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Trichinella britovi in wild boar meat from Italy, 2015–2021: A citizen science approach to surveillance

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ABSTRACT

As a result of the increase of game meat intended for human consumption through Europe, a plethora of food-borne diseases, including trichinellosis, may occur in consumers, posing a relevant public health threat.

Thus, this study aims to a citizen science approach to monitor the occurrence of *Trichinella* spp. in wild boar meat intended for human consumption, evaluating the risk of infection for consumers.

Following the European Regulation 2015/1375 (*laying down specific rules on official controls for Trichinella in meat*), from 2015 to 2021, hunters ($n = 478$) were involved to collect diaphragm pillar samples of wild boars from mainland southern Italy, which were tested for *Trichinella* spp. L_1 larvae via HCl-pepsin digestion and Multiplex PCR.

Overall, 139,160 animals were collected (average of 19,880 per year), being 14 (i.e., 0.01%) tested positive to *Trichinella britovi* by the combined biochemical and molecular approach. An average larval burden of 28.4 L_1 per gram of meat was found (minimum 3.2 - maximum 132.6). A statistically significant difference was found in the prevalence according to hunting seasons ($p < 0.01$, with higher values in 2016 and 2021) and regions of the study area ($p < 0.01$). No statistically significant decrease in the prevalence of *T. britovi* throughout the study period was found ($p = 0.51$), except in Apulia region ($p < 0.01$).

These findings revealed a stable prevalence of *T. britovi* in wild boar meat intended for human consumption, suggesting a risk of infection for consumers, especially hunters and local markets users. Citizen science surveillance models could be promoted to improve trichinellosis control and prevention in a *One Health* perspective.

1. Introduction

Considering that over 70% of emerging zoonoses origin from wildlife, the increasing density of synanthropic animal species in peri-urban areas may enhance the spread of pathogens to pets, farm animals and humans [1]. Is this the case of wild boar populations (*Sus scrofa*) which are getting new ecological niches in urban settlements [2], potentially increasing the chance for zoonoses transmission [3]. Moreover, in the last decades, the human consumption of wild boar meat and meat products has posed further challenges to the control and prevention of food-borne diseases [4], including trichinellosis. This parasitic zoonosis, caused by *Trichinella* spp. (Adenophorea, Trichinellidae), is responsible for life-threatening clinical implications in humans, such as chronic weakness and myalgia, trouble coordinating movements, heart and

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breathing symptoms, and even death in heavy infections [5]. Among the ten distinct *Trichinella* species worldwide identified, four (i.e., *Trichinella spiralis, Trichinella pseudospiralis*, *Trichinella nativa* and *Trichinella britovi*) have been found in European wild boar meat, to date suspected as one of the main source of infection for consumers [6]. In addition, due to the omnivorous diet of wild boars and their wide geographical distribution, these ungulates can act as a reservoir of infection for wild mammals and birds, enabling the spread of *Trichinella* into distant areas of the world [7]. Despite this, in European countries few large-scale studies are available on the occurrence of *Trichinella* spp. in wild boars, even if their meat products are commonly present in local markets and traditional festivals [8], being considered as a delicacy and touristic attractiveness due to the nutritional and culinary properties [9]. According to the European legislation on specific rules for official controls of *Trichinella* in meat (i.e., the Commission Implementing Regulation EU 2015/1375 and 2019/627) [10,11], all quarry susceptible to this parasite should be

