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Updates on *Trichinella* species infection in wild boar (*Sus scrofa*) from Southern Romania

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Abstract

Romanians widely practice hunting; the meat of hunted animals, especially wild boar, can often avoid specific control for various reasons. Consequently, human trichinellosis in Romania is a zoonosis with a high incidence, with the consumption of uncontrolled pork and game being incriminated as the leading cause of human infection. Molecular analysis of Trichinella larvae collected by artificial digestion from wild boars hunted in several areas in Vâlcea County, Romania, revealed only one species, *Trichinella britovi*. The obtained results should alarm the human consumer, especially hunters, regarding the significance of veterinary control of the meat from the hunted animals.

Keywords: wild boar, Romania, molecular analysis, *Trichinella britovi*

1.Introduction

Trichinellosis, a widespread zoonosis affecting mainly carnivore and omnivore mammals, is produced by species of the genus *Trichinella*. Currently, ten recognized species and three undefined genotypes are taxonomically accepted within the genus [1, 2]. Among them, *Trichinella spiralis* is the most well-known; pigs are most susceptible to infection, followed by carnivores and rodents [1, 3, 4].

Two cycles are defined in the epidemiology of trichinellids; the domestic one involves pigs, horses, synanthropic rodents, and domestic carnivores [5, 6]. The sylvatic cycle associates a large number of omnivorous and carnivorous species, rodents, birds, and reptiles. Of the ten accepted species of *Trichinella*, only *T. spiralis* is transmitted and maintained in the domestic cycle worldwide and can also spread in the sylvatic one [7]. However, it is not excluded that the sylvatic species to invade domestic habitats.

Out of the sylvatic genotypes, *T. britovi* is more specific to carnivores in temperate areas in Europe [8]. The flow of sylvatic *Trichinella* species from wildlife to domestic focus and in the opposite direction of *T. spiralis* from domestic to sylvatic animals is provided by multiple mechanisms [7]. Wild and domestic carnivores, through necrophagy and predation, the synanthropic and sylvatic rodents species, and humans by improper hunting habits, can transfer *Trichinella* species between the two cycles.

In Romania, numerous studies have reported an increased prevalence of *Trichinella* infection in wild species [9]. Of these, the wild boar is a significant source of human contamination [10]. Local and regional studies focused on this game species reported a prevalence varying between 0.1 and 23.5% [9]. Both *T. spiralis* and *T. britovi* were molecularly identified across the country. These studies covered Romania's North-Eastern and North-Western, Central, Western, South-Western, and South-Eastern regions [11].

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In wild boars hunted in Southern Romania, we previously reported by artificial digestion a prevalence of 0.21% (3/1364) in a study carried out in Vâlcea County between 2017-2019 [10]. The current research aimed to identify the *Trichinella* species involved in infected wild boars hunted in the mentioned area using molecular analysis.

Materials and methods

2.1.Geographic Location of the Hunting Area

The study was performed between January 2017 and December 2019 in 14 hunting grounds located in Vâlcea County, Southern Romania (Figure 1).

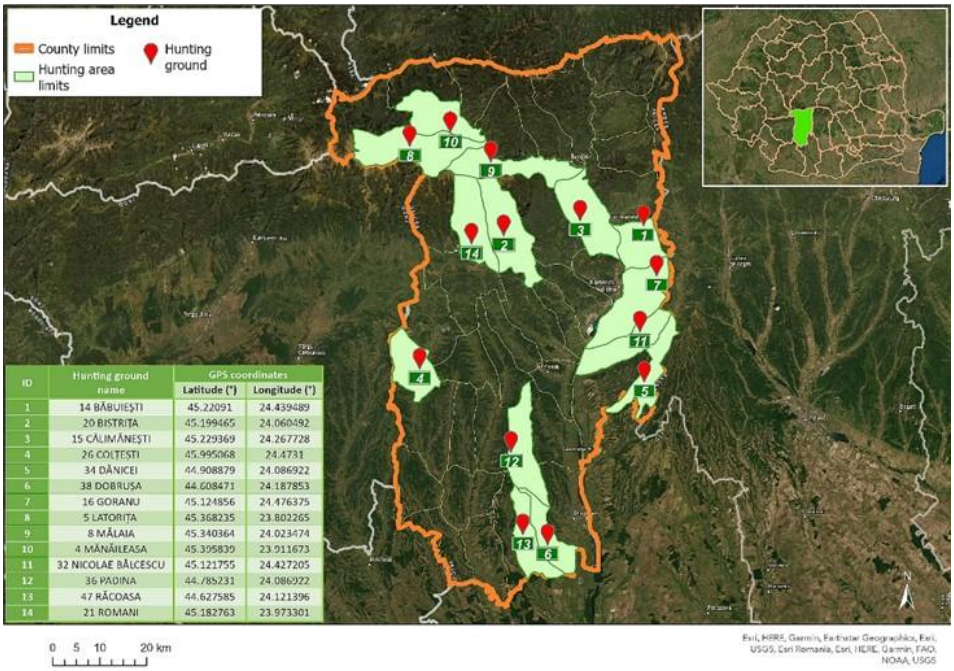
2.2. The collecting of Trichinella sp. larvae

