

# The International Trichinella Reference Centre database. Report on thirty-three years of activity and future perspectives

G. Marucci<sup>a,\*</sup>, D. Tonanzi<sup>a</sup>, M. Interisano<sup>a</sup>, P. Vatta<sup>a</sup>, F. Galati<sup>b</sup>, G. La Rosa<sup>a</sup>

<sup>a</sup> Department of Infectious Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome 00161, Italy

<sup>b</sup> DG-INF, Servizio di Informatica, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome 00161, Italy

## ARTICLE INFO

### Keywords:

*Trichinella*  
Database  
International Trichinella reference Centre

## ABSTRACT

The International Trichinella Reference Centre (ITRC) is the official laboratory of the International Commission on Trichinellosis, of the World Organization for Animal Health and of the European Union Reference Laboratory for Parasites. The ITRC was established in 1988 as a repository of *Trichinella* strains and a source of reference materials and information for international scientific research. To date, more than 8000 *Trichinella* isolates collected throughout the world have been identified at the species or genotype level by the ITRC staff and the information has been stored in a freely accessible database providing the largest collection of data available for scientists involved in the systematics and epidemiology of this parasite. This paper presents a summary of the data collected over 33 years of activity and describes the database functionalities. It finally advocates the potential of the database to improve knowledge of the epidemiology and taxonomy of *Trichinella*, which in turn may help the international surveillance of *Trichinella* species.

## 1. Introduction

The International Trichinella Reference Centre (ITRC) was established in 1988 as the official reference laboratory of the International Commission on Trichinellosis (ICT). It was then recognized by the World Organization for Animal Health (OIE) in 1992, and, since 2006, it actively cooperates with the European Union Reference Laboratory for Parasites (EURLP, ISS, Italy).

The current tasks of the ITRC are: i) *in vivo* maintenance of reference isolates of the species and genotypes recognized in the genus; ii) on-demand identification of *Trichinella* larvae at the species or genotype level; iii) assignment of a unique ISS identification code characterizing each tested isolate iv) preparation and storage of reference material, including L1 larvae, crude antigens and DNA, which are provided under request; vi) collection of genetic and epidemiological data generated by the ITRC; vii) storage and sharing of the data in a publicly accessible database (DB).

Trichinellosis is a world-wide zoonosis acquired by consuming raw or improperly cooked meat or meat-derived products from wild and domestic carnivores and omnivores infected with larvae of nematode worms of the genus *Trichinella*. The genus is partitioned into two clades based on the presence or absence of a collagen wall (capsule) that envelops the worm while in the muscle cell. The encapsulated clade comprises seven species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli*, *T. nelsoni*, *T. patagoniensis* and *T. chanchalensis*) and three genotypes (*Trichinella* T6, *Trichinella* T8 and *Trichinella* T9) infecting mammals (Pozio and Zarlenga, 2021). The non-encapsulated clade includes three species, *T. pseudospiralis* infecting mammals and birds, and *T. papuae* and *T. zimbabwensis*

\* Corresponding author.

E-mail address: [gianluca.marucci@iss.it](mailto:gianluca.marucci@iss.it) (G. Marucci).

<https://doi.org/10.1016/j.fawpar.2022.e00156>

Received 22 December 2021; Received in revised form 11 April 2022; Accepted 11 April 2022

Available online 19 April 2022

2405-6766/© 2022 The Authors. Published by Elsevier Inc. on behalf of International Association of Food and Waterborne Parasitology. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

infecting mammals and reptiles (Pozio et al., 2004).

The ITRC has, to date, successfully identified at the species or genotype level more than 8000 *Trichinella* isolates originating from European and non-European countries, providing the largest collection of data available for scientists involved in systematics and epidemiology of this parasite. All available information such as parasite host, geographical location and coordinates, date of collection, the sender and their affiliation, are recorded, along with the ISS code. In 2002, the original paper-based database was “digitalized” to facilitate data entry and registration and in 2004 replaced by a free access web application to permit retrieving of information by users.

This paper aims to underline the work done by the ITRC in collecting information on the isolates identified in a DB and showing a summary of the epidemiological results suggested by their analysis.

## 2. The database

### 2.1. *Trichinella* isolates

From the end of the 80's, the confirmation of the hypothesis that the *Trichinella* genus was far more polymorphic than originally believed incited more epidemiological studies, but these were of little value to other investigators since authors usually assigned *Trichinella* infections to *T. spiralis* and only rarely they did a more detailed analysis to infer the belonging to one of the other species known at that time. During a meeting held in Orvieto (Italy) in 1993, the International Commission on Trichinellosis (ICT) urged scientists to further investigate the *Trichinella* isolates to species level prior to the publication of infection reports. Because of this, the ITRC flourished and took on an international role in *Trichinella* research because investigators needed to have their newly identified isolates genotyped.

Scientists from different countries and institutions have contributed to the DB growth by providing *Trichinella* larvae collected during routine meat inspection of animals intended for human consumption, epidemiological studies or necropsy of road-killed or hunted wildlife carcasses. Therefore, since the larvae received by the ITRC were collected for different purposes, the data contained in the DB must be analysed cautiously, taking into account the possible bias due, for example, to epidemiological campaigns on individual host or geographic areas.