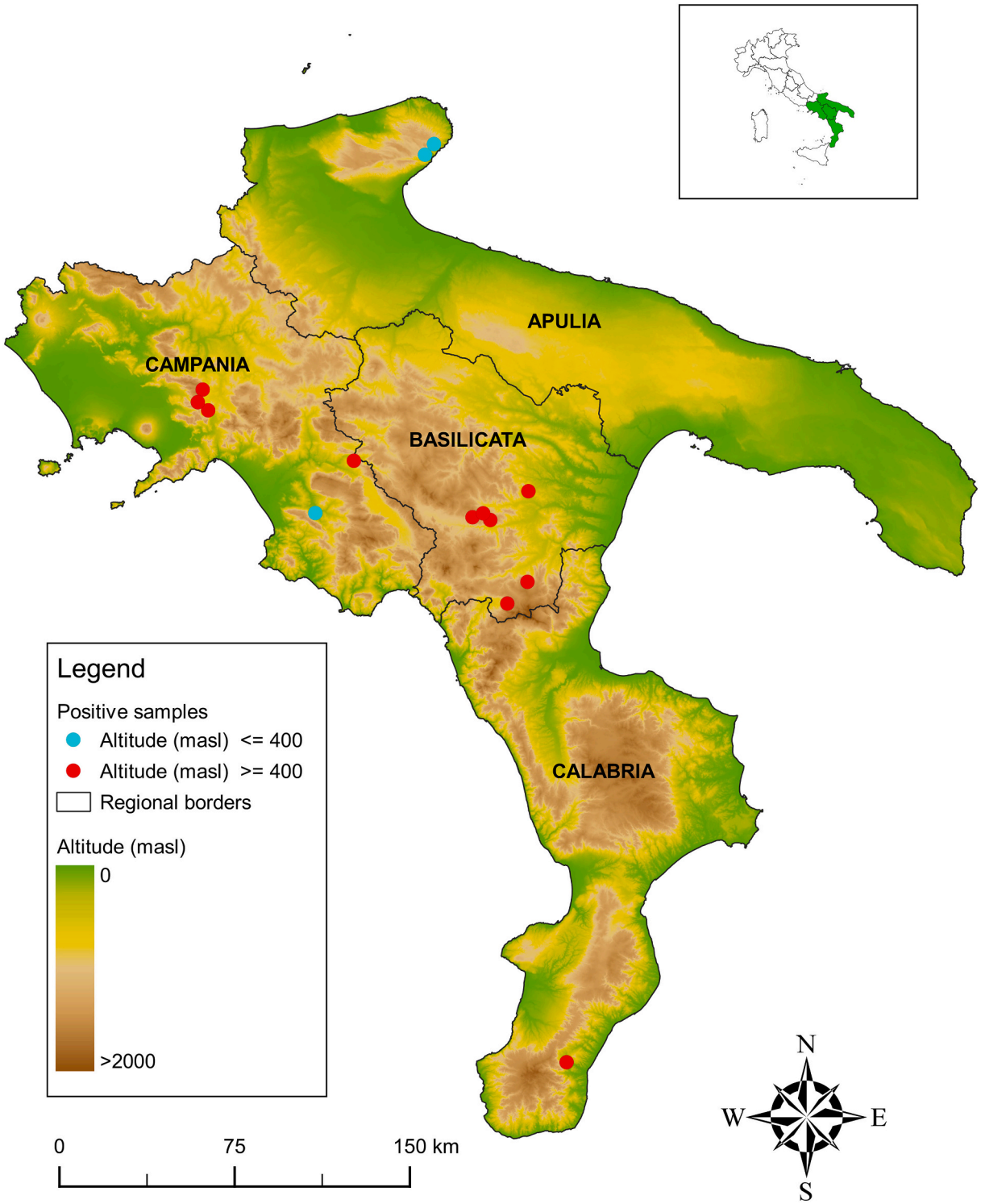

Fig. 1. Map showing the distribution of wild boars (n = 14) tested positive to *Trichinella britovi* according to different altitudes (meters on the sea level, masl) and regions of mainland southern Italy, 2015–2021.

screened for presence of larvae (L_1) in the muscles before being placed on the market for human consumption. However, given the trichinellosis outbreaks due to the ingestion of untested raw/undercooked wild boar game meat products in Europe, such as France [12], Spain and Sweden [13], Serbia [14], Belgium [15], as well as Italy [16], the health education of citizens (especially hunters) on the biology of this parasite and its monitoring in these ungulates is the way forward to prevent foodborne diseases, including trichinellosis.

Therefore, under the frame of a *citizen science* approach involving wild boar hunters of southern Italy, the aim of this study was to assess the occurrence of *Trichinella* species in game meat intended for human consumption for evaluating the risk of infection to consumers.

