Adaptation of *Trichinella* spp. for survival in cold climates☆

Edoardo Pozio

Department of Infectious Diseases, Istituto Superiore di Sanità, Rome, Italy

---

**Article info**

**Article history:**
Received 7 June 2016
Received in revised form 13 July 2016
Accepted 14 July 2016
Available online 16 July 2016

**Keywords:**
Trichinella nativa
Trichinella britovi
Trichinella T6
Freezing
Decaying muscle
Subnivean climate
Temperature
Humidity

---

**Abstract**

Parasites of the genus *Trichinella* are a complex of at least 12 taxa with a broad geographic range, including, Africa, the Americas, Asia, Australasia and Europe, and a broad host spectrum encompassing mammals, birds and reptiles. These entozoic parasites complete their life cycle in only one host; however, there are several biological and epidemiological features, which suggest that the environmental temperature and moisture influence the circulation of these nematodes in nature. An important adaptation of these parasites, which facilitates their transmission, is the physiological mechanism utilized by muscle larvae to promote their survival in decaying carcasses. The greater the persistence of larval viability, the higher the probability of being ingested by a scavenging host. The larval metabolism is basically anaerobic favoring its survival in decaying tissues. The persistence of larvae in putrefying flesh is, of course, also determined by high humidity and low temperatures. This condition has been proposed as the environment of the “free-living” stage, resembling the egg stage of most of other nematode species. The importance of this stage in the natural cycle of these parasites is underscored by the survival of muscle larvae of *Trichinella nativa*, *Trichinella T6* and, to a lesser degree, of *Trichinella britovi* in frozen muscles of carrions for months up to several years. Survival is greatest at temperatures between 0 °C and −20 °C. At lower temperatures, survival time is reduced rapidly, suggesting that the optimal temperature range for survival to freezing corresponds to the temperature under the snow. The subnivean climate is characterized by a stable temperature near freezing because heat released from the soil is trapped by the low thermal conductivity of snow. This adaptation for survival of *Trichinella* spp. in cold climates is under the influence of six intrinsic and extrinsic factors. The survival of larvae of the three *Trichinella* taxa to freezing in host muscles represents a concern for the food safety of humans consuming game meat. The climate change certainly will influence the natural cycle of *Trichinella* taxa circulating in cold climates, due to the increasing temperature, decreasing humidity and snow cover, and increasing exposure to heat stress. A reduction of the distribution area of *T. nativa* and *Trichinella T6*, and an advance toward the north of the distribution area of *T. britovi* can be expected.

© 2016 Published by Elsevier Inc. on behalf of International Association of Food and Waterborne Parasitology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

---

**Contents**

1. Introduction .................................................................................. 5
2. Freeze-resistant taxa ................................................................. 5
   2.1. *Trichinella nativa* ................................................................. 5
   2.2. *Trichinella T6* .................................................................... 6
   2.3. *Trichinella britovi* ............................................................... 6
   2.4. Other *Trichinella* species .................................................... 8

☆ This paper is based on an invited presentation given by the authors in a symposium organized by the International Association for Food and Waterborne Parasitology as part of EMOP XII in Turku, Finland, 20-24 July, 2016.

E-mail address: edoardo.pozio@iss.it.

http://dx.doi.org/10.1016/j.fawpar.2016.07.001

2405-6766/© 2016 Published by Elsevier Inc. on behalf of International Association of Food and Waterborne Parasitology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
3. The parasites' adaptation to the environment ........................................... 8
3.1. Larval survival in decaying carcasses ................................................. 8
3.2. Survival of muscle larvae in frozen muscles ..................................... 8
3.3. Survival of Trichinella spp. larvae in meat derived local food products ........ 10
3.4. The climate change and the freeze-resistant Trichinella taxa ................. 10
4. Future investigations ........................................................................... 10
5. Conclusions ....................................................................................... 11
References ............................................................................................ 11

1. Introduction

Nematode parasites of the genus *Trichinella* are a complex of at least 12 taxa, with a broad geographic range including, Africa, the Americas, Asia, Australasia and Europe, and a broad host spectrum encompassing mammals, birds and reptiles (Pozio and Zarlenga, 2013). Their life cycle differs substantially from those of all the other nematodes, since it comprises two generations in the same host (Gottstein et al., 2009). Two clades, named encapsulated and non-encapsulated, are recognized in the genus *Trichinella*. All species and genotypes infect mammals; however, out of the three species of the non-encapsulated clade, one infects birds in addition to mammals, and two infect reptiles besides mammals (Pozio and Zarlenga, 2013).