### 2.2. Identification methods

The methods used to identify each *Trichinella* isolate are annotated in the DB, reflecting improvements in diagnostic methods over time. The analysis of the alloenzyme patterns (Flockhart et al., 1982; La Rosa et al., 1992) was the first method used (from 1982 to 1992) and was applied to about 250 isolates. Subsequently, from 1992 to 1998, about 400 isolates were identified by the Random Amplification of Polymorphic DNA (RAPD) technique (Bandi et al., 1993). Of importance, the higher sensitivity of RAPD allowed the analysis of single larvae (instead of pools of larvae) and an easier and faster identification of the infecting species.

Since 1999, the multiplex PCR (Zarlenga et al., 1999; Pozio and La Rosa, 2010) has become the diagnostic method of choice for the identification of *Trichinella* species and genotypes, due to its low cost and technical simplicity. The method uses five primer pairs, designed to amplify specific fragments of the ribosomal gene cluster (internal transcribed spacers 1 and 2, and expansion segment V of the large subunit rDNA gene). The amplification pattern, that includes one or two bands, is characteristic of every known species or genotype, except for T8 and *T. chanchalensis*, whose pattern is identical to that of *T. britovi* and *T. nativa*, respectively. *Trichinella* T8 is identified by sequencing part of the internal transcribed spacer 2 (Marucci et al., 2009), while *T. chanchalensis* by PCR-Restriction Fragment Length Polymorphism (Sharma et al., 2020).

Over 7400 *Trichinella* isolates have been identified by multiplex PCR. The method has been further refined by the use of automated DNA extraction and capillary electrophoresis.

### 2.3. The database

The first version of the computerized database was powered by a web application developed in MS Access and published through the Citrix Metaframe platform. In 2020, an upgrade of the database was designed and developed in Asp.net core technology using Microsoft Visual Studio 2019 (Microsoft Corporation, Washington, USA), incorporating existing and novel queries. The associated database was designed, normalized and developed to Fourth Normal Form (4NF) using Microsoft SQL Server Management Studio 18.

Each isolate positively identified at the ITRC is added to the DB, and each record (i.e., each isolate) includes all the information provided by the sender/user and the ITRC staff. The attributes associated with the isolates include: the ISS code assigned by ITRC, the *Trichinella* species identified by ITRC, host species common name, host species scientific name, original name of the sample as assigned by sender, location of origin (including continent, country, province, locality and geographical coordinates), year of collection, year of arrival at ISS, identification method used at ITRC, donor name, donor institution, institution address, relevant notes suggested by the sender or by ITRC. Most of the attributes are searchable through the web application for convenience of the user. The database is currently hosted in the ICT web site: <http://www.trichinellosis.org/>

#### 2.3.1. Geographical distribution

The 8000 records/isolates present in the ITRC database helped to draw an overall picture of the geographical distribution of *Trichinella* species and their correlations with biological and ecological factors. Six continents are represented in the DB, Europe (93%), North America (3.76%), Asia (1.76%), Africa (0.64%), South America (0.48%) and Oceania (0.42%). About 5000 distinct geographical

locations from 64 countries are currently represented (Table 1).

According to this geographical information, *T. spiralis* and *T. pseudospiralis* each occupy a cosmopolitan distribution, while the distribution and relative prevalence of the other *Trichinella* species and genotypes are in accordance with specific ecological factors and/or climate conditions. *T. nativa* and *Trichinella* T6 are present in the coldest area of the world, i.e., *T. nativa* in arctic and subarctic regions of Asia, Europe and North America, and the T6 genotype in arctic and subarctic regions of North America and along the Rocky Mountains.

*T. britovi* and *T. murrelli* are distributed in temperate regions of Asia and Europe and of North America, respectively. *T. nelsoni*, *T. zimbabweensis* and the T8 genotype are confined in Africa South of the Sahara, *T. papuae* is present in the Australasia region and in South East Asia, *T. patagoniensis* in Southern regions of South America, *T. chanchalensis* has been detected in north-western Canada, while the T9 genotype is restricted to Japan (Table 3) (Fig. 1).

### 2.3.2. Host species

A total of 70 different host species are recorded in the DB, including 61 species of mammals, 5 species of birds and 3 species of reptiles (Table 2). Among mammals, the largest number of *Trichinella* isolates were collected from wild boar (*Sus scrofa*, 39%), domestic pig (*Sus scrofa domesticus*, 18%) and red fox (*Vulpes vulpes*, 14%). Other well-represented host species are lynx (*Lynx lynx*, 9%), raccoon dog (*Nyctereutes procyonoides*, 6%), wolf (*Canis lupus lupus*, 5%) and brown bear (*Ursus arctos*, 1%) (Fig. 2). Avian hosts include the little owl (*Athene noctua*), tawny owl (*Strix aluco*), tawny eagle (*Aquila rapax*), black vulture (*Coragyps atratus*) and red kite (*Milvus milvus*), all birds of prey or species with scavenging behavior. Reptile species include Nile crocodile (*Crocodylus niloticus*), saltwater crocodile (*Crocodylus porosus*) and monitor lizard (*Varanus niloticus*). *Trichinella* isolates collected from pork products (sausages, salami, smoked meat, etc.) and from muscle biopsies of patients with trichinellosis are also present in the DB, but in a very small percentage.

### 2.3.3. *Trichinella* species and genotypes

The species most frequently recorded in the database are *T. spiralis* (40.4%), *T. britovi* (37.5%) and *T. nativa* (17.1%), the other species range between a minimum of 0.01% for *T. chanchalensis* to a maximum of 1.5% for the *Trichinella* T6 genotype (Table 3).