2. Methods

2.1. Participants enrolment

All participants enrolled (i.e., hunters, $n = 478$) were fully informed about the research aim and features. In accordance with the European Regulation EC No 853/2004 (*laying down specific hygiene rules for on the hygiene of foodstuffs*) [17], participants were trained on health education and proper handling of wild boar carcasses as recommended in Section 4, Chapter 1. All participants were supported in the field activities by veterinarians specialized in meat inspection of the University of Naples Federico II (Italy) and regional health systems (Aziende Sanitarie Locali, ASL).

2.2. Study area and sampling

This study included all regions (i.e., Apulia, Basilicata, Calabria, Campania) of southern Italy (Fig. 1.), characterized by a typical Mediterranean temperate climate and progressively continental features in mainland and mountainous landscapes. From October 2015 to December 2021, diaphragm pillar samples (approximately 100 g) of individual wild boar carcasses were collected by the participants, stored at ±4 °C in plastic biohazard bags, and delivered to the closer provincial section of the reference laboratory for *Trichinella* spp. of the Department of Animal Health, Experimental Zooprophylactic Institutes of Campania and Calabria (IZSME) and Apulia and Basilicata (IZSPB), for the biochemical examination.

2.3. Biochemical analyses

All diaphragm pillar samples (5 g) were screened for the detection of *Trichinella* spp. larvae using the HCl-pepsin digestion method, in accordance with the specific rules on official controls for *Trichinella* in meat (i.e., Commission Implementing Regulation EU 2015/1375 and 2019/627) [10,11]. Briefly, all samples were first analysed in pools, using 10 samples for each pool; when a positive was found, all samples of the pool were individually tested. In order to assess the average larval burden (i.e., no. larvae per gram of sample, lpg), isolated larvae were observed and counted by stereomicroscopy (Leica S9i, Leica Microsystems GmbH). All larvae were then fixed in 96% ethanol, stored at 2° - 8 °C and delivered to the European Union Reference Laboratory for Parasites (EURLP) of the Istituto Superiore di Sanità (Rome, Italy) for identification at the species level.

2.4. DNA extraction and PCR protocol

DNA was extracted from single larvae following the EURLP internal protocol "Identification of *Trichinella* muscle stage larvae at the species level by Multiplex PCR". Briefly, DNA was purified using DNA IQSystem kit (Promega, USA) and Tissue and Hair Extraction kit (Promega, USA). Five primer sets, targeting specific regions (expansion segment V, ITS1 and ITS2) of the ribosomal DNA repeats, were used to obtain a species-specific electrophoretic DNA banding pattern [18,19].

2.5. Statistical analysis

Exact binomial 95% confidence intervals (CIs) were established for the proportions of infection herein found. The exact Fisher's test was used to assess statistical differences of infection rates among the hunting seasons and regions of the study area. A value of $p < 0.05$ was considered statistically significant. Statistical analyses were performed by using the online software Epitools - Epidemiological Calculators [20]. The distribution of *T. britovi*-positive wild boars according to the altitudes and regional borders of the studied area was determined using ArcGIS (version 10.3; ESRI, Redlands, CA, USA).

3. Results

Overall, the *citizen science* approach herein followed permitted to collect and analyse a total number of 139,160 wild boar diaphragm pillars from mainland southern Italy, with an increasing number of samples recorded in all regions of the study area throughout the observed period (i.e., from 4,449 in 2015 to 22,460 in 2021, average value of 19,880 animals per year) (Table 1). Out of 139,160 wild boars examined, 14 (i.e., 0.01%; 95% CI: <0.01–0.02) scored positive to *Trichinella* spp. (Fig. 2.) by the HCl-pepsin digestion method and larvae were identified as *T. britovi* by the multiplex-PCR assay; no case of co-infection by different *Trichinella* species was found. The preponderance of positive wild boars (i.e., $n = 11/14$; 78.6%; 95% CI: 52.4–92.4) was found in geographic areas of 400 m above sea level (masl) (Fig. 1.). The HCl-pepsin method revealed an average larval burden in diaphragm pillar samples of 28.4 lpg, with minimum and maximum values ranging from 3.2 to 132.6, respectively. During the different hunting seasons, higher prevalence values (i.e., 0.02%; 95% CI: 0.01–0.06) were observed in wild boars collected during 2016 and 2021. Regarding the regions, a statistically significant difference in prevalence was found ($p < 0.01$), being in Apulia the highest infection rate recorded (i.e., 0.6%; 95% CI: 0.2–2.2). No statistically significant decrease in the prevalence of *T. britovi* throughout the study period was found ($p = 0.51$), except in Apulia region ($p < 0.01$). Detailed data on prevalence, confidence intervals and statistical analyses, based on different hunting seasons and regions, are listed in Table 1. The map reporting the distribution of *T. britovi*-positive wild boars, according to different altitudes and regional borders of the study area, is shown in Fig. 1.