The *Trichinella* ancestors diverged from *Trichuris suis* from 532 to 382 million years ago (mya), and the encapsulated and non-encapsulated *Trichinella* taxa diverged about 28–15 mya coinciding with the transition from Oligocene to Miocene. Subsequent diversification leading to extant taxa was circumscribed in the upper and uppermost Miocene during the Tortonian and Messinian periods, commencing about 10 and 7 mya and continuing into the Pliocene and Pleistocene (Korhonen et al., 2016).

Encapsulated *Trichinella* taxa initially expanded into North America across Beringia during a time frame more than 5 mya, before the inception of Northern Hemisphere glaciations. Inception of Northern Hemisphere glaciation cycles and periodic emergence of the Bering land bridge after 2.5 and 2.0 mya led to independent episodes of geographic colonization and host-switching, driving patterns of isolation and expansion of *Trichinella*, linking Eurasia and the Nearctic (Korhonen et al., 2016).

Out of the nine encapsulated taxa, the distribution areas of *Trichinella nativa*, *Trichinella britovi* and the genotype *Trichinella* T6 overlap partially or completely with cold regions and the muscle larval stage of these taxa developed mechanisms to survive in frozen carrions. *T. nativa* and *Trichinella* T6 derive from a common lineage, whereas, *T. britovi* derives from another a little bit separate lineage (Korhonen et al., 2016).

The separation of *T. nativa* and *Trichinella* T6 may have occurred during the last glacial period, when ice overspread the southern regions of North America and *T. nativa* isolates living in the arctic colonized the Rocky Mountains. When the glacial period ended, the ice withdrew and left behind the arctic-derived, freeze-resistant *Trichinella* isolates, which remained restricted to the Rocky Mountains as a glacial relict. This would have resulted in restricted gene flow with arctic isolates, and *Trichinella* T6 could have begun an evolutionary path independent from *T. nativa* (La Rosa et al., 2003).

The importance of resistance to freezing by arctic isolates has become apparent from observations by Brandly and Rausch (1950), who suggested the possibility that the arctic strain of *Trichinella*, today identified as *T. nativa* or *Trichinella* T6, might be more resistant to low temperatures than those circulating in temperate areas. Studying a human outbreak of trichinellosis caused by black bear meat consumption in Alaska, Clark et al. (1972) observed that patients acquired the infection after the consumption of the bear meat frozen at −18 °C for 81 days. Afterwards, the survival of *T. nativa* larvae in frozen muscles of naturally infected carnivores was investigated monitoring the freezing temperature and time of freezing (Dick and Belosevic, 1978; Boev et al., 1979; Dies, 1980; Dick and Chadee, 1981). Eaton (1979) reported that Ozerecevskaya recovered living *Trichinella* larvae from polar bear meat frozen for more than 2 years. Boev et al. (1979) stated that *T. nativa* is a dozen times more resistant to low temperature in comparison with other species. In 1989, it was observed that also *T. britovi* larvae can survive in frozen muscles of carnivores (Pozio et al., 1989).

The aims of the present review were to collate information on the epidemiology and biological mechanisms which allow the survival of these parasites to freezing, and to forecast the impact of global warming and climate change on freeze-resistant *Trichinella* species.

2. Freeze-resistant taxa

2.1. Trichinella nativa

The first report on the circulation of *Trichinella* spp. among mammals in the arctic date back to 1934, when Parnell described the presence of *Trichinella spiralis* in carnivores of north-east Canada. The existence of a sylvatic cycle of *Trichinella* transmission at high latitudes was then recognized in Greenland by Thorborg et al. (1948).

