International Journal for Parasitology: Parasites and Wildlife 19 (2022) 191–195

Contents lists available at ScienceDirect

International Journal for Parasitology: Parasites and Wildlife

journal homepage: www.elsevier.com/locate/ijppaw

Trichinella surveillance program in wild birds, Emilia-Romagna (northern Italy), 2006–2021. First report of *Trichinella pseudospiralis* in western marsh harrier (*Circus aeruginosus*) in Italy

Rugna G [a,*], Marucci G [b], Bassi P [a], Gelmini L [a], D'Annunzio G [a], Torreggiani C [a], Pupillo G [a], Rubini S [a], A. Di Donato [a], Maioli G [a], Garbarino C [a], Tamba M [a]

[a] *Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna, Via A. Bianchi 9, 25124, Brescia, Italy*
[b] *Unit of Foodborne and Neglected Parasitic Diseases, Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161, Rome, Italy*

ARTICLE INFO

Keywords:
Trichinella pseudospiralis
Western marsh harrier
Birds
Trichinella surveillance
Italy

ABSTRACT

The nematode *Trichinella pseudospiralis* is a cosmopolitan parasite capable of infecting both birds and mammals including humans. *T. pseudospiralis* has a limited zoonotic importance in comparison to that of the other *Trichinella* species. However, it has been recognized as the etiological agent of two outbreaks of trichinellosis due to the consumption of wild boar meat. The role played by birds in the epidemiology of *T. pseudospiralis* is still unclear and needs to be deepened. The aim of our work was to show the results of an extensive wild bird surveillance carried out in the Emilia-Romagna (E-R) region, northern Italy, over the last 16 years. As part of the regional wildlife surveillance program, 14,933 raptors and carrion-eating birds' carcasses were necropsied from 2006 to 2021 and tested for the presence of *Trichinella* spp. larvae with only one positive result, an adult female of western marsh harrier. The larvae load (LPG) was evaluated on breast (100 LPG), wings (3.6 LPG), shoulder (2 LPG), head (4.5 LPG), thighs (8 LPG), lower legs (2 LPG) and tongue (0.77 LPG). The results of the present study confirm that also in northern Italy *T. pseudospiralis* has a low prevalence comparing to that of other *Trichinella* species. However, this study demonstrates that *T. pseudospiralis* can reach a high parasitic load in infested birds. The large distribution range, probably facilitated by its ability to infest birds, suggests the need for a continuous monitoring program.

1. Introduction

Nematodes of the genus *Trichinella* are relevant food-borne zoonotic agents causing trichinellosis in humans and show a global distribution in domestic and wild animals (Pozio and Murrell, 2006; Pozio, 2007). In Europe, four species with different biological characteristics, namely *T. spiralis*, *T. nativa*, *T. britovi* and *T. pseudospiralis*, are known to circulate in wild carnivores and some in sylvatic and domestic swine (Pozio, 2019).

The non-encapsulated species *T. pseudospiralis*, discovered for the first time in a raccoon (*Procyon lotor*) in Russia (Garkavi, 1972), is considered a cosmopolitan parasite (Pozio, 2016). This species is the only one of the genus capable of infecting both birds and mammals including humans (Pozio and Zarlenga, 2013; Pozio, 2016).

In the last years, there has been a great increase of the detection of *T. pseudospiralis* in the European Union. This nematode is widely distributed in wildlife (e.g. birds of prey, wild boars, red foxes, raccoon dogs), and its occurrence in domestic pigs and synanthropic rats was also documented (Pozio, 2001; Hurníková et al., 2005; Nöckler et al., 2006; Pozio, 2016; Cybulska et al., 2018), representing a potential source of human infections. The increased use of artificial digestion of muscle samples to detect *Trichinella* spp. larvae (European Commission, 2015), which is much more sensitive than trichinelloscopy in detecting non-encapsulated species (Gottstein et al., 2009; Bilska-Zając et al., 2020), is probably the primary reason for the increased recognition of this parasite (La Rosa et al., 2001). However, its prevalence is generally much lower than that of encapsulated sympatric species (Pozio and Zarlenga, 2013).