All wild boars hunted in the period mentioned above were tested by artificial digestion. The method was performed at the County Sanitary-Veterinary Laboratory (CSVL) Vâlcea, according to the EU Regulation 1375/2015, to examine striated muscle tissue [12].

2.3. DNA extraction and molecular analysis

Five *Trichinella* sp. larvae isolated from each infected boar were used for DNA extraction. The PureLink® Genomic DNA Mini Kit (INVITROGEN®) was used for extraction according to the manufacturer's instructions. The DNA obtained and purified, free of proteins, nucleases, other contaminants, and inhibitors, was stored at -80°C until molecular analysis through PCR.

Molecular identification was carried out in the Parasitology Department, Faculty of Veterinary Medicine, Timisoara, by multiplex PCR according to the methodology described by Pozio and La Rosa [13]. Five pairs of primers were used to identify nine species/ genotypes of *Trichinella*, namely *T. spiralis*, *T. nativa*, *T. britovi*, *T. pseudospiralis*, *T. murelli*, *Trichinella* T6 genotype, *T. nelsoni*, *T. papuae*, and *Trichinella* T11 genotype (Table 1).



ID	Hunting ground name	GPS coordinates	
		Latitude (°)	Longitude (°)
1	14 BĂBUIEȘTI	45.22091	24.439489
2	20 BISTRIȚA	45.199465	24.060492
3	15 CĂLIMĂNEȘTI	45.229369	24.267728
4	26 COLȚEȘTI	45.995068	24.4731
5	34 DĂNICEI	44.908879	24.086922
6	38 DOBRUȘA	44.608471	24.187853
7	16 GORANU	45.124856	24.476375
8	5 LATORIȚA	45.368235	23.802265
9	8 MĂLAIA	45.340364	24.023474
10	4 MĂNĂILEASA	45.395839	23.911673
11	32 NICOLAE BĂLCESCU	45.121755	24.427205
12	36 PADINA	44.785231	24.086922
13	47 RĂCOASA	44.627585	24.121396
14	21 ROMANI	45.182763	23.973301

Figure 1. The location of the hunting grounds where the wild boars were collected

Table 1. The pair primers used in the study.

No	Pairs identified	Primer sequences (5′–3)
I.	5'-GTTCCATGTGAACAGCAGT-3'	5'-CGAAAAACATACGACAACTGC-3'
II.	5'-GCTACATCCTTTTTTTTGTT-3'	5'-AGACACAATATCAACCACAGTACA-3'
III.	5'-GCGGAAGGATCATTATCGTGT-3'	5'-ATGGATTACAAAGAAAAACCATCACT-3'
IV.	5'-GTGAGCGTAATAAAGGTGCAG-3'	5'-TTCATCACACATCTTCCACTA-3'
V.	5'-CAATTGAAAACCGCTTAGCGTGTTT-3'	5'-TGATCTGAGGTCGACATTTCC-3'

Master Mix MyTaqTM Red Mix (BIOLINE®) containing the necessary components in a concentrated form 2x was used to achieve the reaction. The final volume of the PCR reaction was 50 µl, of which 39.0 µl was represented by MyTaqTM Red Mix (BIOLINE®), 1 µl primer reverse, 1 µl primer forward of each pair, dilute to a concentration of 10 pmol/µl according to the protocol described by the manufacturer. The remaining components in the reaction were represented by DNA extracted from the sample to be analyzed and ultrapure water. Also, positive and negative controls were included in the reactions for each analysis.