4. Discussion

This study reports the first large-scale survey using *citizen science* approach for assessing the occurrence of *Trichinella* spp. in wild boar game meat of Italy.

The high number of wild boars screened (i.e., $n = 139,160$; $n = 19,880$ per year) and the wide study area (mainland southern Italy) herein investigated highlight the importance of *citizen science* as a way forward to obtain data of interest to public health. Moreover, the increasing number of samples collected in all investigated regions throughout the observed period (i.e., from 4,449 in 2015 to 22,460 in 2021), clearly indicates a positive feedback by hunters in terms of health education, game meat safety surveillance and trichinellosis outbreaks prevention. Indeed, although the high abundance of wild boar populations in the studied area has been estimated [2], the main reason for this increasing trend of samples in later years is likely the training on the food safety of game meat and potential infection risks, involving a growing number of hunters aware of the importance of testing meat.

The low overall prevalence (i.e., 0.01%) of *T. britovi* in wild boar populations of Italy is consistent with that of Hungary (0.01%) [21] and Croatia (0.07%) [9], although higher rates of infection are reported in these ungulates from Estonia (0.7%) [22] and Latvia (2.2%) [23]. Indeed, this significant difference of infection prevalence in different countries would confirm the greater occurrence of *T. britovi* in the north than in the south of Europe, due to a plethora of environmental

Table 1
Wild boar diaphragm pillar samples ($n = 139,160$) tested for *Trichinella* spp., continental southern Italy, 2015–2021.

Hunting season	Region Apulia		Basilicata		Calabria		Campania		Total		95% CI	*p*-value
	Pos/Tot	%	Pos/Tot	%	Pos/Tot	%	Pos/Tot	%	Pos/Tot	%		
2015	0/25	NA	0/2	NA	0/1,216	NA	0/3,206	NA	0/4,449	NA	NA	NA
2016	2/11	18.2	0/4	NA	0/5,354	NA	1/9,830	0.01	3/15,199	0.02	0.01 - 0.06	$p<<0.01$
2017	0/10	NA	0/690	NA	0/9,163	NA	0/9,316	NA	0/19,179	NA	NA	NA
2018	0/15	NA	2/624	0.3	0/13,517	NA	0/10,726	NA	2/24,882	<0.01	<0.01 - 0.03	$p<<0.01$
2019	0/71	NA	2/1,519	0.1	0/14,502	NA	1/13,278	<0.01	3/29,370	0.01	<0.01 - 0.03	$p<<0.01$
2020	0/109	NA	0/1,174	NA	1/11,270	0.01	1/11,068	0.01	2/23,621	0.01	<0.01 - 0.03	$p=0.990$
2021	0/84	NA	2/1,274	0.2	0/5,233	NA	2/15,869	0.01	4/22,460	0.02	0.01 - 0.05	$p<0.01$
Total	2/325	0.6	6/5,287	0.1	1/60,255	<0.01	5/73,293	0.01	14/139,160	0.01	<0.01 - 0.02	$p<<0.01$
95% CI	0.2 - 2.2		0.05 - 0.2		<0.01 - 0.01		<0.01 - 0.02		<0.01 - 0.02			
p	$p<0.01$		$p=0.580$		$p=0.630$		$p=0.860$		$p=0.51$			

95% CI: 95% confidence interval; NA: not applicable; *p*: *p*-value with statistical significance <0.05; Pos/Tot: number of positive samples on the total examined.