With the advent of molecular tests and identification of multiple single worms from each isolate, it became possible to identify isolates harbouring infections comprising different species (mixed infections). Currently, 237 out of 8065 isolates (2.9%) present in the database are mixed infections. The most abundant species in mixed infections is *T. spiralis* (56.6%), which has been found in coinfection with *T. britovi*, *T. nativa* and *T. pseudospiralis* in Europe, Asia and North America, and in coinfection with *T. murrelli* in North America. The second most involved species is *T. nativa* (39.7%), which has been found in coinfection with *T. britovi* and *T. pseudospiralis* in Europe and with T6 in North America. Less frequent mixed infections involve *T. britovi* + *T. pseudospiralis* (1.7%) observed in Europe and *T. nelsoni* + *Trichinella* T8 (0.8%) observed in South Africa. Two triple infections were also reported, the first involving *T. spiralis* + *T. nativa* + *T. britovi* was detected in a lynx from Finland and in a wolf from Russia, and the second involving *T. spiralis* + *T. nativa* + *T. pseudospiralis* detected in a brown bear from Russia (Table 4).

## 2.4. Hosts-species relationship

According to the host information reported in the DB, which reflects various sampling biases, *T. spiralis* is primarily associated with wild boars (54.7%) and domestic pigs (34.7%). *T. nativa* isolates are mainly associated with lynx (40%), raccoon dog (26%), red fox (11.7%) and wolf (10.3%); *T. britovi* isolates are associated with wild boar (41%), red fox (26.3%), domestic pig (9.2%) and wolf (8.8%); *T. pseudospiralis* infections are associated with wild boar (47.4%), Florida panther (13%) and raccoon dog (12.4%). As regards to non-European *Trichinella* species, T6 is mainly reported in wolverine (68.8%) and less frequently in wolf (7.2%) and grizzly (6.4%); *T. murrelli* in coyote (37.3%), bobcat (25.3%) and raccoon (14.6%); *T. nelsoni* in spotted hyena (33.3%), lion (33.3%) and leopard (11.1%); T8 in lion (62.5%), spotted hyena (25%) and leopard (12.5%); *T. papuae* in saltwater crocodile (55%), wild pig (35%); *T. zimbabweensis* in Nile crocodile (78.9%) and monitor lizard (15.8%); *T. patagoniensis* in cougar (80%) and mountain lion (20%) (Fig. 3).

## 3. Discussion

The new ITRC application was designed to streamline existing queries and maintenance of the database and ease the construction/

**Table 1**  
Number of different localities of origin of the *Trichinella* isolates reported in the database by continent.

Continent	No of countries	No of locality of origin	Total number of isolates
Africa	9	38	52
Asia	12	62	142
Oceania	3	6	34
Europa	34	4689	7495
North America	4	127	303
South America	2	27	39
<b>Total</b>	<b>64</b>	<b>4949</b>	<b>8065</b>

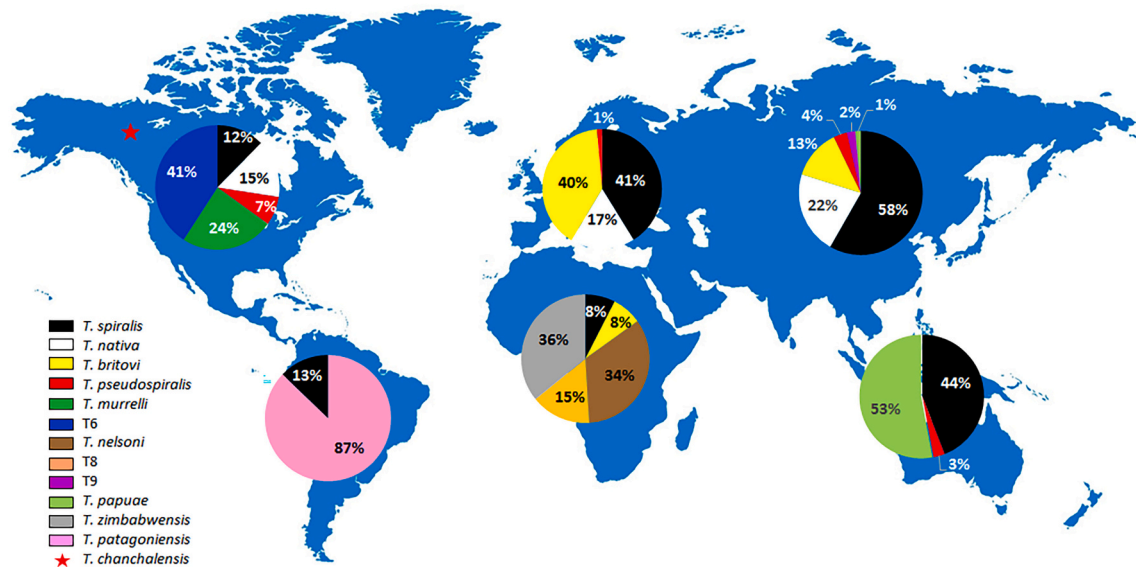


Fig. 1. *Trichinella* species geographical distribution. The relative percentage of species in each continent is reported in the pie chart.

addition of novel queries and data. With this new application, the connection to the database has been made much more secure, even though the database does not contain sensitive data. Only pre-established queries are available to users and addition/deletion and modification of data contained in the database is now strictly controlled by the administrators of the application. At the moment there are various ways of addressing the data in the DB and additional novel ways being considered is a dynamic process that will permit expanded interrogation of the database with little effort. The new design of the database and application also permits easy expansion of the data in the DB, via the addition of novel tables and relations.