The amplification program was made with the My Cycler thermo cycler (BioRad®). This program included the following steps: DNA denaturation cycle performed at 94°C for 5 minutes; 35 cycles of distortion at 94°C for 20 seconds, hybridization at 58°C for 30 seconds, and extension at 72°C for 1 minute; incubation of samples at 4°C.

The control of the amplicons was achieved by horizontal electrophoresis in a submerged system of electrophoresis in agarose gel 1.5% at 120 V and 90 mA for 60 minutes. HyperLadder IV DNA represented the standard of DNA molecular size - Bioline® 100 bp, which contains ten strips from 100 bp to 1000 bp.

After the samples migrated on the agarose gel, the gel image with DNA fragments was captured using a UV photosystem.

3.Results

Multiplex PCR demonstrated the presence of *T. britovi* in all three positive samples, two species-specific bands (127 and 253 bp) being revealed (Figure 2); no mixed infections were confirmed.

4.Discussion

Although *T. spiralis* and *T. britovi* are spread in the same environments in Europe, it is colloquially recognized that *T. britovi* is more prevalent in sylvatic carnivores, whereas *T. spiralis* is prevalent in both wild boars and domestic swine [8]. The results of the presented study seem to deny this statement since all three samples isolated from infected wild boars were confirmed as *T. britovi*. However, it is often reported *T. britovi* infects wild boars across Europe.

In Southern European countries, *T. britovi* was recently first reported in a wild boar originating from a defined risk area in Portugal [14].

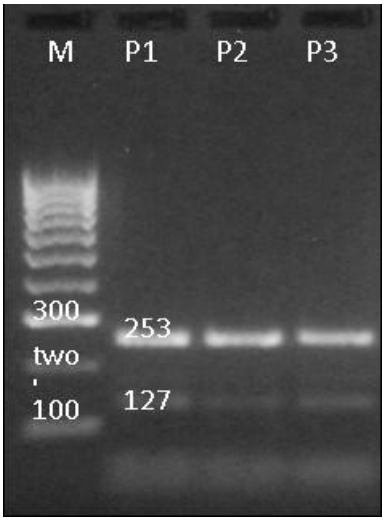


Figure 2. PCR-based detection of *Trichinella britovi* in infected wild boars; M-molecular marker, P1, 2, 3 – analyzed samples

In Northeastern Spain, the species was identified in 0.009% (3/33206) tested wild boars; however, *T. spiralis* was the dominant species (0.32%, 109/33206) in the area [15]. *T. britovi* is also reported in wild boar in Bosnia and Herzegovina [16]. In Bulgaria, *T. britovi* affected 97.83% of the examined wild boars, whereas *T. spiralis*, only 2.17% [17]. In Croatia, *T. britovi* was identified in 14 of the 38 (36.8%) molecularly tested samples from wild boars examined between 2010–2017 [18]. In Greece, even if all examined wild boars hunted during 2019–2020 were negative for *Trichinella* spp., [19] human infections with *T. britovi* are reported, wild boar meat or semi-raw pork being incriminated [20]. In Italy, *T. britovi* is reported in wild boar in different regions [21] being also involved in numerous human outbreaks, with the meat of this species as the source of infection [22, 23, 24]. Additionally, *T. britovi* was isolated from wild boar meat linked to a human trichinellosis outbreak in Serbia, suggesting the significant role of wild boars as reservoirs of this species in the country [25].

Trichinella britovi was also frequently reported in wild boars from Northern and Western Europe. Although *T. nativa* is the dominant species in the wildlife of the Nordic countries, an outbreak of human trichinellosis is described in Sweden with the source of wild boar meat contaminated with *T. britovi* [26].

The presence of *T. britovi* in wild boar is also indirectly confirmed in France through reporting some human outbreaks with the meat of this species as the source [27, 28]. A 38-year study in Latvia established an overall prevalence of 2.5% infected wild boar, *T. britovi* being the predominant (90%) species [29]. In Estonia, *T. britovi* has registered a total prevalence of 0.7%, but mixed infections with *T. spiralis* (0.01%) and *T. nativa* (0.02%) were also identified [30]. In Lithuania, 72% (31/43) of the examined wild boars were infected with *T. britovi* [31].

Trichinella britovi infection in wild boars is also reported in Central European countries. In Poland, *T. britovi* was identified in 23.8% of the wild boars' infected carcasses [32]. In Hungary, *T. britovi* was dominant in wild boars since 64.7% of the infected animals were infected with mentioned species, only 29.4% with *T. spiralis*, and 5.9% with *T. pseudospiralis* [33]. In Slovakia, *T. britovi* is also the predominant etiological agent of the sylvatic cycle, reported in 99% of the infected wild boars [34].