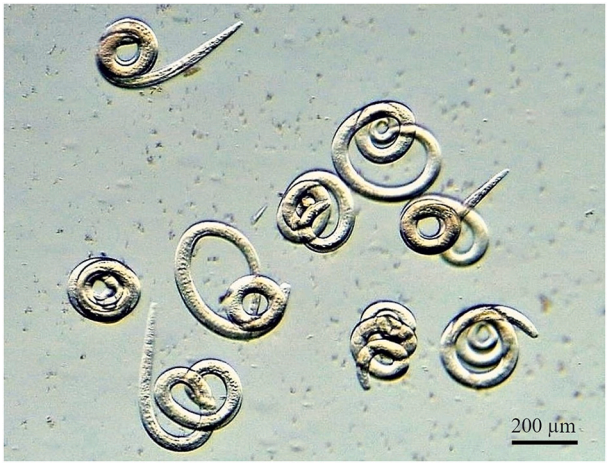

Fig. 2. *Trichinella* spp. L_1 larvae found by HCl-pepsin method in a diaphragm pillar sample of a wild boar collected in southern Italy between 2015 and 2021.

conditions which favour the survival of larvae in decaying muscles of animal carcasses [24]. However, the finding of *T. britovi* as the only *Trichinella* spp. in wild boars of the study area corroborates this species as the main circulating in the Mediterranean basin [25], unlike the central European countries where *T. spiralis* is notoriously widespread [9]. In fact, although *T. spiralis* and *T. britovi* occasionally occur in sympatry, their territorial separation could be due to the biological ability of the first species to inhibit infections in the host by the second one [9]. This hypothesis would agree with the experimental demonstrations of inhibition by *T. spiralis* towards *T. nativa* and *T. pseudospiralis*, indicating that co-infections do not occur when a host is primarily infected by *T. spiralis* [18,26]. Finally, the preponderance of positive wild boars (i.e., $n = 11/14$; 78.6%) from areas of at least 400masl in the study area confirms the wider circulation of *T. britovi* in wildlife at high altitudes, due to a less anthropic pressure and, consequently, increased carnivorous and scavenging behaviours in such areas [27].

Regarding the larval burden of *T. britovi* in wild boar meat, the average value herein found (i.e., 28.4 lpg) suggests a high risk of human infection, not only for hunters and their families and friends who commonly represent the main users of these food products [8,28]. In fact, as revealed by a recent questionnaire survey from Portugal, a high percentage of hunters (i.e., 93%) give away/sell meat or homemade products (e.g., raw sausages), often untested for *Trichinella* spp. (i.e., 80% of cases), introducing at least 12 t of potentially infected meat on the European market [8]. Consequently, it is not surprising that untested wild boar meat is responsible for 55% of global cases of human trichinellosis [28], being also implicated in several outbreaks of infection in European countries in the last decades [12–16]. The data above, combined to the increased consumption of wild boar meat in Europe [29], emphasize the crucial role of public health stakeholders in trichinellosis educational and surveillance programs towards hunters and consumers to prevent the risk of infection, as recommended by the European Regulation EC No 853/2004 [17] and the Commission Implementing Regulation EU 2015/1375 and 2019/627 [10,11]. In addition, the higher average of larvae in wild boars (i.e., 28.4 lpg) compared to other wildlife, e.g. brown bears (*Ursus arctos*, 4.1 lpg), lynxes (*Lynx lynx*, 4.3 lpg), badgers (*Meles meles*, 11.7 lpg) [22], European polecats (*Mustela putorius*, 24.6 lpg) [30], red foxes (*Vulpes vulpes*, 2.3 lpg) [31] and wolves (*Canis lupus*, 7.6 lpg) [32] may indicate a repeated ingestion of infected meat by these ungulates via scavenging, eventually enhanced through the availability of wildlife carcasses from road accidents and improper disposal of their offal by hunters during field activities [6,33]. Indeed, the inappropriate discarding of carcasses on the hunting ground of the study area (e.g., foxes culled by hunters to reduce the predation towards other huntable species, such as hares *Lepus europaeus* - personal communication) would increase the availability of large amount of potential sources of infection to wild boars, promoting the parasitic circulation [33,34]. This hypothesis furtherly underscores the importance of training hunters on transmission pathways of zoonotic parasites, as well as proper game meat handling procedures in the field.