* Corresponding author.
E-mail addresses: gianluca.rugna@izsler.it (R. G), patrizia.bassi@izsler.it (B. P), luca.gelmini@izsler.it (G. L), giulia.dannunzio@izsler.it (D. G), camilla.torreggiani@izsler.it (T. C), giovanni.pupillo@izsler.it (P. G), silva.rubini@izsler.it (R. S), alessandra.didonato@izsler.it (A. Di Donato), giulia.maioli@izsler.it (M. G), chiaraanna.garbarino@izsler.it (G. C), marco.tamba@izsler.it (T. M).

https://doi.org/10.1016/j.ijppaw.2022.09.006
Received 31 August 2022; Received in revised form 21 September 2022; Accepted 21 September 2022
Available online 30 September 2022

Therefore, *T. pseudospiralis* has a limited zoonotic importance in comparison to that of the other *Trichinella* species. However, it has been recognized as the etiological agent of two outbreaks of trichinellosis due to the consumption of wild boar meat, which occurred in France and Italy in 1999 and 2015, respectively (Ranque et al., 2000; Gómez-Morales et al., 2021).

The number of mammals tested for *Trichinella* spp. by digestion has been much higher than that of birds, thus the role played by birds in the epidemiology of *T. pseudospiralis* is still unclear and needs to be deepened (Pozio, 2016).

The aim of our work was to show the results of an extensive wild bird surveillance carried out in the Emilia-Romagna (E-R) region, northern Italy, over the last 16 years.

2. Material and methods

2.1. Sampling

According to the Commission Regulation (EC) no. 2075/2005 (European Commission, 2005), a wildlife monitoring program in the E-R region has been implemented in 2006 with the aim to provide data for the risk assessment of *Trichinella* spp. presence in domestic pigs.

The program has been carried out by the official veterinary services in collaboration with hunters, forest guards and staff employed in Wildlife Rescue Centres and identifies as indicator species of *Trichinella* presence all carnivores, birds of prey, and other bird species with necrophagous behaviour, such as corvids. As a food safety measure, the detection of *Trichinella* spp. is also carried out in all hunted wild boars (Regione Emilia-Romagna, 2022).

2.2. Diagnostic procedures in birds

From each bird, 10 g of muscles from the head were tested for the presence of parasite larvae by the magnetic stirrer method for sample digestion according to the Commission Regulation (EC) no. 1375/2015 (European Commission, 2015). This diagnostic procedure is considered as the gold standard for the *Trichinella* spp. larvae detection and does not differ from the ISO 18743:2015 procedure (International Organization for Standardization, 2015). In case of a positive result, larvae were recovered, stored in 90% ethyl alcohol and forwarded to the European Union Reference Laboratory for Parasites (EURLP, Istituto Superiore di Sanità, Rome, Italy) for species identification.

2.3. Trichinella species identification and isolate typing

DNA was extracted from single larvae using DNA IQ System 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 in a multiplex PCR to obtain a species-specific electrophoretic DNA banding pattern (Zarlenga et al. 1999; Pozio and La Rosa 2010). PCR products were purified using QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and sent to Eurofins Scientific (Brussels, Luxembourg) for standard Sanger sequencing.

The PCR-amplified ESV sequence was compared to GenBank database, by Basic Local Alignment Search Tool (BLAST), to search for similar sequences. To investigate the polymorphism of ESV microsatellite locus, the sequence was aligned with published sequences of *T. pseudospiralis* isolates belonging to Palearctic, Nearctic, and