This species is usually characterized as the arctic or freeze-resistant species and is widespread among wildlife of the arctic and sub-arctic areas of the Holarctic region (i.e., Canada, Greenland, Alaska and New Hampshire in the United States, Belorussia, Estonia, Finland, Latvia, Lithuania, Norway, Russia, Sweden, China, Kazakhstan, Kyrgyzstan, and Tajikistan) (Fig. 1). The southern distribution boundary has been tentatively identified between the isotherms −5° to −4 °C in January (Shaikenov and Boev, 1983; Shaikenov, 1992; Pozio and La Rosa, 2000). However, isolated foci of *T. nativa* were detected south of these isotherms in Germany and Poland (Chmuryzynska et al., 2013). The spread of *T. nativa* in the Arctic was favored by the use of the icepack as a bridge among lands by wild animals (Fig. 1). Furthermore, the high capacity of the polar bear to survive on drifting ice for weeks, allowed the detection of *T. nativa* infected polar bears hundreds of kilometers far from the ice pack (Skirnisson et al., 2010).
More than 700 isolates originating from terrestrial and marine carnivores living in arctic and subarctic areas have been identified as *T. nativa*; whereas, only eight isolates from wild boar living in arctic and subarctic areas have been identified as *T. nativa* so far (Pozio and Kapel, 1999) (Table 1). These epidemiological data are in agreement with the results of experimental infections, which show that swine (both domestic pig and wild boar) are not suitable hosts for *T. nativa* (Kapel and Gamble, 2000; Kapel, 2001; Nöckler et al., 2005; Hill et al., 2009). There are several reports of nematodes putatively identified as *Trichinella* larvae in muscles of rodents and lagomorphs from arctic and subarctic regions (Rausch, 1970). However, since the advent of molecular methods for species confirmation, there has not been any documentation of natural infections of *T. nativa* in these mammals, suggesting that nematode larvae belonging to other genera may have been erroneously considered as *Trichinella* larvae (Pozio, 2005; Marucci et al., 2013). The importance of sylvatic carnivores as reservoirs of *T. nativa* in nature is attested to by the finding that this parasite survives in host musculature for at least 20 years (Kumar et al., 1990). *T. nativa* is also the etiological agent of trichinellosis in human populations living in arctic and subarctic regions, who acquire the infection from eating raw meat from reservoir hosts such as walrus, bear and other game animals (Rausch, 1970; Margolis et al., 1979; MacLean et al., 1989; Forbes, 2000; Serhir et al., 2001; Schellenberg et al., 2003; Møller et al., 2005; Houzé et al., 2009; Møller et al., 2010; Bukina and Odoevskaia, 2013; Jenkins et al., 2013; Dupouy-Camet et al., 2016).

2.2. *Trichinella T6*

*Trichinella T6* is a North American genotype (Pozio et al., 1992a) widespread in carnivores. More than 150 *Trichinella* T6 isolates originating from terrestrial carnivores (Table 1) living in arctic and subarctic areas have been identified so far (w3.iss.it/site/Trichinella/). This genotype has never been detected in swine. These epidemiological data are in agreement with the results of experimental infections, which show that swine (both domestic pig and wild boar) are not suitable hosts for *T. nativa* (Kapel and Gamble, 2000; Kapel, 2001; Nöckler et al., 2005; Hill et al., 2009). The distribution range is confined to the arctic and subarctic regions of the United States and Canada, since *Trichinella T6* infected animals originated from Alaska, Colorado, Idaho, Montana, Ohio, Pennsylvania, Wyoming, Nunavut, and Ontario (Worley et al., 1990; Pozio, 2000; Pozio, 2001; La Rosa et al., 2003; Gajadhar and Forbes, 2010; Reichard et al., 2008; Reichard et al., 2016) (Fig. 1). A few human infections have been documented from the consumption of cougar and black bear meat in the United States (Idaho) and Canada (Ontario), respectively (Dworkin et al., 1996; ITRC).

2.3. *Trichinella britovi*

Among sylvatic species, *T. britovi* has a wide geographical range, occurring in wildlife of the temperate areas of the Palearctic region, from the Iberian peninsula to Kazakhstan (Pozio, 2000, 2001) and extending southward to Northern and Western Africa.
The northern geographic boundary appears to be determined by the isotherms $-6^\circ$ to $-5^\circ$C in January (Shaikenov and Boev, 1983; Shaikenov, 1992; Pozio, 2000). In Palearctic regions, this species is sympatric with *T. nativa* between the isotherms $-4^\circ$C and $-6^\circ$C, and there have been several reports of mixed infections in the same host from Estonia, Finland, Latvia, Lithuania and Sweden (Pozio, 2000; Oivanen et al., 2002; Malakauskas et al., 2007; Deksne et al., 2016).

This species is prevalent among sylvatic carnivores of the families Canidae, Felidae and Mustelidae, but it has also been documented in an Eurasian beaver, horses, three rodent species, swine, brown bears, and Viverridae in Africa (Table 1) (w3.iss.it/site/Trichinella/).

In *Trichinella* spp. infected domestic pigs and wild boar of Europe, *T. britovi* accounts for 20.3% and 43.8% of the infections, respectively.

