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Original Article

Study of *Trichinella* spp. Seroprevalence in Horse Population of West Azerbaijan, Northwestern Iran

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Abstract

Background: We aimed to determine the seroprevalence of *Trichinella* in horses in West Azerbaijan Province, Iran a region known for its wildlife interactions.

Methods: The study was conducted in March 2016 across four cities in West Azerbaijan: Urmia, Chaldoran, Sardasht, and Khoy, Iran. A total of 184 equines, ranging from three to over ten years of age, were randomly sampled. Blood samples were collected and were analyzed using the ID Screen® *Trichinella* Indirect Multi-species ELISA to detect antibodies.

Results: Of the 184 serum samples, 4 (4.3%) from northern counties and 2 (2.2%) from southern counties tested positive for trichinellosis. Chi-square and Fisher's exact tests indicated no significant correlations between age, gender, or residential location and disease prevalence. The logistic regression model was not statistically significant ($P = 0.754$), revealing that these demographic factors do not substantially influence trichinellosis risk in the studied equines.

Conclusion: This study provides crucial insights into the low seroprevalence of *Trichinella* in horses in West Azerbaijan, suggesting that equines may not be primary reservoirs of the parasite despite overlapping habitats with infected wildlife.

Introduction

Nematodes infect a broad variety of hosts, including plants, insects, and vertebrates; they make up a large fraction of parasitic eukaryotic parasites (1). Trichinellosis, caused by the parasitic nematode Trichinella, presents significant threats to both public health and the economy (2).This disease exhibits a broad spectrum of hosts, encompassing carnivores, omnivores, and even specific herbivores; its primary association is with the consumption of improperly cooked pork (3, 4).While pig consumption is commonly linked to human cases, there is growing concern about its effects on wildlife and the potential for transmission to domestic animals (4-6).

In Iran, *Trichinella* infections have been reported in Stray dogs, golden Jackals, rodents, wild boars, leopards, Mongoose, jungle cats, brown bears and foxes from various regions (7-9) (Fig. 1). Mobedi et al. reported the initial occurrence of *Trichinella* infection in wild animals in Iran from 1967 to 1971, specifically in the Caspian Sea region (10). A total of four *Trichinella* species have been discovered in Iran from twelve conducted research. These species are *T. britovi, T. spiralis, T. murrelli,* and *T. nelsoni* (11). These nematodes are predominantly present in carnivorous animals such as foxes, jackals, leopards, and wild boars. Additionally, rodents can act as hosts for these parasites. T. britovi is particularly common in wild animals in Iran (12).

Although the Islamic dietary regulations forbid the consumption of pork, there are growing concerns regarding the spread of trichinellosis in Iran due to increased cross-border interactions, wildlife hunting, and the prevalence of *Trichinella* species in wild boars. The danger is heightened by the illegal hunting and consumption of wild boar meat by specific populations, particularly religious minorities like Jews, Christians and Zoorostians etc., even though the number of cases may be small (12). Moreover, the scavenging behavior of wild boars puts them extremely vulnerable to parasite diseases, hence increasing the likelihood of disease transmission (12, 13). To decrease the threat of trichinellosis, it is crucial to implement educational programs for hunters and consumers, particularly in high-risk regions of Iran, due to the significant risk of infection from contaminated wild boar carcasses (11-13) .Rostami, Khazan, et al. reported a seroprevalence rate of 2.1% in a single human case of trichinellosis in Mazandaran Province, located in northern Iran (14) (Fig. 1).