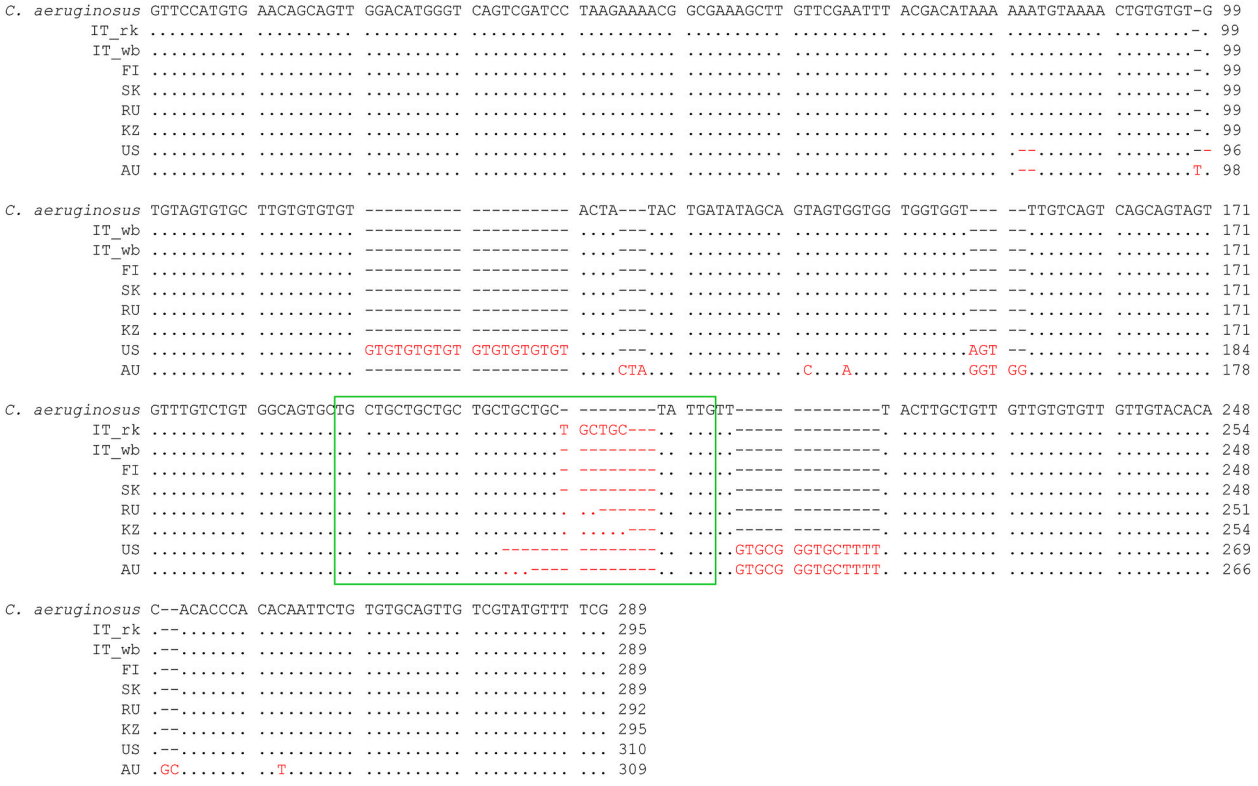

Fig. 1. Alignment of homologous ESV sequences of *T. pseudospiralis* isolates belonging to Palearctic, Nearctic and Australian populations. *C. aeruginosus*, isolate from the western marsh harrier (ISS8343); IT_rk, isolate from a red kite (*Milvus milvus*) of the Basilicata region (ISS7768); IT_wb, isolate from a wild boar hunted in Northern Italy (ISS2851); FI, isolate from a raccoon dog (*Nyctereutes procyonoides*) of Finland (ISS681); SK, isolate from a peregrine falcon (*Falcus peregrinus*) of the Slovak Republic (MN963194); RU, isolate from a raccoon (*Procyon lotor*) of Southern Russia (ISS13); KZ, isolate from a tawny eagle (*Aquila rapax*) of Kazakhstan (ISS176); US, isolate from a black vulture (*Coragypus atratus*) of USA (ISS470); AU, isolate from a tiger cat (*Dasyurus maculatus*) of Australia (ISS141). Conserved bases are represented by dots; gaps are represented by dashes; different residues are highlighted in red; TGC microsatellite region is boxed in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Australian populations (Fig. 1). A total of six Palearctic isolates (northern Italy, southern Italy, Finland, Kazakhstan, southern Russia, Slovak Republic), one Nearctic isolate (Alabama), and one Australian isolate (Tasmania) were used in the alignment. All the analyses were done by using the bioinformatics platform CLC Workbench 8.0.1 (Qiagen, Hilden, Germany).