In Romania, *T. britovi* infection in wild boars is reported in the North-Eastern part of the country, where the recorded prevalence was 1.46% (156/10,695) [35]. Three samples collected from wild boars in Central Romania were confirmed as *T. britovi* [36]. In Eastern and Western Romania, the overall prevalence of *Trichinella* sp. in wild boars was 1.66% (93/5596), with *T. britovi* being identified in 34 (36.5%) of the infected animals [37]. Here, we also confirmed the presence of the species in the southern part of the country.

Trichinella britovi is the most widely distributed species within the temperate climate; apart from Europe, it is also spread in Asia and Northern and Western Africa [24]. In hunted wild boars in Northern Iran, the overall prevalence of *Trichinella* spp. infection was 5.7% (2/35), *T. britovi* being the most prevalent species circulating in wild boars of Iran [38]. The human outbreaks caused by consuming hunted wild boars' meat confirmed the presence of *T. britovi* in Turkey [39, 40].

Analyzing the data presented above, the hypothesis of parasitism, to the same extent, of wild carnivores and wild boars by *T. britovi* emerges, thus disproving the statement that this species is more prevalent in wild carnivores. Indeed, following the recognition of *T. britovi* as an independent species, it was initially identified in many wild carnivore species, leading to the idea of a specific carnivore species. However, equal parasitism of wild boar and carnivores by *T. britovi* demonstrates that the existing inter-relationships within the sylvatic focus are much more complex, requiring further investigations.

Conclusions

The presence of *T. britovi* in wild boars across Romania is a certainty. Thus, wild boar meat poses a high zoonotic risk, especially for private domestic use and the estrangement of untested meat. Consequently, it is essential to raise hunters' awareness of the risk that the consumption of wild boar meat can represent. Adjusting the health measures to minimize the infection risk of humans appears to be significant in reducing consumer risk. Concluding, the surveillance and control of Trichinella infection in wild boars must be maintained and promoted.

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Compliance with Ethics Requirements: Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human or animal subjects (if exist) respect the specific regulation and standards.

References

1. Pozio E. (2020). Scientific achievements of the last 60 years: From a single to a multispecies concept of the genus *Trichinella*. *Veterinary Parasitology*, 30, 109042.
2. Zarlenga D, Thompson P and Pozio E. (2020). *Trichinella* species and genotypes. *Research in Veterinary Science*, 133, 289-296.
3. Borza C, Kundnani NR, Darabus G, Mederle N, Pascu C, Abhinav S, Pavel R and Herman V. (2021). Trichinellosis: a constant present worldwide involved in the public health. *Revista Română de Medicină Veterinară*, 31, 92-96.
4. Marin AM, Dărăbuș G, Herman V, Morariu S, Olariu TR, Dărăbuș RG, Sîrbu B and Mederle N. (2021). Study on the prevalence and larval burden of the nematode *Trichinella* spp. in red foxes from hunting grounds in Timis County. *Revista Română de Medicină Veterinară*, 31, 23-26.