Based on statistics, the absence of a significant decrease in prevalence throughout the study period ($p = 0.51$), except in Apulia, indicates that *T. britovi* is well established in wild boar populations of southern Italy, suggesting the need to intensify the link among sanitary stakeholders and consumers [35]. In addition, the statistically significant higher prevalence of *T. britovi* in Apulia than in other regions of the study area (i.e., 0.6%; $p < 0.01$), could be related to several aspects affecting the parasite circulation in a given environment (i.e., hunting management, wildlife density, prey-predator regulation, overlap among wild, synanthropic and domestic life cycle) [30] which need to be further investigated.

5. Conclusions

These findings revealed a stable prevalence of *T. britovi* in wild boar meat intended for human consumption, suggesting a risk of infection for consumers, especially hunters and local markets users. In a *One Health* perspective, citizen science projects and a deeper cooperation between scientific stakeholders (e.g., physicians, veterinarians, biologists) and data providers, such as citizens and wildlife managers, are advised to establish technically sound surveillance models for the prevention of trichinellosis.

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Ethical approval

Not required.

CRediT authorship contribution statement

Giovanni Sgroi: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Nicola D'Alessio:** Writing – review & editing, Supervision, Funding acquisition. **Gianluca Marucci:** Methodology, Validation, Resources, Writing – review & editing. **Laura Pacifico:** Formal analysis. **Francesco Buono:** Formal analysis. **Georgiana Deak:** Writing – review & editing. **Aniello Anastasio:** Data curation. **Maria Interisano:** Methodology. **Pasquale Fraulo:** Data curation. **Antonella Pesce:** Data curation. **Valerio Toscano:** Data curation. **Antonella Cristina Romano:** Data curation. **Mariateresa Toce:** Data curation. **Lucia Palazzo:** Data curation. **Esterina De Carlo:** Data curation. **Alessandro Fioretti:** Data curation. **Vincenzo Veneziano:** Conceptualization, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

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References

[1] S.S. Morse, J.A. Mazet, M. Woolhouse, C.R. Parrish, D. Carroll, W.B. Karesh, et al., Prediction and prevention of the next pandemic zoonosis, Lancet. 380 (2012) 1956–1965, https://doi.org/10.1016/S0140-6736(12)61684-5.

[2] C. Pittiglio, S. Khomenko, D. Beltran-Alcrudo, Wild boar mapping using population-density statistics: from polygons to high resolution raster maps, PLoS One 13 (2018), e0193295, https://doi.org/10.1371/journal.pone.0193295.

[3] G. Sgroi, R. Iatta, R.P. Lia, N. D'Alessio, R.R.S. Manoj, V. Veneziano, et al., Spotted fever group rickettsiae in *Dermacentor marginatus* from wild boars in Italy, Transbound. Emerg. Dis. 68 (2021) 2111–2120, https://doi.org/10.1111/tbed.13859.

[4] G. Sgroi, M. Viscardi, M. Santoro, G. Borriello, N. D'Alessio, F. Boccia, et al., Genotyping of *toxoplasma gondii* in wild boar (*Sus scrofa*) in southern Italy: epidemiological survey and associated risk for consumers, Zoonoses Public Health 67 (2020) 805–813, https://doi.org/10.1111/zph.12762.

[5] Centers for Disease Control and Prevention, Parasites - Trichinellosis (also known as Trichinosis). https://www.cdc.gov/parasites/trichinellosis, 2020 (accessed 10 November 2022).

[6] E. Pozio, Factors affecting the flow among domestic, synanthropic and sylvatic cycles of *Trichinella*, Vet. Parasitol. 93 (2000) 241–262, https://doi.org/10.1016/s0304-4017(00)00344-7.

[7] E. Pozio, D.S. Zarlenga, New pieces of the *Trichinella* puzzle, Int. J. Parasitol. 43 (2013) 983–997, https://doi.org/10.1016/j.ijpara.2013.05.010.