3. Results

As part of the wildlife monitoring program in the E-R region, northern Italy, 14,933 raptors and carrion-eating birds' carcasses were necropsied from 2006 to 2021 and tested for the presence of *Trichinella* spp. larvae with one positive result (Table 1).

On the 24th of March 2021, an adult female western marsh harrier (*Circus aeruginosus* Linnaeus, 1758), dead after an attempted rescue by a Wildlife Rescue Centre, was collected in the Modena municipality, E-R region, Italy; 34 m above sea level.

After the detection of *Trichinella* sp. larvae in head muscles (e.g. masseter and neck muscles), samples from other muscle groups as breast (5 gr), thighs (5 g), lower legs (5 g), wings (5 g), and the whole tongue (1.3 g) were also collected and tested for the presence of *Trichinella* sp. larvae. Following digestion, larvae were recovered, enumerated and the larvae per g of tissue (LPG) value was calculated. All the body parts tested positive for the presence of *Trichinella* sp. larvae with LPG values ranging from a minimum of 0.77 for the tongue to a maximum of 100 for breast muscles (Table 2). The larvae were identified as *T. pseudospiralis* by multiplex PCR. The amplified ESV sequence (GenBank accession No. OP325399) was identical to the homologous sequence obtained from *T. pseudospiralis* larvae isolated from a wild boar in Italy as well as to those isolated from a raccoon dog in Finland and from a peregrine falcon in Slovak Republic. When compared with other Palearctic isolates, including one collected from a red kite in Southern Italy, the sequence showed to differ only for the number of repeats of the microsatellite TGC (Fig. 1).

Table 1
Birds tested for *Trichinella* spp. in the Emilia-Romagna region, 2006–2021.

Order	Common name	Scientific name	No. of tested birds	No. of Positive
Accipitriformes	Northern goshawk	*Accipiter gentilis*	15	
	Eurasian sparrowhawk	*Accipiter nisus*	114	
	Golden eagle	*Aquila chrysaetos*	1	
	Buteo	*Buteo buteo*	212	
	Western marsh harrier	*Circus aeruginosus*	9	1
	Hen harrier	*Circus cyaneus*	1	
	Greater spotted eagle	*Clanga clanga*	1	
	Red kite	*Milvus milvus*	1	
	European honey buzzard	*Pernis apivorus*	1	
Charadriiformes	European herring gull	*Larus argentatus*	32	
	Mediterranean gull	*Larus melanocephalus*	1	
	Black-headed gull	*Larus ridibundus*	56	
Falconiformes	Peregrine falcon	*Falco peregrinus*	1	
	Hawk	*Falco* spp.	32	
	Eurasian hobby	*Falco subbuteo*	7	
	Common kestrel	*Falco tinnunculus*	346	
	Red-footed falcon	*Falco vespertinus*	1	
Passeriformes	Hooded crow	*Corvus cornix*	3829	
	Eurasian jackdaw	*Corvus monedula*	7	
	Crow	*Corvus* spp.	60	
	Eurasian jay	*Garrulus glandarius*	1369	
	Eurasian magpie	*Pica pica*	8324	
Pelecaniformes	Great egret	*Ardea alba*	1	
	Grey heron	*Ardea cinerea*	23	
	Purple heron	*Ardea purpurea*	1	
	Cattle egret	*Bubulcus ibis*	3	
	Little egret	*Egretta garzetta*	8	
	Least bittern	*Ixobrychus exilis*	1	
	Dwarf bittern	*Ixobrychus sturmii*	1	
	Black-crowned night heron	*Nycticorax nycticorax*	2	
Strigiformes	Short-eared owl	*Asio flammeus*	1	
	Long-eared owl	*Asio otus*	108	
	Little owl	*Athene noctua*	210	
	Eurasian scops owl	*Otus scops*	73	
	Tawny owl	*Strix aluco*	39	
	Barn owl	*Tyto alba*	42	

4. Discussion

Since its first description in a raccoon (Garkavi, 1972), *T. pseudospiralis* was detected in a large variety of domestic animals and wildlife including pigs, birds, wild boars, red foxes, racoon dogs (Pozio, 2016). Recently, the nematode was detected for the first time in a wolf from Italy (Ricchiuti et al., 2021). Birds are suitable host for this non-encapsulated species and are suspected to be involved in the maintenance and dissemination of the parasite in the sylvatic cycle. However, there have been more reports of natural infections of *T. pseudospiralis* in mammalian than in avian hosts, although these values may be biased given that wild birds are rarely tested for the presence of *Trichinella* spp. larvae (Pozio, 2016).