The data recorded in the database by ITRC staff, during 33 years of activity, involves more than 8000 worldwide *Trichinella* isolates. The number of isolates received and tested at ITRC has increased over time from an average of 70 isolates per year from 1984 to 2007 to an average of 366 per years from 2008 up to present (data not shown). The largest increase in the isolate collection rate occurred from 2005 after the enforcement of COMMISSION REGULATION (EC) No 2075/2005, which lays down specific rules on official controls of meat products intended for human consumption, of animal species that may be infested with nematodes of the genus *Trichinella*. The application of the regulation at the European level led to a large increase of controls on carcasses of domestic pigs and wild boar, whose meat products are often consumed raw and that may represent a risk for human health. This explains why these two species represent, together, 56% of the *Trichinella* hosts recorded in the DB. Other well-represented animal hosts include species like red fox, racoon dog and wolf that are subject to hunting or epidemiological surveys, as they are considered indicator species for the presence of *Trichinella* in European wildlife (Vlado Teodorović et al., 2014; Kärssin et al., 2017).

Since 2006, the activity of ITRC overlaps that of the EURLP, whose tasks include supporting the Reference Laboratories of EU member states in the identification of *Trichinella* isolates collected during surveillance programs. As a consequence, the ITRC received many European *Trichinella* isolates, which in turn explains why the majority of *Trichinella* isolates recorded in the DB are represented by *T. spiralis*, *T. nativa* and *T. britovi*, three of the four *Trichinella* species present in Europe.

The correlation between *Trichinella* species and animal hosts, which emerge from DB analysis, does not always match the acknowledged epidemiological picture. Indeed, while the predominant association of *T. spiralis* with wild boars and domestic pigs (89.4%) agrees with literature (EFS Agency, 2021; Pozio, 2021), the large number of *T. britovi* isolates associated with wild boars (41%), rather than with red foxes (26.3%), does not reflect the real epidemiological situation in which the red fox is the main natural host of this species (Bilska-Zajac et al., 2020; Deksne et al., 2016; Kärssin et al., 2017). This is due to the fact that the *T. britovi* isolates recorded in the DB mainly originated from surveillance studies with a specific focus on wild boar (Bilska-Zajac et al., 2013; Kirjušina et al., 2015; Balić et al., 2020; Vieira-Pinto et al., 2021). Likewise, the high association of *T. pseudospiralis* with the Florida panther and that of T6 with the wolverine in isolates collected from North America (Reichard et al., 2008; Reichard et al., 2015), reflects data derived from surveillance studies focused on specific host species because of their interest as possible *Trichinella* indicators in a specific geographical region.

The ITRC database is strictly linked to the testing activity carried out by ITRC staff, since the ISS code correlates each *Trichinella* species or genotype identified at the ITRC with the isolate information recorded in the DB. Thanks to this, the DB has significantly contributed to our knowledge of the epidemiology (Pozio, 1998; Pozio, 2001; Pozio and Zarlenga, 2005; Pozio et al., 2009a; Pozio, 2016) and taxonomy (Pozio et al., 2001; Pozio et al., 2009b; Korhonen et al., 2016) of the *Trichinella* genus. Of particular relevance, new *Trichinella* species have been proposed, including *T. britovi* (Pozio et al., 1992a, 1992b), *T. murrelli* (Pozio and La Rosa, 2000), *T. papuae* (Pozio et al., 1999), *T. zimbabwensis* (Pozio et al., 2002) and *T. patagoniensis* (Krivokapich et al., 2012), as well as the new genotypes referred to as T6 (Pozio et al., 1992a, 1992b), T8 (Pozio et al., 1992a, 1992b) and T9 (Nagano et al., 1999). Moreover, the

**Table 2**List of host species and number of *Trichinella* isolates recorded in the database for each species.

Genus	Species	Common name	No. of isolates
Acinonyx	Jubatus	cheetah	1
Alopex	Lagopus	arctic fox	29
Apodemus	Sylvaticus	field mouse	2
Aquila	Rapax	tawny eagle	1
Athene	Noctua	little owl	1
Canis	Aureus	golden jackal	48
Canis	Latrans	coyote	28
Canis	Lupus	wolf	426
Canis	lupus familiaris	Domestic/stray dog	34
Castor	Fiber	european beaver	1
Chaetophractus	Villosus	armadillo	3
Coragypus	Atratus	black vulture	1
Crocodylus	Niloticus	nile crocodile	15
Crocodylus	Porosus	saltwater crocodile	11
Crocuta	Crocuta	spotted hyena	7
Dasyurus	Maculatus	spotted-tailed quolls	1
Equus	Caballus	domestic horse	13
Erignathus	Barbatus	bearded seal	1
Erinaceus	Europaeus	hedgehog	1
Felis	Catus	domestic/stray cat	16
Felis	Concolor	mountain lion	4
Felis	Serval	serval	1
Felis	Silvestris	wild cat	13
Genetta	Genetta	genetta	1
Gulo	Gulo	wolverine	106
Herpestes	auropunctatus	Small Asian Mongoose	1
Homo	Sapiens	man	24
Hyaena	Hyaena	striped hyena	1
Lutra	Lutra	river otter	3
Lynx	Lynx	lynx	706
Lynx	Rufus	bobcat	20
Martes	Americana	american marten	1
Martes	Foina	stone marten	13
Martes	Martes	pine marten	10
Martes	Pennanti	fisher	1
Martes	Zibellina	sable	2
Meles	Meles	badger	20
Milvus	Milvus	red kite	1
Mustela	Erminea	ermine	1
Mustela	Vison	american mink	1
Nandinia	Binotata	african palm civet	2
Nyctereutes	procyonoides	raccoon dog	487
Otocyon	Megalotis	bat eared fox	1
Panthera	Leo	lion	11
Panthera	Pardus	leopard	3
Panthera	Tigris	tiger	1
Phacochoerus	Aethiopicus	warthog	1
Prionailurus	bengalensis euptailurus	Amur cat	4
Procyon	Lotor	raccoon	12
Puma	Concolor	mountain lion	17
Puma	concolor coryi	florida panther	19
Rattus	Norvegicus	brown rat	49
Rattus	Rattus	black rat	5
Strix	Aluco	tawny owl	1
Sus	Scrofa	domestic pig	1443
Sus	Scrofa	wild boar	3111
Urocyon	cinereoargenteus	gray fox	1
Ursus	Americanus	black bear	23
Ursus	Arctos	brown bear	115
Ursus	arctos horribilis	grizzly	10
Ursus	Maritimus	polar bear	15
Ursus	thibetanus laniger japonicus	Japanese black bearr	1
Ursus	thibetanus laniger	Himalayan bear	1
Varanus	Niloticus	monitor lizard	3
Viverra	Civetta	true civet	1
Vulpes	Corsac	corsac fox	1
Vulpes	Vulpes	red fox	1094
Vulpes	Vulpes	silver fox	3
Vulpes	Lagopus	arctic fox	2