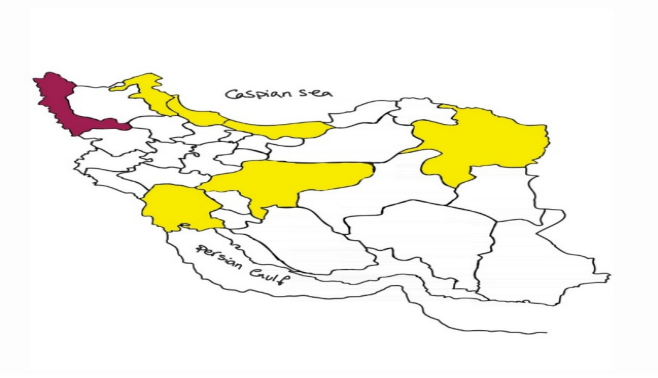

Fig. 1: Map of Iran which shows the six provinces where previously *Trichinella* spp. infected animals have been found from 1967 up until now. (Gilan, Mazandaran, Khorasan-e-Razavi, Rasht, Ardabil and Khuzestan, and from this study, Urmia) (Animals were brown bear; golden jackal; jungle cat; leopard; mongoose; Persian gerbil; red fox; stray dog; wild boar and now Horse)

Although there is an enormous amount of data on the global occurrence of trichinellosis in humans and animals, there is an absence of knowledge addressing its prevalence specifically in Iranian horse populations (11). The objective of this study is to determine the seroprevalence of *Trichinella* in horses located in the West Azerbaijan province of Iran, which is recognized for its large population of wild boars. Prior studies have demonstrated the existence of gastrointestinal nematodes, specifically *Trichinella* species, in horse populations in Iran (15). It is crucial to investigate the existence of *Trichinella* in the population of wild boars and horses due to their overlapping habitats and the possibility for horses to act as intermediate hosts for other parasites.

We using ELISA techniques, aimed to improve our comprehension of the possible spread of this parasite in horses and its implications for epidemiology (16). This study enhances the knowledge of the epidemiology of trichinellosis and its impact on public and animal health by providing clarification on the potential involvement of horses as carriers of the parasite.

Materials and Methods

Study area

This study was carried out in the West Azerbaijan Province, located in Northwestern Iran, during March 2016. Four cities in the West Azerbaijan province in Northwestern Iran were chosen for sample collection: Urmia (37°32'59.99"N,45°05'60.00"E), Chaldoran (39°03'32.40"N,44°23'4.79"E), Sardasht (36°12'60.00"N,45°28'59.99"E) and Khoy (38°33'5.39"N,44°56'31.19"E) (Fig. 2).

Fig. 2: The cities which were studied in this article

Sample collection

A total of 184 equines were selected randomly from a diverse range of rural farms located in the chosen urban areas. The equine participants ranged in age from approximately three to over 10 years old, and covered a diverse spectrum of breeds. Horses were provided with fresh feed as part of their customary farming circumstances.

The blood sample was collected from the jugular vein of each horse in the field. The specimens were quickly delivered to the laboratory while stored on ice. After the centrifugation process was finished, the serum was separated and transferred into Eppendorf tubes. The hemolyzed samples were discarded. Before conducting ELISA analysis, the serum samples were stored in the freezer.

Indirect ELISA for detection of antibodies

Collected serums were tested with ID Screen® *Trichinella* Indirect Multi-species (Sensitivity = 90.47%, n= 42, Specificity = 100%, CI95%: 98.95 –100%). Anti-*Trichinella* anti-

bodies were assayed by indirect ELISA as it was described in protocols. This method was chosen due to this test's high specificity and sensitivity (16). The results were analyzed by calculating the S/P% value according to the following formula:

$$S/P\,\% = \frac{\mathrm{OD}_{\mathrm{Sample}} - OD_{NC}}{\mathrm{OD}_{\mathrm{PC}} - OD_{NC}} \times 100$$

The samples with an S/P% value equal to or lower than 50% were considered negative, while those with an S/P% value equal to or greater than 60% were considered positive. The serum samples with S/P% greater than 50% but lower than 60% were interpreted as suspicious.

Statistical Analysis

The statistical analyses were performed using SPSS version 25 (IBM Corp., Armonk, NY, USA). Descriptive statistics, such as the mean, standard deviation, and frequencies, were computed to characterize the demographic features of the horses and the distribution of positive serum trichinosis cases. A logistic regression model was used to analyze the correlation between demographic characteristics and positive serum trichinellosis. The Bonferroni correction was used to make up for multiple comparisons in evaluating the importance of individual demographic variables in predicting the probability of positive serum trichinellosis.