The reproductive capacity index values (RCI) of *T. pseudospiralis* isolates in experimentally infected chickens were apparently low (La Rosa et al., 2001), while rodents were more suitable hosts for the parasite, suggesting that the role played by mammals as reservoirs of this species is greater than that of avian hosts. Anyhow, this does not preclude the possibility that carrion-eating avian species may function as better hosts for *T. pseudospiralis*, resulting in a higher RCI, and therefore, enhancing the geographical dissemination of the species, which is known to be present in at least three continents (Pozio and Murrell, 2006).

The role of raptorial birds as a host of *T. pseudospiralis* has been documented in eight species belonging to five Families, namely: *i)* Family Strigidae: a tawny owl (*Strix aluco*) and a little owl (*Athene noctua*); *ii)* Family Corvidae: a crow (*Corvus frugilegus*); *iii)* Family Accipitridae: a tawny eagle (*Aquila rapax*), a western marsh harrier (*Circus aeruginosus*) and a red kite (*Milvus milvus*); *iv)* Family Tytonidae: a masked owl (*Tyto novaehollandiae*); *v)* Family Cathartidae: a black vulture (*Coragyps atratus*) (Pozio, 2016; Marucci et al., 2021).

A previous study on a larger number of scavenging birds evaluated the presence of *Trichinella* spp. in free-ranging corvids in central Italy. None of the 769 tested birds was positive (Mancianti et al., 2020).

Over a period of 16 years, the monitoring program of the E-R region involved several thousand birds belonging to six different orders and found only one positive result. In the same period and region, *T. pseudospiralis* larvae were also detected in only one out of thousands of

Table 2
T. pseudospiralis larvae distribution in the tested muscle groups of the western marsh harrier of this study. LPG = larvae per gram of tissue digested.

Muscle group	Total number of larvae	LPG
Breast	500	100
Wings	18	3.6
Shoulder	10	2
Head (masseter and neck muscles)	45	4.5
Thighs	40	8
Lower legs	10	2
Tongue	1	0.77

screened wild boars (Merialdi et al., 2011), confirming the fact that the detection of this parasite is sporadic (Pozio, 2016).

The *T. pseudospiralis*-infected subject identified in our study was rescued by a Wildlife Rescue Centre because it was unable to fly. The routine necropsy showed a wing fracture and cranial trauma as the only pathological findings. After the positive result, the subject was more thoroughly examined to determine the distribution and load of *T. pseudospiralis* larvae among different muscular groups. The higher LPG (value 100) was detected in the breast muscles, while the average number of larvae in other muscular districts was significantly lower (Table 2).

The distribution of muscular larvae of *Trichinella* spp. is mainly influenced by both the species of nematode and the host species (Kapel et al., 1998; Cybulska et al., 2018). According to Kapel et al. (2005), the predilection muscles of *T. pseudospiralis* larvae in experimentally infected red foxes were the diaphragm, the upper forelimb and the filet, whereas a lower larval recovery was observed in the lower hindlimb, the masseter, the lower forelimb and the tongue. On the contrary, in an experimentally infection in swine, the highest larval burden was detected in the tongue and the diaphragm (Pozio et al., 2020).

The intensity of *Trichinella* spp. in wild animals is generally lower than 10 LPG, while intensities higher than 50 LPG are considered exceptional (Dick and Pozio, 2001). Interestingly, a previous report on *T. pseudospiralis* in *Circus aeruginosus* from Tasmania showed a parasite load of 650 LPG. In the same report, a 6-day-old marsh harrier was experimentally infected with muscle tissue containing *T. pseudospiralis* larvae (34 LPG) derived from a naturally infected Tasmanian devil, highlighting that this bird was a suitable host for *T. pseudospiralis* (Obendorf and Clarke, 1992). To the best of our knowledge there are no studies on pathological changes in birds infected by *Trichinella* larvae. Therefore, we are not able to establish whether the observed fractures were caused by injuries due to a pre-existing pathological flying difficulty linked to the high larval burden in breast muscles.

