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

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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 envelopes 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

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infesting 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 did they do 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. For example, T. spiralis is, 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. zimbabwensis* 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*, 6%) 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. zimbabwensis* 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/
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, raccoon 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 (Biłska-Zając et al., 2020; Deksnē 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 (Biłska-Zając 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

Fig. 1. Trichinella species geographical distribution. The relative percentage of species in each continent is reported in the pie chart.
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               |
| Alopec            | 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         | europeean beaver | 1               |
| Chaetophractus    | Villonus      | armadillo        | 3               |
| Coragyps          | Atratus       | black vulture    | 1               |
| Crocodylus        | Niloticus     | nile crocodile   | 15              |
| Crocodylus        | Porosus       | saltwater crocodile | 11             |
| Crocuta           | Crocuta       | spotted hyena    | 7               |
| Dasyurus          | Maculatus     | spotted-tailed guolls | 1             |
| Eguus             | Caballus      | domestic horse   | 13              |
| Erignathus        | Barbatus      | bearded seal     | 1               |
| Erineceus         | Europaeps     | 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         | auro punctatus | 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             |
| Nycterereutes     | procyonoides  | racoon dog       | 487             |
| Otocyon           | Megalotis     | bat eared fox    | 1               |
| Panthera          | Leo           | lion             | 11              |
| Panthera          | Pardus        | lepard           | 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            | Norviegicus   | brown rat        | 49              |
| Rattus            | Rattus        | black rat        | 5               |
| Strix             | Aluco         | tawny owl        | 1               |
| Sus               | Scrofa        | domestic pig     | 1443            |
| Sus               | Scrofa        | wild boar        | 3111            |
| Urocyon           | cinereoargentus | 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 bear | 1           |
| Ursus             | thibetanus laniger | Himalayan bear | 1               |
| Varamus           | 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               |
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.

### Table 3

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

### Table 4

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

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