A great number of human cases of trichinellosis were caused by this parasite in Europe (e.g., Spain, France, Italy, Greece, Romania, Bulgaria, Slovak Republic) mainly by the consumption of wild boar meat, pork from backyard or free-ranging pigs, horses, and rarely by the consumption of meat from carnivores (fox, dog, jackal) (Dubinsky et al., 2001; Rodríguez de las Parras et al., 2004; De Bruyne et al., 2006; Nezri et al., 2006; Kurdova-Mintcheva et al., 2009; Murrell and Pozio, 2011; Boutsini et al., 2014; Fichi et al., 2015; Pozio, 2015).

| Natural hosts | Trichinella species/genotype |
|---------------|-----------------------------|
| Canidae       |                             |
| Arctic fox, Alopex lagopus | Tn, T6                     |
| Corsac fox, Vulpes corsac   | Tn                          |
| Domestic dog, Canis familiaris | Tn, Tb                     |
| Golden jackal, Canis aureus | Tn, Tb                      |
| Grey fox, Urocyon cinereoargenteus | T6                        |
| Raccoon dog, Nyctereutes procyonoides | Tn, Tb                  |
| Red fox, Vulpes vulpes      | Tn, T6, Tb                  |
| Wolf, Canis lupus           | Tn, T6, Tb                  |
| Castoridae               |                             |
| Eurasian beaver, Castor fiber | Tb                              |
| Equidae                 |                             |
| Horse, Equus caballus     | Tb                          |
| Felidae                 |                             |
| Bob cat, Lynx rufus       | T6                          |
| Cougar, Puma concolor     | Tn, T6                      |
| Domestic cat, Felis catus  | Tn, T6                      |
| Leopard, Panthera pardus  | Tb                          |
| Leopard cat, Felis euptilura | Tn                    |
| Lynx, Lynx lynx           | Tn, Tb                      |
| Siberian tiger, Panthera tigris | Tn                    |
| Wild cat, Felis silvestris | Tn, Tb                    |
| Muridae                 |                             |
| Black rat, Rattus rattus  | Tb                          |
| Brown rat, Rattus norvegicus | Tb                      |
| Field mouse, Apodemus sylvaticus | Tb                |
| Mustelidae              |                             |
| American marten, Martes americana | Tn                    |
| Badger, Meles meles      | Tn, Tb                      |
| Fisher, Martes pennanti  | T6                          |
| Otter, Lutra lutra       | Tn, Tb                      |
| Pine marten, Martes martes | Tn, Tb                     |
| Sable, Martes zibellina  | Tn                          |
| Stone marten, Martes foina | Tb                      |
| Weasel, Mustela erminea  | Tn                          |
| Wolverine, Gulo gulo     | Tn, T6                      |
| Odobenidae              |                             |
| Walrus, Odobenus rosmarus | Tn                          |
| Phocidae                |                             |
| Bearded seal, Erignathus barbatus | Tn                |
| Grey seal, Halichoerus grypus | Tn                      |
| Ringed seal, Phoca hispida | Tn                   |
| Suidae                  |                             |
| Domestic pig            | Tb                          |
| Wild boar, Sus scrofa    | Tn, Tb                      |
| Ursidae                 |                             |
| Black bear, Ursus americanus | Tn, T6              |
| Brown bear, Ursus arctos | Tn, T6                     |
| Grizzly bear, Ursus arctos horribilis | Tn, T6 |
| Polar bear, Ursus maritimus | Tn                      |
| Viverridae              |                             |
| African palm civet, Nandinia binotata | Tb                  |
| Small Asian mongoose, Herpestes auropunctatus | Tb          |
| True civet, Viverra civetta | Tn                      |

(Nezri et al., 2006; Pozio et al., 2005) (Fig. 1). The northern geographic boundary appears to be determined by the isotherms $-6^\circ$ to $-5^\circ$C in January (Shaikenov and Boev, 1983; Shaikenov, 1992; Pozio, 2000). In Palearctic regions, this species is sympatric with *T. nativa* between the isotherms $-4^\circ$C and $-6^\circ$C, and there have been several reports of mixed infections in the same host from Estonia, Finland, Latvia, Lithuania and Sweden (Pozio, 2000; Oivanen et al., 2002; Malakauskas et al., 2007; Deksne et al., 2016). This species is prevalent among sylvatic carnivores of the families Canidae, Felidae and Mustelidae, but it has also been documented in an Eurasian beaver, horses, three rodent species, swine, brown bears, and Viverridae in Africa (Table 1) (w3.iss.it/site/Trichinella/). In *Trichinella* spp. infected domestic pigs and wild boar of Europe, *T. britovi* accounts for 20.3% and 43.8% of the infections, respectively. A great number of human cases of trichinellosis were caused by this parasite in Europe (e.g., Spain, France, Italy, Greece, Romania, Bulgaria, Slovak Republic) mainly by the consumption of wild boar meat, pork from backyard or free-ranging pigs, horses, and rarely by the consumption of meat from carnivores (fox, dog, jackal) (Dubinsky et al., 2001; Rodríguez de las Parras et al., 2004; De Bruyne et al., 2006; Nezri et al., 2006; Kurdova-Mintcheva et al., 2009; Murrell and Pozio, 2011; Boutsini et al., 2014; Fichi et al., 2015; Pozio, 2015).
2.4. Other Trichinella species