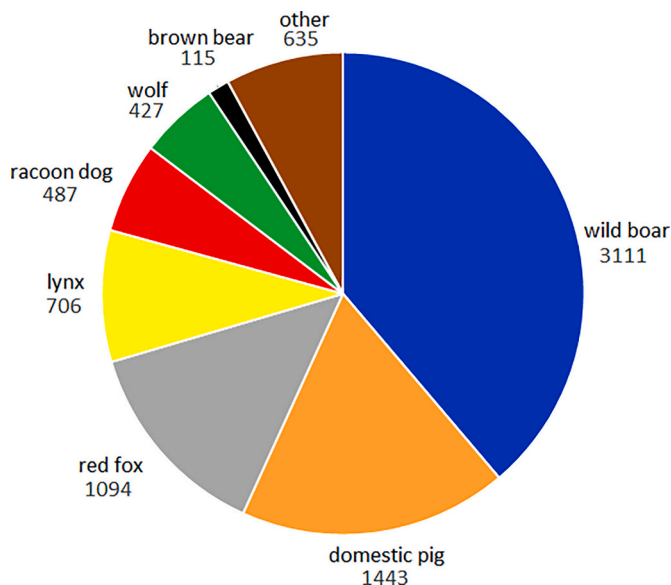


Fig. 2. Main mammalian host species reported in the database and number of associated infections.

Table 3

Number of *Trichinella* infections reported in the database by species and their distribution.

Species/genotype	No of infections (%)	Distribution
<i>T. spiralis</i>	3342 (40.41)	Cosmopolitan
<i>T. nativa</i>	1418 (17.15)	Arctic and subarctic regions of Asia, Europe and North America
<i>T. britovi</i>	3099 (37.47)	Temperate areas of Asia and Europe, North and western Africa
<i>T. pseudospiralis</i>	137 (1.66)	Cosmopolitan
<i>T. murrelli</i>	75 (0.91)	Temperate regions of North America
<i>Trichinella</i> T6	125 (1.51)	Subarctic regions of North America
<i>T. nelsoni</i>	18 (0.22)	South Africa, Kenya, Tanzania
<i>Trichinella</i> T8	8 (0.10)	South Africa and Namibia
<i>Trichinella</i> T9	3 (0.04)	Japan
<i>T. papuae</i>	20 (0.24)	Papua New Guinea, Thailand, Malaysia
<i>T. zimbabwensis</i>	19 (0.23)	Zimbabwe and South Africa
<i>T. patagoniensis</i>	5 (0.06)	Argentina
<i>T. chanchalensis</i>	1 (0.01)	Canada