The western marsh harrier is a medium-sized diurnal raptor belonging to the Family Accipitridae Vieillot, 1816. The marsh harrier diet consists mainly of small mammals such as mice (*Apodemus* spp.) and voles (*Arvicola* spp.), and fish, insects, eggs and medium-sized birds (Alves et al., 2014). This feeding behaviour makes this bird a susceptible species for *Trichinella* spp. infection. The species has an extremely wide breeding range from Europe and north western Africa to Central Asia and the northern parts of the Middle East. The species is a partial migrant, with populations in Southern and Western Europe, North Africa and at the south of its range in Asia being generally resident (Ferguson-Lees and Christie 2001; Agostini and Panuccio, 2010). In Italy, breeding sites are mainly located in coastal wetlands of the northern Adriatic and inland marshes of the Po Valley (Nardo, 1993; Martelli and Rigacci, 2005; Spina and Volponi, 2008).

Biological, biochemical, and molecular analyses have revealed three different populations of *T. pseudospiralis* in the Palearctic, Nearctic and Australian regions (Zarlenga et al. 1996; La Rosa et al., 2001). The ESV sequence of the larvae infecting the western marsh harrier of this study showed to be in some cases identical, in others highly similar, to the homologous sequence obtained from *T. pseudospiralis* larvae isolated from different hosts in Europe. The difference concerned only the numbers of repeat of the TGC microsatellite. Since the TGC microsatellite length polymorphism represents a peculiar characteristic of the Palearctic isolates of *T. pseudospiralis* (La Rosa et al., 2001), the high variability of the region can explain the difference between the sequences obtained from the larvae belonging to the two Italian birds of prey.

5. Conclusions

The results of the wildlife monitoring program of the Emilia-Romagna region confirm that also in Italy *T. pseudospiralis* has a low prevalence comparing to that of other *Trichinella* species (e.g. *T. britovi*), especially in carnivores (Garbarino et al., 2017; Ricchiuti et al., 2021). However, this study demonstrates that *T. pseudospiralis* can reach a high parasitic load in infested birds. Although the low prevalence of this species represents a limited zoonotic risk for humans, the sporadic but at the same time ongoing isolation of this parasite suggests a stable presence in the Italian territory. The large distribution range, probably facilitated by its ability to infest birds, suggests the need for a continuous monitoring program involving both domestic and wild animals.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors are grateful to all the local hunters, forest guards, and staff from the Wildlife Rescue Centres for their help in collecting samples. We would also like to express our gratitude for Dr. Elio Licata and Dr. Stefano Palminteri from the Veterinary Public Health Services for the implementation and coordination of the wildlife surveillance program in the Emilia-Romagna region.

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Agostini, N., Panuccio, M., 2010. Western marsh harrier (*Circus aeruginosus*) migration through the Mediterranean Sea: a review. J. Raptor Res. 44 (2), 136–142.

Alves, M., Ferreira, J., Torres, I., Fonseca, C., Matos, M., 2014. Habitat use and selection of the marsh harrier *Circus aeruginosus* in an agricultural-wetland mosaic. ARDEOLA 61 (2), 351–366.

Bilska-Zając, E., Różycki, M., Grądziel-Krukowska, K., Bełcik, A., Mizak, I., Karamon, J., Sroka, J., Zdybel, J., Cencek, T., 2020. Diversity of *Trichinella* species in relation to the host species and geographical location. Vet. Parasitol. 279, 109052.

Cybulska, A., Skopek, R., Kornacka, A., Popiołek, M., Piróg, A., Laskowski, Z., Moskwa, B., 2018. First detection of *Trichinella pseudospiralis* infection in raccoon (*Procyon lotor*) in Central Europe. Vet. Parasitol. 254, 114–119.