Experimental infections in different animal hosts have shown that also muscle larvae of some other Trichinella species can survive freezing in muscles of livestock and laboratory animals. T. spiralis larvae in horse muscles survived freezing at \(-18^\circ C\) for 4 weeks (Hill et al., 2007). T. spiralis, T. murrelli and T. pseudospiralis larvae in pig muscles survived freezing at \(-6.5^\circ C\) for 106 h (Hill et al., 2008). However, these times of survival and the freezing temperature could have more importance for the human safety than for the natural cycle of these parasites. From a food safety perspective, it is important to recognize that freezing may not be effective in killing parasites in certain host muscle.

In frozen mouse muscles, the survival time of larvae of T. nativa, T. britovi and Trichinella T6, increased with the larval age (from 2 up to 12 months) and was longer at \(-10^\circ C\) than at \(-30^\circ C\) (Pozió et al., 1994). The survival of T. spiralis, T. murrelli and Trichinella T8, was trifling. In frozen rat muscles, only T. nativa larvae survived at \(-18^\circ C\) for 1 week; at \(-5^\circ C\), T. spiralis, T. nativa, and T. britovi larvae survived 4 weeks; whereas, T. pseudospiralis, T. murrelli and Trichinella T6 larvae survived at \(-5^\circ C\) only 1 week (Malakauskas and Kapel, 2003).

3. The parasites' adaptation to the environment

3.1. Larval survival in decaying carcasses

An important adaptation of Trichinella spp. muscle larvae, which facilitates the transmission, is a physiological mechanism to survive in decaying carcasses; the greater the persistence of larval viability, the higher the probability of being ingested by a scavenging host. In spite of the larva-induced angiogenic process that develops around the nurse cell after larval penetration of the muscle cell, larval metabolism is basically anaerobic (Despommier, 1990), which favors its survival in decaying tissues. In fact, Trichinella spp. of the encapsulate clade are dispersed in a way analogous to many nematodes which have hardy eggs or a free-living stage in water or soil. Trichinella spp. have a similar free-living stage, with populations of larvae in collagen capsules in a special biotope, the carcass. These populations are maintained in a way analogous to helmint egg populations as, for example, Capillaria hepatica. The concept of a free-living stage in the carcass is of high epidemiological significance, since it reduces the importance of predation (Madsen, 1974). Of course, it does not mean that the larval survival cannot occur via predation, but on a broad scale it is of less epidemiological importance as compared with scavenging.

The persistence of larvae in putrefying flesh is also influenced by the environment: high humidity and low temperatures favor survival even when the muscle tissue is completely liquefied. This adaptive mechanism of survival is a biological character displayed by all taxa in the genus Trichinella; the survival in host carcasses is longer for the encapsulated than for the non-encapsulated species (Stewart et al., 1990; Owen and Reid, 2007). I personally collected infective T. nativa and T. britovi larvae in their collagen capsules, without any remnant of muscle tissues, from the bottom of vials, which had contained skinned and eviscerated mouse carcasses that were more than three months old, when the vials had been forwarded from Vladivostok to Rome, by regular mail in 1988. On the bottom of the vials, collagen capsules containing the infective larvae and some mouse bones were in a green liquid.

3.2. Survival of muscle larvae in frozen muscles

In the natural cycle of the parasite, the importance of the larva survivability in animal carcasses is further proved by the resistance of muscle larvae in frozen muscles for one (T. britovi) or more years (T. nativa and Trichinella T6) (Table 2). The anaerobic metabolism favoring the survival in putrefying flesh, along with the ability of larvae of some species to survive freezing, are two separate mechanisms that strongly increase the larval survival in nature. Survival is greatest at temperatures between 0 °C and \(-20^\circ C\). For example, Trichinella T6 larvae in carnivore muscles frozen at \(-6.5\) to \(-20^\circ C\) survived for at least 4 months (Worley et al., 1986), whereas, Trichinella T6 larvae in carnivore muscles frozen at \(-20^\circ C\) to \(-30^\circ C\) for 30 days, did not survive (Worley et al., 1990).