5. Bilska-Zajac E Rozycki M Korpysa-Dzirba W Belcik A Zietek-Barszcz A Wlodarczyk-Ramus M Gontarczyk A and Cencek T. (2021). *Trichinella* Outbreaks on Pig Farms in Poland in 2012-2020. *Pathogens*, 10, 1504.
6. Diaz JH, Warren RJ and Oster MJ. (2020). The Disease Ecology, Epidemiology, Clinical Manifestations, and Management of Trichinellosis Linked to Consumption of Wild Animal Meat. *Wilderness & Environmental Medicine*, 31, 235-244.
7. Pozio E. (2000). Factors affecting the flow among domestic, synanthropic and sylvatic cycles of Trichinella. *Veterinary Parasitology*, 93, 241-62.
8. Pozio E, Rinaldi L, Marucci G, Musella V, Galati F, Cringoli G, Boireau P and La Rosa G. (2009). Hosts and habitats of *Trichinella spiralis* and *Trichinella britovi* in Europe. *International Journal Parasitology*, 39, 71-9.
9. Gherman CM, Boros Z, Băieș MH, Cozma-Petruț A and Cozma V. (2022). A review of *Trichinella* species infection in wild animals in Romania. *Food Waterborne Parasitology*, 28:e00178.
10. Moraru MMF, Herman V, Oprescu I, Fodor JT, Morariu S, Ilie M, Marin AM and Mederle N. (2022). Wild boar - the source of human contamination with *Trichinella* spp. in Romania. *Revista Română de Medicină Veterinară*, 32, 38-42.
11. Nicorescu IM, Ionita M, Ciupescu L, Buzatu CV, Tanasuica R and Mitrea IL. (2015). New insights into the molecular epidemiology of *Trichinella* infection in domestic pigs, wild boars, and bears in Romania. *Veterinary Parasitology*, 212, 257-61.
12. Commission Implementing Regulation (EU) 2015/1375 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat (codification). Available online: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015R1375 (accessed on 12.12.2022).
13. Pozio E and La Rosa G. (2003). PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. *Methods in Molecular Biology*, 216, 299-309.
14. Vieira-Pinto M, Fernandes ARG, Santos MH and Marucci G. (2021). *Trichinella britovi* infection in wild boar in Portugal. *Zoonoses Public Health*, 68, 103-109.
15. Díaz A, Tejedor MT, Padrosa A and Quilez J. (2021). Prevalence of *Trichinella spiralis* and *Trichinella britovi* in wild boars in the northeast of Spain. *European Journal of Wildlife Research*, 67, 20.
16. Santrac V, Nedic DN, Maric J, Nikolic S, Stevanovic O, Vasilev S, Cvetkovic J and Sofronic-Milosavljevic L. (2015). The first report of *Trichinella pseudospiralis* presence in domestic swine and *T. britovi* in wild boar in Bosnia and Herzegovina. *Acta Parasitology*, 60, 471-5.
17. Lalkovski N. (2019). Species composition of *Trichinella* in domestic and wild animals in Bulgaria. *Bulgarian Journal of Veterinary Medicine*, 22, 99-104.
18. Balić D, Marucci G, Agičić M, Benić M, Krovina Z, Miškić T, Aladić K and Škrivanko M. (2020). *Trichinella* spp. in wild boar (*Sus scrofa*) populations in Croatia during an eight-year study (2010-2017). *One Health*, 11, 100172.
19. Dimzas D, Chassalevris T, Ozolina Z, Dovas CI and Diakou A. (2021). Investigation of the Food-Transmitted Parasites *Trichinella* spp. and *Alaria* spp. in Wild Boars in Greece by Classical and Molecular Methods and Development of a Novel Real-Time PCR for Alaria spp. Detection. *Animals* (Basel), 11, 2803.
20. Dimzas D, Diakou A, Koutras C, Gómez Morales MA, Psalla D, Keryttopoulos P, Deligianni D, Kontotasios K and Pozio E. (2019). Human trichinellosis caused by *Trichinella britovi* in Greece, and literature review. *Journal of Helminthology*, 13, 94:e33.
21. Pozio E, La Rosa G and Gomez Morales MA. (2001). Epidemiology of human and animal trichinellosis in Italy since its discovery in 1887. *Parasite*, 8(2 Suppl), S106-8.
22. Turiac IA, Cappelli MG, Olivieri R, Angelillis R, Martinelli D, Prato R and Fortunato F. (2017). Trichinellosis outbreak due to wild boar meat consumption in southern Italy. *Parasites&Vectors*, 10, 107.
23. Stroffolini G, Rossi L, Faraoni S, Calcagno A, Bonora S, DI Perri G and Calleri G. (2022). *Trichinella britovi* outbreak in north-west Italy, 2019-2020: beware of wild boar. *International Journal of Infectious Diseases*, 116, S77-S78.
24. Troiano G and Nante N. (2019). Human Trichinellosis in Italy: an epidemiological review since 1989. *Journal of Preventive Medicine and Hygiene*, 60, E71-E75.
25. Dmitric M, Debeljak Z, Vidanovic D, Sekler M, Vaskovic N, Matovic K and Karabasil N. (2018). *Trichinella britovi* in Game Meat Linked to Human Trichinellosis Outbreak in Serbia. *Journal of Parasitology*, 104, 557-559.
26. Gallardo MT, Mateos L, Artieda J, Wesslen L, Ruiz C, García MA, Galmés-Truyols A, Martin A, Hernández-Pezzi G, Andersson Y, Gárate T and Christensson D. (2007). Outbreak of trichinellosis in Spain and Sweden due to consumption of wild boar meat contaminated with *Trichinella britovi*. *Euro Surveillance*, 12, E070315.1.
27. Gari-Toussaint M, Tieulié N, Baldin J, Dupouy-Camet J, Delaunay P, Fuzibet JG, Le Fichoux Y, Pozio E and Marty P. (2005). Human trichinellosis due to *Trichinella britovi* in southern France after consumption of frozen wild boar meat. *Euro Surveillance*, 10, 117-8.