Dick, T.A., Pozio, E., 2001. *Trichinella* spp. and trichinellosis. In: Samuel, W.M., Pybus, M.J., Kocan, A.A. (Eds.), Parasitic Diseases of Wild Mammals, second ed. Iowa State University Press, Ames, IA, pp. 380–396.

European Commission, 2005. Commission Regulation (EC) No 2075/2005 of 5 December 2005 laying down specific rules on official controls for *Trichinella* in meat (Text with EEA relevance). Off. J. L338, 68–82, 2005.

European Commission, 2015. Commission Regulation (EC) No 1375/2015 of 10 August 2015 laying down specific rules on official controls for *Trichinella* in meat (Text with EEA relevance). Off. J. L212, 7–34, 2015.

Ferguson-Lees, J., Christie, D.A., 2001. Raptors of the World. Houghton Mifflin Harcourt.

Garbarino, C., Interisano, M., Chattante, A., Marucci, G., Merli, E., Arrigoni, N., Cammi, G., Ricchi, M., Tonanzi, D., Tamba, M., La Rosa, G., Pozio, E., 2017. *Trichinella spiralis* a new alien parasite in Italy and the increased risk of infection for domestic and wild swine. Vet. Parasitol. 246, 1–4.

Garkavi, B.L., 1972. Species of *Trichinella* isolates from wild carnivores. Veterinariya 10, 90–91.

Gómez-Morales, M.A., Mazzarello, G., Bondi, E., Arenare, L., Bisso, M.C., Ludovisi, A., Amati, M., Viscoli, C., Castagnola, E., Orefice, G., Magnè, F., Pezzotti, P., Pozio, E., 2021. Second outbreak of *Trichinella pseudospiralis* in Europe: clinical patterns, epidemiological investigation and identification of the etiological agent based on the Western blot patterns of the patients' serum. Zoonoses and Public Health 68 (1), 29–37.

Gottstein, B., Pozio, E., Nöckler, K., 2009. Epidemiology, diagnosis, treatment, and control of trichinellosis. Clin. Microbiol. Rev. 22 (1), 127–145.

Hurníková, Z., Snábel, V., Pozio, E., Reiterová, K., Hrcková, G., Halásová, D., Dubinský, P., 2005. First record of *Trichinella pseudospiralis* in the Slovak Republic found in domestic focus. Vet. Parasitol. 128 (1–2), 91–98.

ISO 18743, 2015. Microbiology of the Food Chain - Detection of *Trichinella* Larvae in Meat by Artificial Digestion Method. International Organization for Standardization, Geneva, Switzerland. First edition 2015-09-15.

Kapel, C.M., Webster, P., Lind, P., Pozio, E., Henriksen, S.A., Murrell, K.D., Nansen, P., 1998. *Trichinella spiralis, T. britovi*, and *T. nativa*: infectivity, larval distribution in muscle, and antibody response after experimental infection of pigs. Parasitol. Res. 84 (4), 264–271.

Kapel, C.M., Webster, P., Gamble, H.R., 2005. Muscle distribution of sylvatic and domestic *Trichinella* larvae in production animals and wildlife. Vet. Parasitol. 132 (1–2), 101–105.

La Rosa, G., Marucci, G., Zarlenga, D.S., Pozio, E., 2001. *Trichinella pseudospiralis* populations of the Palearctic region and their relationship with populations of the Nearctic and Australian regions. Int. J. Parasitol. 31 (3), 297–305.

Mancianti, F., Terracciano, G., Sorichetti, C., Vecchio, G., Scarselli, D., Perrucci, S., 2020. Epidemiologic survey on *Toxoplasma gondii* and *Trichinella pseudospiralis* infection in corvids from Central Italy. Pathogens 9 (5), 336.

Martelli, D., Rigacci, L., 2005. Aggiornamento dello status del falco di palude in Italia. Avocetta 29, 117.

Marucci, G., Romano, A.C., Interisano, M., Toce, M., Pietragalla, I., Collazzo, G.P., Palazzo, L., 2021. *Trichinella pseudospiralis* in a red kite (*Milvus milvus*) from Italy. Parasitol. Res. 120 (6), 2287–2290.