At lower temperatures, the survival time is reduced, suggesting that the optimal temperature range for survival at freezing temperatures corresponds to the temperature under the snow. This habitat has been named as the "subnivium". The "subnivium" can be described as the "below snow", which provides environmental stability (Keppel and Wardell-Johnson, 2012). The warmer and more stable conditions within the subnivium are principally driven by snow duration, density, and depth (Fig. 2).

The subnivean climate is characterized by a stable temperature near freezing because heat released from the soil is trapped by the low thermal conductivity of snow. In addition to hollow spaces beneath logs and other objects that intercept snowfall, a base layer of loose, granular snow exists at the interface between the snowpack and the soil surface. This loose snow is formed by water vapor transport. As heat from the soil slowly migrates upward through the snow layer, it creates a vertical gradient of decreasing temperature (Keppel and Wardell-Johnson, 2012).

Concurrently, sublimation causes the air within the snowpack to become saturated, and because warmer air holds more water, a gradient of water vapor concentration also forms. As saturated air diffuses upward, it encounters colder temperatures and the water vapor condenses (Pauli et al., 2013). The result is continual transport of water molecules upward through the snow, reducing snow crystal size at the base and forming a thermally stable zone of loose, granular snow. This well-insulated and thermally stable environment is reflected by smaller changes in subnivium temperature relative to changes in air temperature (Fig. 2). As snow depth decreases or snow density increases, the insulation provided by the snow is reduced and temperatures in the subnivium become colder and more variable until they track ambient temperatures.
In Latvia, a correlation between the incidence of *Trichinella* spp. in wild boar and the number of snow cover days during the period 1976–1993, was observed. The higher the number of snow cover days, the higher the *Trichinella* spp. incidence in wild boar and vice versa (Kirjušina et al., 2015).

*Trichinella nativa* isolates originating from carnivores from the coldest geographical regions expressed the highest tolerance to freezing as showed by a high Arctic Alaskan isolate which was more resistant to freezing than isolates from Norway. Furthermore, temperature fluctuations around freezing point (−5 °C) for up to seven weeks with seven overnight (+21 °C) thawing events, had little effect on larval infectivity. A negative effect of the initial repeated freeze–thaw events was observed once the larvae were exposed to longer periods of subsequent deep freezing (Davidson, 2008).

Laboratory and field studies suggest that several intrinsic and extrinsic factors concur to the freezing resistance of *Trichinella* spp. larvae in host muscles: i. the genetic structure of the parasite, i.e. only muscle larvae belonging to three taxa (*T. nativa*, *T. britovi* and *Trichinella* T6) survive in frozen muscles for periods of time which can be important for the natural cycle; ii. the host species, i.e. the survival of larvae of the three taxa is longer in frozen muscles of carnivores than in frozen muscles of swine or laboratory rodents; iii. The age of muscle larvae, which is also related to the development of the nurse cell and the collagen capsule, i.e. the older the