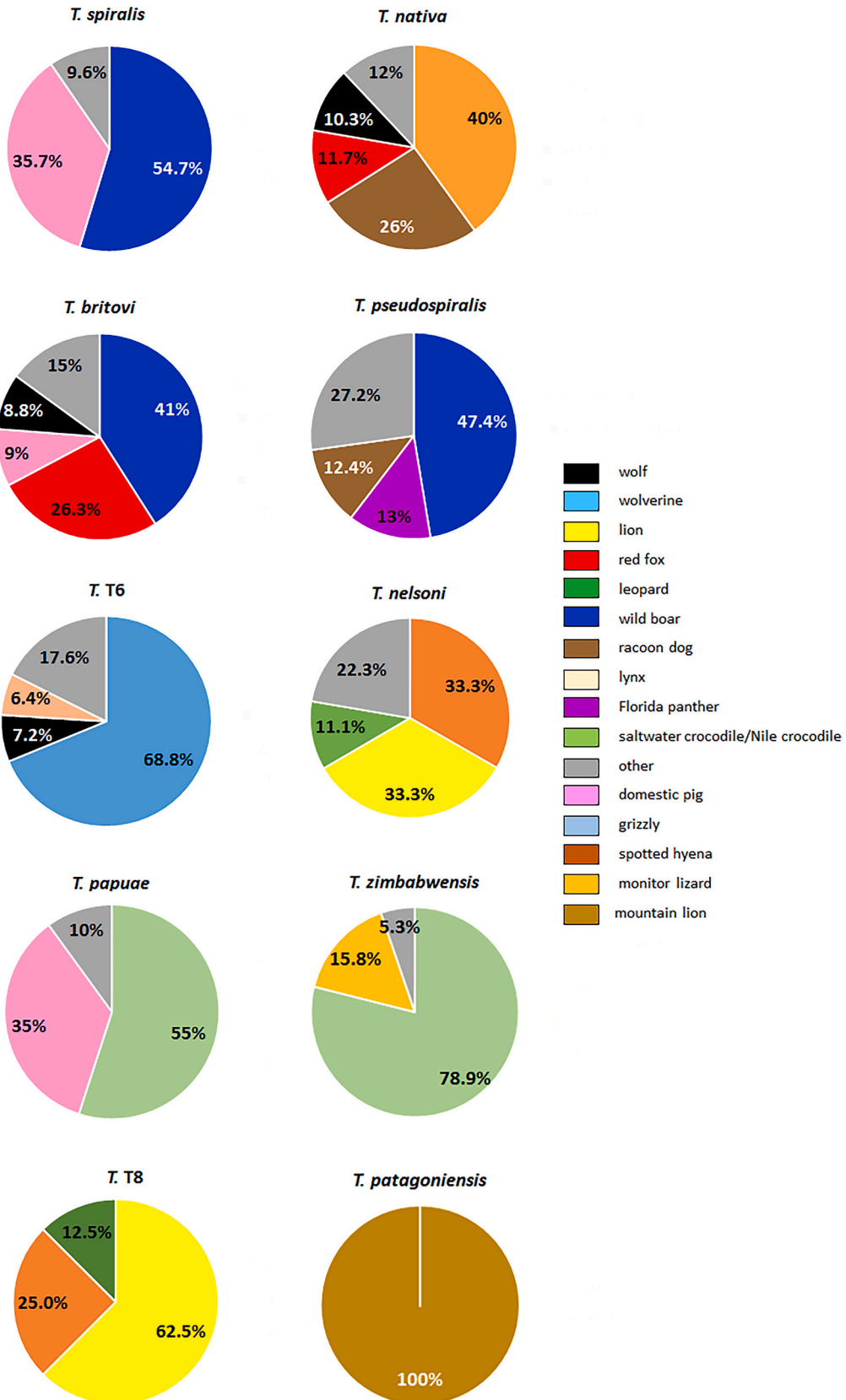
Table 4

*Trichinella* multiple infections reported in the database and geographical area of origin.

Involved species	Number of isolates (%)	Geographical origin
<i>T. spiralis</i> + <i>T. nativa</i>	18 (7.6)	Asia, Europe and North America
<i>T. spiralis</i> + <i>T. britovi</i>	108 (45.6)	Europe
<i>T. spiralis</i> + <i>T. pseudospiralis</i>	7 (3)	Europe and North America
<i>T. spiralis</i> + <i>T. murrelli</i>	1 (0.4)	USA
<i>T. nativa</i> + <i>T. britovi</i>	85 (35.9)	Europe
<i>T. nativa</i> + <i>T. pseudospiralis</i>	6 (2.5)	Europe
<i>T. nativa</i> + <i>T. T6</i>	3 (1.3)	North America
<i>T. britovi</i> + <i>T. pseudospiralis</i>	4 (1.7)	Europe
<i>T. nelsoni</i> + <i>T. T8</i>	2 (0.8)	South Africa
<i>T. spiralis</i> + <i>T. nativa</i> + <i>T. Britovi</i>	2 (0.8)	Finland and Russia
<i>T. spiralis</i> + <i>T. nativa</i> + <i>T. pseudospiralis</i>	1 (0.4)	Russia

DB, via the use of the ISS code, allows to easily recover the information on *Trichinella* isolates promoting their use as reference material for scientific studies.

The free access to the database allows the use of the data contained therein by the international scientific community, not only for research purposes but also for the control of this infection. The large amount of data reported in the database can be of help to increase knowledge about the geographical distribution of *Trichinella* species and the hosts involved in maintaining the sylvatic cycle of this parasite contributing to more effective control programs for this zoonosis.



(caption on next page)

**Fig. 3.** Hosts more frequently associated to *Trichinella* species. Data on *Trichinella* T9 and *T. chanchalensis* are not showed because only three and one isolate are recorded in the database respectively.

#### 4. Conclusions

The ITRC database achieved the goal of providing readily available and comprehensive international data on the occurrence and distribution of *Trichinella* species and can be considered a fundamental tool for surveillance of *Trichinella* nematodes giving support to epidemiological studies and control programs. This has been possible thanks to the contribution of numerous international scientific donors.

In the future, the active participation of countries for which data are limited or not yet available, will allow a more complete view of this parasitic infection in individual geographic regions, countries or continents.