[8] M. Vieira-Pinto, A.R.G. Fernandes, M.H. Santos, G. Marucci, *Trichinella britovi* infection in wild boar in Portugal, Zoonoses Public Health 68 (2021) 103–109, https://doi.org/10.1111/zph.12800.

[9] D. Balić, G. Marucci, M. Agičić, M. Benić, Z. Krovina, T. Miškić, et al., *Trichinella* spp. in wild boar (*Sus scrofa*) populations in Croatia during an eight-year study (2010-2017), One Health. 11 (2020), 100172, https://doi.org/10.1016/j.onehlt.2020.100172.

[10] European Union, Commission Implementing Regulation (EU) 2015/1375 laying down specific rules on official controls for *Trichinella* in meat of 2015. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32015R1375, 2015 (accessed 10 August 2022).

[11] European Union, Commission Implementing Regulation (EU), 2019/627 laying down uniform practical arrangements for the performance of official controls on products of animal origin intended for human consumption of 2019. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX:32019R0627, 2019 (accessed 10 August 2022).

[12] M. Gari-Toussaint, N. Tieulié, J. Baldin, J. Dupouy-Camet, P. Delaunay, J. G. Fuzibet, et al., Human trichinellosis due to *Trichinella britovi* in southern France after consumption of frozen wild boar meat, Euro Surveill. 10 (2005) 117–118, https://doi.org/10.2807/esm.10.06.00550-en.

[13] M.T. Gallardo, L. Mateos, J. Artieda, L. Wesslen, C. Ruiz, M.A. García, et al., Outbreak of trichinellosis in Spain and Sweden due to consumption of wild boar meat contaminated with *Trichinella britovi*, Euro Surveill. 12 (2007), https://doi.org/10.2807/esw.12.11.03154-en. E070315.1.

[14] R. Barruet, A. Devez, J. Dupouy-Camet, G. Karadjian, D. Plavsa, G. Chydériotis, et al., A common source for a trichinellosis outbreak reported in France and Serbia in 2017, Euro Surveill. 25 (2020) 1900527, https://doi.org/10.2807/1560-7917.ES.2020.25.24.1900527.

[15] P. Messiaen, A. Forier, S. Vanderschueren, C. Theunissen, J. Nijs, M. Van Esbroeck, et al., Outbreak of trichinellosis related to eating imported wild boar meat, Belgium, 2014, Euro Surveill. 15 (2016) 21, https://doi.org/10.2807/1560-7917.ES.2016.21.37.30341.

[16] I.A. Turiac, M.G. Cappelli, R. Olivieri, R. Angelillis, D. Martinelli, R. Prato, et al., Trichinellosis outbreak due to wild boar meat consumption in southern Italy, Parasit. Vectors 10 (2017) 107, https://doi.org/10.1186/s13071-017-2052-5.

[17] European Union, Regulation (EC) No 853/2004 of the European Parliament and of the Council laying down specific hygiene rules for food of animal origin of 29 April 2004. https://eur-lex.europa.eu/legal-content/en/ALL/?uri=CELEX%3A32004R0853, 2004 (accessed 10 August 2022).

[18] D.S. Zarlenga, M.B. Chute, A. Martin, C.M.O. Kapel, A multiplex PCR for unequivocal differentiation of six encapsulated and three non-encapsulated genotypes of *Trichinella*, Int. J. Parasitol. 29 (1999) 1859–1867, https://doi.org/10.1016/s0020-7519(99)00107-1.

[19] E. Pozio, G. La Rosa, Trichinella, in: D. Liu (Ed.), Molecular Detection of Foodborne Pathogens, CRC Press Taylor & Francis Group, Boca Raton, 2010.

[20] E.S.G. Sergeant, Epitools: Epidemiological Calculators, Ausvet, 2018. https://epitools.ausvet.com.au (accessed 20 November 2022).

[21] Z. Tolnai, Z. Széll, G. Marucci, E. Pozio, T. Sréter, Environmental determinants of the spatial distribution of *Trichinella britovi* and *Trichinella spiralis* in Hungary, Vet. Parasitol. 204 (2014) 426–429, https://doi.org/10.1016/j.vetpar.2014.04.024.