28. De Bruyne A, Ancelle T, Vallée I, Boireau P and Dupouy-Camet J. (2006). Human trichinellosis acquired from wild boar meat: a continuing parasitic risk in France. *Euro Surveillance*, 11, E060914.5
29. Kirjušina M, Deksne G, Marucci G, Bakasejevs E, Jahundoviča I, Daukšte A, Zdankovska A, Bērziņa Z, Esīte Z, Bella A, Galati F, Krūmiņa A and Pozio E. (2015). A 38-year study on *Trichinella* spp. in wild boar (*Sus scrofa*) of Latvia shows a stable incidence with an increased parasite biomass in the last decade. *Parasites&Vectors*, 8, 137.
30. Kärssin A, Häkkinen L,Vilem A, Jokelainen P and Lassen B. (2021). *Trichinella* spp. in Wild Boars (*Sus scrofa*), Brown Bears (*Ursus arctos*), Eurasian Lynxes (*Lynx lynx*) and Badgers (*Meles meles*) in Estonia, 2007–2014. *Animals*, 11, 183.
31. Malakauskas A, Paulauskas V, Järvis T, Keidans P, Eddi C and Kapel CMO. (2007). Molecular epidemiology of *Trichinella* spp. in three Baltic countries: Lithuania, Latvia, and Estonia. *Parasitology Research*, 100, 687–693.
32. Bilska-Zając E, Różycki M, Chmurzyńska E, Marucci G, Cencek T, Karamon J and Bocian L. (2013). *Trichinella* species circulating in wild boar (*Sus scrofa*) populations in Poland. *International Journal Parasitology Parasites Wildlife*, 2, 211-3.
33. Széll Z, Marucci G, Ludovisi A, Gómez-Morales MA, Sréter T and Pozio E. (2012). Spatial distribution of *Trichinella britovi*, *T. spiralis* and *T. pseudospiralis* of domestic pigs and wild boars (*Sus scrofa*) in Hungary. *Veterinary Parasitology*, 183, 393-6.
34. Hurníková Z and Dubinský P. (2009). Long-term survey on *Trichinella* prevalence in wildlife of Slovakia. *Veterinary Parasitology*, 159, 276-80.
35. Iacob O, Chiruță C and Mareș M. (2022). *Trichinella spiralis* and *T. britovi* in North-Eastern Romania: A Six-Year Retrospective Multicentric Survey. *Veterinary Sciences*, 9, 509.
36. Blaga R, Gherman C, Cozma V, Zocevic A, Pozio E and Boireau P. (2009). *Trichinella* species circulating among wild and domestic animals in Romania. *Veterinary Parasitology*, 159, 218-21.
37. Nicorescu IM, Ionita M, Ciupescu L, Buzatu CV, Tanasuica R and Mitrea IL. (2015). New insights into the molecular epidemiology of *Trichinella* infection in domestic pigs, wild boars, and bears in Romania. *Veterinary Parasitology*, 212, 257-61.
38. Rostami A, Khazan H, Kazemi B, Kia EB, Bandepour M, Taghipour N and Mowlavi G. (2017). Prevalence of *Trichinella* spp. Infections in Hunted Wild Boars in Northern Iran. *Iranian Journal of Public Health*, 46, 1712-1719.
39. Heper Y, Yilmaztepe F, Komitova R, Akalin H, Vutova K and Helvaci S. (2005). A trichinosis outbreak caused by wild boar meat in Turkey. *Parasite*, 12, 191-2.
40. Akkoc N, Kuruuzum Z, Akar S, Yuce A, Onen F, Yapar N, Ozgenc O, Turk M, Ozdemir D, Avci M, Guruz Y, Oral AM and Pozio E. (2009). Izmir Trichinellosis Outbreak Study Group. A large-scale outbreak of trichinellosis caused by *Trichinella britovi* in Turkey. *Zoonoses Public Health*, 56, 65-70.