#### 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.

#### References

- Balić, D., Marucci, G., Agičić, M., Benić, M., Krovina, Z., Mišić, T., Aladić, K., Škrivanko, M., 2020. *Trichinella* spp. in wild boar (*Sus scrofa*) populations in Croatia during an eight-year study (2010–2017). *One Health* 11 (Sep 29), 100172. <https://doi.org/10.1016/j.onehlt.2020.100172>. PMID: 33392373.
- Bandi, C., La Rosa, G., Comincini, S., Damiani, G., Pozio, E., 1993. Random amplified polymorphic DNA technique for the identification of *Trichinella* species. *Parasitology*. 107 (Pt 4), 419–424. Nov. PMID: 8278221.
- Bilska-Zajac, E., Różycki, M., Chmurzyńska, E., Marucci, G., Cencek, T., Karamon, J., Bocian, L., 2013. *Trichinella* species circulating in wild boar (*Sus scrofa*) populations in Poland. *Int. J. Parasitol. Parasites Wildl.* 2, 211–213. <https://doi.org/10.1016/j.ijppaw.2013.05.004>. Jun 7. PMID: 24533337.
- Bilska-Zajac, E., Różycki, M., Grądziel-Krukowska, K., Belcik, 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 (Mar), 109052. <https://doi.org/10.1016/j.vetpar.2020.109052>.
- Deksne, G., Segliņa, Z., Jahundoviča, I., Esīte, Z., Bakasejevs, E., Bagrade, G., Keidāne, D., Interisano, M., Marucci, G., Tonanzi, D., Pozio, E., Kirjušina, M., 2016. High prevalence of *Trichinella* spp. in sylvatic carnivore mammals of Latvia. *Vet. Parasitol.* 231, 118–123. <https://doi.org/10.1016/j.vetpar.2016.04.012>. Nov 15.
- EFS Agency, 2021. The European Union one health 2019 zoonoses report. *EFSA J.* 19 (2), 6406.
- Flockhart, H.A., Harrison, S.E., Dobinson, A.R., James, E.R., 1982. Enzyme polymorphism in *Trichinella*. *Trans. R. Soc. Trop. Med. Hyg.* 76 (4), 541–545. [https://doi.org/10.1016/0035-9203\(82\)90159-6](https://doi.org/10.1016/0035-9203(82)90159-6) (PMID: 6926776).
- Kärssin, A., Häkkinen, L., Niin, E., Peik, K., Vilem, A., Jokelainen, P., Lassen, B., 2017. *Trichinella* spp. biomass has increased in raccoon dogs (*Nyctereutes procyonoides*) and red foxes (*Vulpes vulpes*) in Estonia. *Parasit. Vectors* 10, 609. <https://doi.org/10.1186/s13071-017-2571-0>.
- Kirjušina, M., Deksne, G., Marucci, G., Bakasejevs, E., Jahundoviča, I., Daukste, A., Zdankovska, A., Bērziņa, Z., Esīte, Z., Bella, A., Galati, F., Krūmiņa, A., 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. *Parasit. Vectors* 8 (Mar 1), 137. <https://doi.org/10.1186/s13071-015-0753-1>. PMID: 25886306; PMCID: PMC4351677.
- Korhonen, P.K., Pozio, E., La Rosa, G., Chang, B.C., Koehler, A.V., Hoberg, E.P., Boag, P.R., Tan, P., Jex, A.R., Hofmann, A., Sternberg, P.W., Young, N.D., Gasser, R.B., 2016. Phylogenomic and biogeographic reconstruction of the *Trichinella* complex. *Nat. Commun.* 7 (Feb 1), 10513. <https://doi.org/10.1038/ncomms10513>. PMID: 26830005; PMCID: PMC4740406.
- Krivokapich, S.J., Pozio, E., Gatti, G.M., Prous, C.L., Ribicich, M., Marucci, G., La Rosa, G., Confalonieri, V., 2012. *Trichinella patagoniensis* n. sp. (Nematoda), a new encapsulated species infecting carnivorous mammals in South America. *Int. J. Parasitol.* 42 (10), 903–910. <https://doi.org/10.1016/j.ijpara.2012.07.009>. Sep. Epub 2012 Aug 17. PMID: 22921601.
- La Rosa, G., Pozio, E., Rossi, P., Murrell, K.D., 1992. Allozyme analysis of *Trichinella* isolates from various host species and geographical regions. *J. Parasitol.* Aug 78 (4), 641–646. PMID: 1635022.
- Marucci, G., La Grange, L.J., La Rosa, G., Pozio, E., 2009. *Trichinella nelsoni* and *Trichinella* T8 mixed infection in a lion (*Panthera leo*) of the Kruger National Park (South Africa). *Vet. Parasitol.* 159 (3–4), 225–228. <https://doi.org/10.1016/j.vetpar.2008.10.041>. Feb 23. PMID: 19041185.
- Nagano, I., Wu, Z., Matsuo, A., Pozio, E., Takahashi, Y., 1999. Identification of *Trichinella* isolates by polymerase chain reaction—restriction fragment length polymorphism of the mitochondrial cytochrome c-oxidase subunit I gene. *Int. J. Parasitol.* 29, 11131120.
- Pozio, E., 1998. Trichinellosis in the European union: epidemiology, ecology and economic impact. *Parasitol. Today* 14 (1), 35–38. [https://doi.org/10.1016/s0169-4758\(97\)01165-4](https://doi.org/10.1016/s0169-4758(97)01165-4). Jan. PMID: 17040688.
- Pozio, E., 2001. New patterns of *Trichinella* infection. *Vet. Parasitol.* 98 (1–3), 133–148. [https://doi.org/10.1016/s0304-4017\(01\)00427-7](https://doi.org/10.1016/s0304-4017(01)00427-7). Jul 12. PMID: 11516583.
- Pozio, E., 2016. *Trichinella pseudospiralis* an elusive nematode. *Vet. Parasitol.* 231, 97–101. <https://doi.org/10.1016/j.vetpar.2016.03.021>. Nov 15. PMID: 27113211.
- Pozio, E., 2021. Epidemiology. In: Bruschi, F. (Ed.), *Trichinella and Trichinellosis*. Academic Press, pp. 185–263.
- Pozio, E., La Rosa, G., 2000. *Trichinella murrelli* n. sp. etiological agent of sylvatic trichinellosis in temperate areas of North America. *J. Parasitol.* 86 (1), 134–139. Feb. PMID: 10701576.
- 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., Zarlenga, D.S., 2005. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. *Int. J. Parasitol.* Oct 35 (11–12), 1191–1204. <https://doi.org/10.1016/j.ijpara.2005.07.012>. PMID: 16153648.
- Pozio, E., Zarlenga, D.S., 2021. Taxonomy of the *Trichinella* genus. In: Bruschi, F. (Ed.), *Trichinella and Trichinellosis*. Academic Press, pp. 35–76.
- Pozio, E., La Rosa, G., Rossi, P., Murrell, K.D., 1992a. Biological characterizations of *Trichinella* isolates from various host species and geographic regions. *J. Parasitol.* 78, 647653.
- Pozio, E., La Rosa, G., Murrell, K.D., Lichtenfels, J.R., 1992b. Taxonomic revision of the genus *Trichinella*. *J. Parasitol.* Aug 78 (4), 654–659. PMID: 1635024.
- Pozio, E., Owen, I.L., La Rosa, G., Sacchi, L., Rossi, P., Corona, S., 1999. *Trichinella papuae* n.sp. (Nematoda), a new non-encapsulated species from domestic and sylvatic swine of Papua New Guinea. *Int. J. Parasitol.* 29 (11), 1825–1839. Nov. PMID: 10616929.
- Pozio, E., Zarlenga, D.S., La Rosa, G., 2001 Jun. 2001b. The detection of encapsulated and non-encapsulated species of *Trichinella* suggests the existence of two evolutive lines in the genus. *Parasite*. 8 (2 Suppl), S27–S29. <https://doi.org/10.1051/parasite/200108s2027>. PMID: 11484376.
- Pozio, E., Foggini, C.M., Marucci, G., La Rosa, G., Sacchi, L., Corona, S., Rossi, P., Mukaratirwa, S., 2002. *Trichinella zimbabwensis* n.sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. *Int. J. Parasitol.* 32 (14), 1787–1799. Dec 19. PMID: 12464425.
- Pozio, E., Marucci, G., Casulli, A., Sacchi, L., Mukaratirwa, S., Foggini, C.M., La Rosa, G., 2004. *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. *Parasitol* 128, 333342 (PMID: 15074882).