| Table 2 Duration of survival of muscle larvae in wild, domestic and laboratory animal muscles frozen at different temperatures. |
|-------------------------------------------------------------|
| **Trichinella species or genotype** | **Host** | **Temperature °C** | **Time of survival to freezing** | **References** |
|-----------------------------------|---------|-------------------|-------------------------------|----------------|
| *T. nativa*                       | Arctic fox | −15° | 14 months | Chadee and Dick (1982) |
|                                   | −18° | 4 years | Kapel et al. (1999) |
|                                   | −18° | 3 years | Dick and Pozio (2001) |
|                                   | −12° to −16° | 12 months | Odoevskaya et al. (2015) |
| Black bear                        | −18° | 81 days | Clark et al. (1972) |
| Exp. infected pig                 | −6.6° | 106 h | Hill et al. (2009) |
| Laboratory mice                   | −10° | 3 weeks | Pozio et al. (1994) |
| Laboratory rat                    | −18° | 1 week | Malakauskas and Kapel (2003) |
| Laboratory rat                    | −5° | 4 weeks | Malakauskas and Kapel (2003) |
| Marten                            | −18° | 5 months | Dick and Chadee (1981) |
| Polar bear                        | −18° | 5 years | Dick and Pozio (2001) |
|                                   | −18° | 6 months | Chadee and Dick (1982) |
|                                   | −15° | 1 year | Dick and Belosevic (1978) |
| Raccoon                           | −18° | 9 months | Dick (1983) |
| Walrus                            | −20° | 20 months | Leclair et al. (2004) |
| Wolf                              | −18° | 18 months | Dies (1980) |
| Wolverine                         | −18° | 6 months a | Dick and Chadee (1981) |
| Exp. infected pig                 | −6.6° | 106 h | Hill et al. (2009) |
| Grizzly bear                      | −6.5° to −20° | 34 months | Worley et al. (1986) |
| Laboratory mice                   | −10° | 5–13 days | Pozio et al. (1994) |
| Laboratory rat                    | −5° | 1 week | Malakauskas and Kapel (2003) |
| Wolverine                         | −6.5° to −20° | 4 months | Worley et al. (1986) |
| *Trichinella T6*                  | Exp. infected wild boar | −21° | 56 h | Lacour et al. (2013) |
| Exp. infected pig                 | −5° | 4 weeks | Pozio et al. (2006) |
| Laboratory mice                   | −10° | 4–7 days | Pozio et al. (1994) |
| Laboratory rat                    | −5° | 1 week | Malakauskas and Kapel (2003) |
| Red fox                           | −15° | 11 months | Dick and Pozio (2001) |
| Wild boar                         | −20° | 3 weeks | Pozio et al. (1992b) |
| Wild boar                         | −35° b | 1 week | Gari-Toussaint et al. (2005) |
| Wolf                              | −20° | 6 months | Pozio et al. (1989) |

a Completely thawed and refrozen after 1 month of freezing at −15 °C.
b I believe that even if the rated temperature of the home freezer was −35 °C, the wild boar meat did not reach this temperature for 1 week.

In Latvia, a correlation between the incidence of *Trichinella* spp. in wild boar and the number of snow cover days during the period 1976–1993, was observed. The higher the number of snow cover days, the higher the *Trichinella* spp. incidence in wild boar and vice versa (Kirjušina et al., 2015).

*Trichinella nativa* isolates originating from carnivores from the coldest geographical regions expressed the highest tolerance to freezing as showed by a high Arctic Alaskan isolate which was more resistant to freezing than isolates from Norway. Furthermore, temperature fluctuations around freezing point (−5 °C) for up to seven weeks with seven overnight (+21 °C) thawing events, had little effect on larval infectivity. A negative effect of the initial repeated freeze–thaw events was observed once the larvae were exposed to longer periods of subsequent deep freezing (Davidson, 2008).

Laboratory and field studies suggest that several intrinsic and extrinsic factors concur to the freezing resistance of *Trichinella* spp. larvae in host muscles: i. the genetic structure of the parasite, i.e. only muscle larvae belonging to three taxa (*T. nativa*, *T. britovi* and *Trichinella* T6) survive in frozen muscles for periods of time which can be important for the natural cycle; ii. the host species, i.e. the survival of larvae of the three taxa is longer in frozen muscles of carnivores than in frozen muscles of swine or laboratory rodents; iii. The age of muscle larvae, which is also related to the development of the nurse cell and the collagen capsule, i.e. the older the

---

Fig. 2. Minimum daily temperatures under deep snow (black line), shallow snow (dashed line), and ambient temperature (grey line) in Minnesota (adapted from Pauli et al., 2013).
larva in the muscle, the longer the survival time in frozen muscles. However in a laboratory rat model, muscle larvae of 10 to 20 weeks tolerated freezing better than early and late stages of infection (5 and 40 weeks post infection, respectively); iv. the freezing temperature, i.e. at temperatures below −20 °C, the survival of larvae in frozen muscles decreases quickly; v. the humidity, i.e. the higher the % of humidity, the longer the survival time; and vi. the duration of freezing (Pozio et al., 1994; Malakauskas and Kapel, 2003).