Results

A total of 92 serum samples were collected from each region. Four samples (4.3%) from the northern counties tested positive for trichinellosis, whereas 2 samples (2.2%) from the southern counties were positive. To evaluate potential correlations between age, gender, and residential location with the prevalence of trichinellosis, Chi-square tests and Fisher's exact tests were performed. The results indicated no statistical significance, suggesting that these variables do not have a strong predictive capacity for the disease's occurrence.

Further analysis using multivariable logistic regression was conducted to explore the impact of these factors on the likelihood of trichinellosis. The overall model was not statistically significant, with a Chi-square value of 1.196 and a *P*-value of 0.754, indicating that the model did not provide substantial predictive power. The Nagelkerke R^2 value was 2.6%, reflecting that the model explained a minimal portion of the variance in trichinellosis cases, while correctly classifying 96.7% of instances.

Regarding age, the analysis demonstrated that for each three-year increase, the odds of trichinellosis increased by a factor of 1.389; however, this association was not statistically significant ($P = 0.556$), indicating that age does not significantly influence the likelihood of infection.

Similarly, gender was examined as a potential determinant of susceptibility to trichinellosis. The results indicated that male horses had a 1.216-fold higher likelihood of infection compared to females, yet this difference was not statistically significant ($P = 0.825$), suggesting that sex does not substantially impact the overall occurrence of the disease.

Additionally, the analysis of residential location revealed that horses from Khoy and Chaldoran counties exhibited a 1.378-fold increase in infection risk relative to those from Urmia and Sardasht counties, though this difference was also not statistically significant ($P = 0.400$). This finding implies that geographical location does not significantly influence the prevalence of trichinellosis in the studied population.

Although patterns related to age, gender, and location were observed, none of these characteristics exhibited a significant impact on the probability of trichinellosis in the horses assessed in this study (Table 1).

Table 1: Distribution of trichinellosis in horses from West Azerbaijan Province

Variable		Trichinellosis Positive (S/P ≥ 50) N(%)	Trichinellosis Negative (S/P < 50) N(%)	Number
Age		0 (0.0)	17 (100.0)	17
3 years and younger		3 (4.0)	72 (96.0)	75
4-6 years		2 (2.9)	66 (97.1)	68
7-9 years		1 (4.2)	23 (95.8)	24
10 years and older				
Gender	Male	3 (3.5)	83 (96.5)	86
	Female	3 (3.0)	95 (97.0)	98
Living Area	Khoy County	1 (2.0)	45 (98.0)	46
	Chaldoran County	3 (6.5)	43 (93.5)	46
	Urmia County	2 (4.3)	44 (95.7)	46
	Sardasht County	0 (0.0)	46 (100.0)	46

Discussion

This study gives crucial insights on the seroprevalence of *Trichinella* in equine populations in West Azerbaijan, Iran, contributing significantly to the understanding of trichinellosis epidemiology in a region with high wildlife activity. Although *Trichinella* infections are well-documented in carnivorous and omnivorous species in Iran, this research reveals the comparatively low incidence of trichinellosis infection in local equines (7). These findings highlight crucial implications for public health, animal health, and the larger epidemiological context of disease transmission in the region.

Our findings corroborate previous studies (9, 17-19) indicating a significant frequency of *Trichinella* in animal species, especially carnivores, in Iran. Research has recorded *Trichinella* britovi infections in Persian leopards and golden jackals in northern areas, demonstrating the extensive prevalence of the parasite in carnivorous mammals (18). Likewise, *Trichinella* species have been identified in foxes, jackals, and wild boars in northern Iran (9, 17-19). Our findings indicate that, despite their common presence in wildlife, horses are often less vulnerable to *Trichinella* infection, perhaps due to nutritional and ecological conditions that restrict their exposure to the parasite.

