9. Caron Y, Bory S, Pluot M, et al. Human outbreak of trichinellosis caused by *Trichinella papuae* nematodes, central Kampong Thom province, Cambodia. Emerg Infect Dis. 2020;26(8):1759-1766.

10. Devleesschaever B, Praet N, Speybroeck N, et al. The low global burden of trichinellosis: Evidence and implications. Int J Parasitol. 2015;45(2-3):95-99.

11. Murrell KD, Pozio E. Worldwide occurrence and impact of human trichinellosis, 1986–2009. Emerg Infect Dis. 2011;17(12):2194-2202.

12. Messiaen P, Fortier A, Vanderschueren S, et al. Outbreak of trichinellosis related to eating imported wild boar meat, Belgium, 2014. Euro Surveill. 2016;21(37):30341.

13. Rostami A, Khazan H, Kazemi B, et al. Prevalence of *Trichinella* spp. Infections in hunted wild boars in northern Iran. Iran J Public Health. 2017;46(12):1712-1719.

14. Wolfe MS. The eosinophilic patient with suspected parasitic infection. The Travel and Tropical Medicine Manual. Elsevier; 2017:598-609.

15. Boros Z, Vallée I, Panait L, et al. Seroprevalence of *Trichinella* spp. In wild boars (*Sus scrofa*) from Bihor county, western Romania. Helminthologia. 2020;57(3):235-240.

16. Faber M, Schink S, Mayer-Scholl A, et al. Outbreak of trichinellosis due to wild boar meat and evaluation of the effectiveness of post-exposure prophylaxis, Germany, 2013. Clin Infect Dis. 2015;60(12):e98-e104.

17. Fichi G, Stefanelli S, Pagani A, et al. Trichinellosis outbreak caused by meat from a wild boar hunted in an Italian region considered to be at negligible risk for *Trichinella*. Zoonoses Public Health. 2015;62(4):285-291.

18. Greene YG, Padovani T, Rudroff JA, et al. Notes from the field: Trichinellosis caused by consumption of wild boar meat-Illinois, 2013. MMWR Morb Mortal Wkly Rep. 2014;63(20):451.

19. Rostami A, Khazan H, Kia EB, et al. Molecular identification of *Trichinella* spp. In wild boar, and serological survey of high-risk populations in Iran. Food Control. 2018;90:40-47.

20. Mowlavi G, Marucci G, Mobedi I, et al. *Trichinella britovi* in a leopard (*Panthera pardus saxicolor*) in Iran. Vet Parasitol. 2009;164(2-4):350-352.

21. Mirjalali H, Rezaei S, Pozio E, et al. *Trichinella britovi* in the jackal canis aureus from south-west Iran. J Helminthol. 2014;88(4):385-8.

22. Borj H, Sadeghi H, Razmi G, et al. *Trichinella* infection in wildlife of northeast of Iran. Iran J Parasitol. 2012;7(4):57-61.

23. Ellazian M, Tamidji Y, Akbarzadeh M. A survey on human trichinosis in Iran. Archives of Razi Institute. 1976;28:63-65.

24. Moein M. First report of human trichinosis in Iran. J Med the Univ Med School. 1966;5:259-267.

25. Kia E, Meamar A, Zahabian F, et al. An outbreak of human trichinellosis due to consumption of boar meat infected with *Trichinella* sp. Iran J Infect Dis Trop Med. 2008;41:35-38.

26. Lassen B, Janson M, Viltrop A, et al. Serological evidence of exposure to globally relevant zoonotic parasites in the Estonian population. PLoS One. 2016;11(10):e0164142.

27. Moskwa B, Bien J, Cabaj W, et al. The comparison of different ELISA procedures in detecting anti-*Trichinella* IgG in human infections. Vet Parasitol. 2009;159:312-315.

28. Svitben M, Meštrović T, Čičmak LS. The value of systematic screening for *Trichinella* antibodies among individuals with eosinophilia in recognizing outbreak events: A seroprevalence study from Croatia. Ann Parasitol. 2019;65(2):177-189.

29. Miladinović-Tasić N, Ignjatović A. Serological diagnosis of trichinellosis in patients in southern and eastern Serbia in the period from 2007 to 2018. Veterinarski Glasnik. 2019;73:157-167.

30. Latz A, Völger J. Development and performance evaluation of enzyme linked immunosorbent assay and lineblot for serological diagnosis of leishmaniasis in dogs: Ps1. 113. Triop Med Int Health. 2015;20

31. Holzbauer SM, Agger WA, Hall RL, et al. Outbreak of *Trichinella spiralis* infections associated with a wild boar hunted at a game farm in Iowa. Clin Infect Dis. 2014;59(12):1750-1756.

32. Dupouyet C, Bruschi F. Management and diagnosis of human trichinellosis. 2007:37-68.
33. Fröscher W, Gullotta F, Saathoff M, et al. Chronic trichinosis. Eur Neurol. 1988;28:221-226.
34. Wang Z, Cui J. The epidemiology of human trichinellosis in China during 1964-1999. Parasite. 2001;8:S63-S66.
35. Wang Z, Cui J, Xu B. The epidemiology of human trichinellosis in China during 2000-2003. Acta Trop. 2006;97(3):247-251.
36. Cui J, Wang Z, Xu B. The epidemiology of human trichinellosis in China during 2004-2009. Acta Trop. 2011;118(1):1-5.
37. Cohen M, Costantino SN, Calcagno MA, et al. Trichinella infection in wild boars (Sus scrofa) from a protected area of Argentina and its relationship with the presence of humans. Vet Parasitol. 2010;169(3-4):362-366.
38. Akkoc N, Kuruuzum Z, Akar S, et al. A large-scale outbreak of trichinellosis caused by Trichinella britovi in Turkey. Zoonoses Public Health. 2009;56(2):65-70.
39. Chomel BB, Kasten R, Adams C, et al. Serosurvey of some major zoonotic infections in children and teenagers in Bali, Indonesia. Southeast Asian J Trop Med Public Health. 1993;24(2):321-326.
40. Owen IL, Gomez Morales MA, Pezzotti P, et al. Trichinella infection in a hunting population of Papua New Guinea suggests an ancient relationship between Trichinella and human beings. Trans R Soc Trop Med Hyg. 2005;99(8):618-624.
41. Conlan JV, Vongxay K, Khamlome B, et al. Patterns and risks of Trichinella infection in humans and pigs in northern Laos. PLoS Negl Trop Dis. 2014;8(7):e3034.
42. Møller LN, Koch A, Petersen E, et al. Trichinella infection in a hunting community in East Greenland. Epidemiol Infect. 2010;138(9):1252-1256.
43. De-la-Rosa J, Aranda J, Padilla E, et al. Prevalence and risk factors associated with serum antibodies against Trichinella spiralis. Int J Parasitol. 1998;28(2):317-321.
44. Møller LN, Krause TG, Koch A, et al. Human antibody recognition of Anisakidae and Trichinella spp. In Greenland. Clin Microbiol Infect. 2007;13(7):702-708.