- Pozio, E., Rinaldi, L., Marucci, G., Musella, V., Galati, F., Cringoli, G., Boireau, P., La Rosa, G., 2009a. Hosts and habitats of *Trichinella spiralis* and *Trichinella britovi* in Europe. *Int. J. Parasitol.* Jan 39 (1), 71–79. <https://doi.org/10.1016/j.ijpara.2008.06.006>. Epub 2008 Jul 26. PMID: 18708065.
- Pozio, E., Hoberg, E., La Rosa, G., Zarlenga, D.S., 2009b. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. *Infect. Genet. Evol.* 9 (4), 606–616. <https://doi.org/10.1016/j.meegid.2009.03.003>. Jul. Epub 2009 Mar 24. PMID: 19460327.
- Reichard, M.V., Torretti, L., Snider, T.A., Garvon, J.M., Marucci, G., Pozio, E., 2008. *Trichinella* T6 and *Trichinella nativa* in Wolverines (*Gulo gulo*) from Nunavut, Canada. *Parasitol. Res.* 103 (3), 657–661. <https://doi.org/10.1007/s00436-008-1028-y>. Aug. Epub 2008 Jun 1. 18516722.
- Reichard, M.V., Criffield, M., Thomas, J.E., Paritte, J.M., Cunningham, M., Onorato, D., Logan, K., Interisano, M., Marucci, G., Pozio, E., 2015. High prevalence of *Trichinella pseudospiralis* in Florida panthers (*Puma concolor coryi*). *Parasit. Vectors* 8, 67. <https://doi.org/10.1186/s13071-015-0674-z>. Feb 4.
- Sharma, R., Thompson, P.C., Hoberg, E.P., Brad Scandrett, W., Konecni, K., Harms, N.J., Kukka, P.M., Jung, T.S., Elkin, B., Mulders, R., Larter, N.C., Branigan, M., Pongracz, J., Wagner, B., Kafle, P., Lobanov, V.A., Rosenthal, B.M., Jenkins, E.J., 2020. Hiding in plain sight: discovery and phylogeography of a cryptic species of *Trichinella* (Nematoda: Trichinellidae) in wolverine (*Gulo gulo*). *Int. J. Parasitol.* Apr 50 (4), 277–287. <https://doi.org/10.1016/j.ijpara.2020.01.003>. PMID: 32171846.
- Vieira-Pinto, M., ARG, Fernandes, Santos, M.H., Marucci, G., 2021. *Trichinella britovi* infection in wild boar in Portugal. *Zoonoses Public Health* 68 (2), 103–109. <https://doi.org/10.1111/zph.12800>. Mar. Epub 2021 Jan 17. PMID: 33455082.
- Vlado Teodorović, V., Vasilev, D., Čirović, D., Marković, M., Čosić, N., Djurić, S., Djurković-Djaković, O., 2014. The wolf (*Canis lupus*) as an indicator species for the sylvatic trichinella cycle in the Central Balkans. *J. Wildl. Dis.* 50 (4), 911–915. <https://doi.org/10.7589/2013-12-333>.
- 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.