The ELISA approach has demonstrated its significance in identifying *Trichinella* infections, especially in areas with intricate host-parasite interactions. ELISA's excellent sensitivity and specificity make it the best method for diagnosing infections in numerous species, including horses (16). Our study contributes to the increasing amount of literature that supports the use of ELISA in monitoring *Trichinella* in livestock, which is critical for countries like Iran and other Islamic nations where restrictions on diets limit the consumption of pork, but there remains a risk of infection through the consumption of equine meat.

In Iran and Turkey, both Islamic countries, *Trichinella* infections have been observed in domestic animals and wildlife, with occasional uncommon incidents among individuals due to illegal consumption of wild boar and horse meat (7, 14, 20, 21). The application of the ELISA approach in these circumstances is particularly important due to the economic

and public health consequences of horsemeat consumption, which has traditionally been associated with *Trichinella* outbreaks in Europe (22).

The precision and cost-efficiency of ELISA makes it an effective diagnostic instrument in resource-constrained areas (12).Despite the low prevalence of *Trichinella* in equines, ongoing surveillance via ELISA is essential, particularly in regions with significant human and wildlife interaction, such as West Azerbaijan, characterized by high wild boar activity and frequent human-animal encounters. One important factor to consider in this study is the potential for false-positive or false-negative results when using the ELISA method for the detection of *Trichinella*-specific antibodies in horses. While ELISA is widely regarded for its high sensitivity and specificity, there are limitations that could affect the accuracy of the results. In particular, cross-reactivity with antibodies from other parasitic infections could potentially lead to false positives. Furthermore, the timing of blood sample collection is crucial; if samples were collected too long after infection, antibody levels may have waned, resulting in false negatives. This is particularly relevant given that horses can experience transient antibody responses, which may not be detectable beyond a certain post-infection period. Future studies should consider using additional diagnostic methods, such as PCR, to detect *Trichinella* DNA in tissue samples, which would help confirm active infections and mitigate the risk of false results.

The low seroprevalence of *Trichinella* in horses in West Azerbaijan is a positive outcome for public health and the livestock sector. Although horses may not be primary reservoirs for *Trichinella* in this area, the risk from wildlife, especially wild boars, is considerable. Wild boars are recognized vectors of *Trichinella* and are often hunted and consumed in certain regions of Iran, heightening the potential of disease transmission (18). This presents a public health issue, especially in populations who consume wild boar despite religious prohibitions on pork.

Due to the likelihood of *Trichinella* transmission from ingesting contaminated meat, especially from wild boar and horses, it is essential to persist in educating hunters and communities in high-risk regions about the hazards of consuming undercooked or contaminated meat. This study highlights the significance of focused public health programs, particularly in areas like as West Azerbaijan, where human-wildlife interactions are prevalent.

While *Trichinella* infections in horses have been sparsely reported, several global studies suggest that the prevalence in horses is generally low but still poses significant risks to public health, especially in regions where horsemeat is consumed. For instance, outbreaks of *Trichinella* due to the consumption of horsemeat have been documented in Europe, notably in France and Italy. These outbreaks demonstrated that, although rare, the infection can be transmitted through improperly prepared meat products (23-26).

Conclusion

This study contributes to the growing body of knowledge on the epidemiology of *Trichinella* infections in Iran. Although the prevalence of *Trichinella* in horses in West Azerbaijan appears low, the potential risk posed by wildlife, particularly wild boars, remains a concern for public health. The absence of significant demographic predictors of infection emphasizes the need for further research and expanded surveillance to understand fully the transmission dynamics of *Trichinella* in Iran. Educational programs targeting hunters and communities involved in wildlife consumption, coupled with continued monitoring using sensitive diagnostic tools like ELISA, will be crucial in mitigating the risks of *Trichinella* transmission.

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Conflict of Interest

The authors declare that there is no conflict of interests.

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