Merialdi, G., Bardasi, L., Fontana, M.C., Spaggiari, B., Maioli, G., Conedera, G., Vio, D., Londero, M., Marucci, G., Ludovisi, A., Pozio, E., Capelli, G., 2011. First reports of *Trichinella pseudospiralis* in wild boars (*Sus scrofa*) of Italy. Vet. Parasitol. 178 (3–4), 370–373.

Nardo, A., 1993. Il Falco di palude *Circus aeruginosus* in alcune aree della provincia di Venezia. Convegno Faunisti Veneti-Montebelluna. Mezzavilla F., Stival E. (red.) Atti 1, 123–126.

Nöckler, K., Reckinger, S., Pozio, E., 2006. *Trichinella spiralis* and *Trichinella pseudospiralis* mixed infection in a wild boar (Sus scrofa) of Germany. Vet. Parasitol. 137 (3–4), 364–368.

Obendorf, D.L., Clarke, K.P., 1992. *Trichinella pseudospiralis* infections in free-living Tasmanian birds. J. Helm. Soc. Wash. 59 (1), 144–147.

Pozio, E., 2001. New patterns of *Trichinella* infection. Vet. Parasitol. 98, 133–148.

Pozio, E., 2007. World distribution of *Trichinella* spp. infections in animals and humans. Vet. Parasitol. 149 (1–2), 3–21.

Pozio, E., 2016. *Trichinella pseudospiralis* an elusive nematode. Vet. Parasitol. 231, 97–101.

Pozio, E., 2019. *Trichinella* and trichinellosis in Europe. Vet. Glas. 73 (2), 65–84.

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

Pozio, E., Murrell, K.D., 2006. Systematics and epidemiology of *Trichinella*. Adv. Parasitol. 63, 367–439.

Pozio, E., Zarlenga, D.S., 2013. New pieces of the *Trichinella* puzzle. Int. J. Parasitol. 43 (12–13), 983–997.

Pozio, E., Merialdi, G., Licata, E., Della Casa, G., Fabiani, M., Amati, M., Cherchi, S., Ramini, M., Faeti, V., Interisano, M., Ludovisi, A., Rugna, G., Marucci, G., Tonanzi, D., Gómez-Morales, M.A., 2020. Differences in larval survival and IgG response patterns in long-lasting infections by *Trichinella spiralis*, *Trichinella britovi* and *Trichinella pseudospiralis* in pigs. Parasites Vectors 13 (1), 520.

Ranque, S., Faugère, B., Pozio, E., La Rosa, G., Tamburrini, A., Pellissier, J.F., Brouqui, P., 2000. *Trichinella pseudospiralis* outbreak in France. Emerg. Infect. Dis. 6 (5), 543–547.

Regione Emilia-Romagna, 2022. Sorveglianza e monitoraggio della fauna selvatica. Available at: https://www.alimenti-salute.it/documentazione-regionale/90355. (Accessed 21 September 2022).

Ricchiuti, L., Petrini, A., Interisano, M., Ruberto, A., Salucci, S., Marino, L., Del Riccio, A., Cocco, A., Badagliacca, P., Pozio, E., 2021. First report of *Trichinella pseudospiralis* in a wolf (*Canis lupus italicus*). Int. J. Parasitol Parasites Wildl 15, 195–198.

Spina, F., Volponi, S., 2008. Atlante della Migrazione degli Uccelli in Italia. Volume I - Non-Passeriformi. Istituto Superiore per la Protezione e la Ricerca Ambientale (ISPRA). Available at: https://www.isprambiente.gov.it/it/pubblicazioni/pubblicazioni-di-pregio/atlante-della-migrazione-degli-uccelli-in-italia, 27/08/2022.

Zarlenga, D.S., Aschenbrenner, R.A., Lichtenfels, J.R., 1996. Variations in microsatellite sequences provide evidence for population differences and multiple ribosomal gene repeats within *Trichinella pseudospiralis*. J. Parasitol. 82 (4), 534–538.

Zarlenga, D.S., Chute, M.B., Martin, A., Kapel, C.M., 1999. A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. Int. J. Parasitol. 29 (11), 1859–1867.