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Original Article

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

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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, periorbital 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 km^2 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.

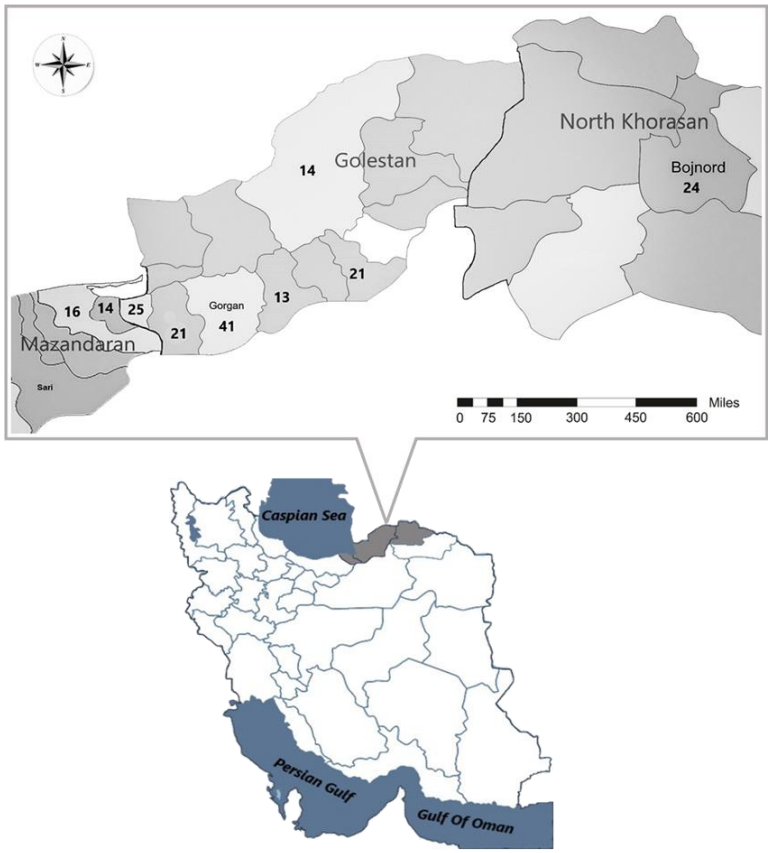

Fig. 1: Map of Iran and the study area. The values show the number of participants in each county

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

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).

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)

Out of 189 individuals, five (2.6%) had anti-T*richinella* 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).

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

Study zone	***ELISA Results***		
	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

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,

and Western blot confirmed the infection in 2.7% of ELISA positive cases (26). The seropositivity 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* seropositivity. 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 ($P \leq 0.05$). 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, Iran. Among the limitations in our study was that some individuals with other social problems, e.g., drug-addictions feared disclosing their identity and were hesitant to give blood samples.

Conclusion

Eating wild boar meat intentionally or unknowingly 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 trichinellosis epidemiology in Iran.

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Conflict of interest

The authors declare that there is no conflict of interests.

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