[22] A. Kärssin, L. Häkkinen, A. Vilem, P. Jokelainen, B. Lassen, *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 (2021) 183, https://doi.org/10.3390/ani11010183.

[23] M. Kirjušina, G. Deksne, G. Marucci, E. Bakasejevs, I. Jahundoviča, A. Daukšte, et al., 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, Parasit. Vectors 8 (2015) 137, https://doi.org/10.1186/s13071-015-0753-1.

[24] F. Bruschi, E. Pozio, Trichinella britovi, Trends Parasitol. 36 (2020) 227–228, https://doi.org/10.1016/j.pt.2019.10.003.

[25] E. Pozio, V. Di Marco Lo, D. Presti, A. Vicari, F.P. Ludovisi, M. Amati Ciarello, et al., The detection of anti-*Trichinella* antibodies in free-ranging Nebrodi Regional Park black pigs from Sicily, Italy, suggests the circulation of *Trichinella britovi* in the island, Vet. Parasitol. Reg. Stud. Reports. 24 (2021), 100578, https://doi.org/10.1016/j.vprsr.2021.100578.

[26] H.K. Ooi, M. Kamiya, M. Ohbayashi, Cross resistance against challenge infection in mice infected with *Trichinella spiralis* or *T. pseudospiralis*, Jpn. J. Vet. Res. 35 (1987) 87–97, https://doi.org/10.14943/jjvr.35.2.87.

[27] E. Pozio, Trichinellosis in the European Union: epidemiology, ecology and economic impact, Parasitol. Today 14 (1998) 35–38, https://doi.org/10.1016/s0169-4758(97)01165-4.

[28] A. Rostami, H.R. Gamble, J. Dupouy-Camet, H. Khazan, F. Bruschi, Meat sources of infection for outbreaks of human trichinellosis, Food Microbiol. 64 (2017) 65–71, https://doi.org/10.1016/j.fm.2016.12.012.

[29] D. Fulgione, M. Buglione, The boar war: five hot factors unleashing boar expansion and related emergency, Land. 11 (2022) 887, https://doi.org/10.3390/land11060887.

[30] Z. Boros, A.M. Ionică, G. Deak, A.D. Mihalca, G. Chișamera, I.C. Constantinescu, et al., *Trichinella* spp. infection in European polecats (*Mustela putorius* Linnaeus, 1758) from Romania, Helminthologia. 58 (2021) 323–327, https://doi.org/10.2478/helm-2021-0032.

[31] E. Bilska-Zając, M. Różycki, K. Grądziel-Krukowska, A. Bełcik, I. Mizak, J. Karamon, et al., Diversity of *Trichinella* species in relation to the host species and geographical location, Vet. Parasitol. 279 (2020), 109052, https://doi.org/10.1016/j.vetpar.2020.109052.

[32] R. Beck, A. Beck, J. Kusak, Z. Mihaljević, S. Lucinger, T. Zivicnjak, et al., Trichinellosis in wolves from Croatia, Vet. Parasitol. 159 (2009) 308–311, https://doi.org/10.1016/j.vetpar.2008.10.068.

[33] G. Sgroi, A. Varcasia, G. Dessì, N. D'Alessio, L. Pacifico, F. Buono, et al., Massive *Taenia hydatigena* cysticercosis in a wild boar (*Sus scrofa*) from Italy, Acta Parasitol. 64 (2019) 938–941, https://doi.org/10.2478/s11686-019-00110-3.
[34] M.A. Gomez-Morales, M. Selmi, A. Ludovisi, M. Amati, E. Fiorentino, L. Breviglieri, et al., Hunting dogs as sentinel animals for monitoring infections with *Trichinella* spp. in wildlife, Parasit. Vectors 9 (2016) 154, https://doi.org/10.1186/s13071-016-1437-1.
[35] Enetwild, Report of the first Annual General Meeting of ENETWILD Parma, 16–18 January 2018. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/sp.efsa.2018.EN-1400, 2018.