3.3. Survival of Trichinella spp. larvae in meat derived local food products

Many outbreaks of human trichinellosis in the Canadian Arctic have occurred following ingestion of uncooked walrus meat in various forms including raw, frozen or aged into a product named “igunaq” (Margolis et al., 1979; Serhir et al., 2001; Proulx et al., 2002). Experimental studies made with seal meat, showed that T. nativa larvae survived in igunaq (meat and blubber placed in a seal skin bag and allowed to ferment), nikku (air-dried meat), and raw frozen sausage (meat, fillers, salt, and spices), for at least 5 months under controlled laboratory conditions (Forbes et al., 2003). T. nativa larvae recovered from walrus meat stored at −20 °C for up to 20 months remained infective for guinea pigs. However, none of the 4–5 and 10-month-old igunaq preparations made with the same walrus meat, contained infective T. nativa larvae, suggesting that the degradation process that occurred in the field can be sufficient to inactivate larvae (Leclaire et al., 2004). T. nativa larvae survived up to 1 year in a fermented meat product, called ‘kopalkhen’ of the Chukcha autonomous Okrug of the Russian Federation, made with fox meat (instead of the most commonly used walrus meat) and frozen at −12° to −16 °C (Odoevskaya et al., 2015).

3.4. The climate change and the freeze-resistant Trichinella taxa

Climate change is a geographically heterogeneous and dynamic process; as a result, future changes to the subnivium will be region-specific. Throughout the Northern Hemisphere, the impact of climate change is predicted to be most prominent during the winter months, resulting in a shorter snow season and decreased snow depth (Trenberth et al., 2007; Brown and Robinson, 2011; Fountain et al., 2012). These climatic changes will likely modify the defining qualities of the subnivium, resulting in broad-scale shifts in distributions of T. nativa, Trichinella T6 and T. britovi.

In the Arctic, the fraction of total annual precipitation that falls as snow is declining (Groisman et al., 2004). Snow cover dynamics in mountain regions are often characterized by large regional and altitudinal variations (Räisänen, 2007), although regional models predict that snow–season length and snow depth are very likely to decrease (Christensen et al., 2007). Although the actual atmospheric lapse rate varies, under normal atmospheric conditions, the average atmospheric lapse rate results in a temperature decrease of 6.4 °C/km of altitude above ground level. The yearly average temperature over the equator is 25 °C, whereas the same over the poles is −20 °C. Hence, the higher the latitude, the lower the temperature (at the same level asl). We can roughly estimate a decrease of 0.5 °C per degree of latitude.

As a result of climatic warming, the eventual extinction of arctic mammals is now a frequent suggestion. That the extent of sea-ice in arctic and subarctic regions has been decreasing for nearly 50 yr has been reported. The winter climate of the Northern Hemisphere is changing rapidly. From 1906 to 2005, global mean surface temperatures increased by 0.74 °C ± 0.18 °C, with the majority of increases occurring over inland areas at northern latitudes during winter and spring (Trenberth et al., 2007). This warming has led to substantial changes in the extent, duration, and characteristics of the areas of snow, freshwater and sea ice, glaciers, and frozen ground (Fountain et al., 2012). The decrease in sea-ice during recent years has been sufficient to indicate that climatic warming may have a devastating effect on mammals throughout the Arctic and on their helminth fauna including Trichinella species circulating among carnivore mammals (Rausch et al., 2007).

4. Future investigations

The investigations on the survival of Trichinella spp. larvae at freezing temperatures tell us that some taxa of the genus Trichinella developed mechanisms to survive freezing; however, there is still the lack of a systematic study to deeply investigate the influence of the intrinsic and extrinsic factors identified to date. For example, no information is available on the biochemical mechanisms, which allow the survival of Trichinella spp. larvae to freezing. Freeze-resistant organisms supercool their bodies and prevent body fluids from freezing by masking or eliminating particles that induce ice formation (Storey and Storey, 1996). Alternatively, freeze-tolerant organisms survive ice formation by manufacturing proteins that promote extracellular freezing, thereby reducing hazardous intracellular water crystal formation.

The distribution area of T. britovi extends from about the 10th to the 60th parallel north, but no information is available if the freezing resistance detected in some T. britovi isolates from Europe is a biological character present in all the isolates of this species or if this feature is restricted only to isolates circulating in the northern regions or in mountain regions of the distribution area. In other words, does only the pressure of the environmental temperature allow the selection of freeze-resistant isolates? Based on the results for T. nativa obtained by Davidson (2008), I can argue that only the pressure of the environmental temperature allow the selection of freeze-resistant isolates.

The optimal freezing temperature range, which allows the longest survival time of Trichinella larvae in host carcasses could be between −5 °C and −20 °C, i.e. the temperature of the subnivean climate, which provides environmental stability (Keppel and Wardell-Johnson, 2012). This hypothesis is in agreement with the experimental results obtained on T. nativa isolates (Davidson, 2008).
5. Conclusions

Climate change certainly will influence the natural cycle of Trichinella taxa circulating in cold climates, due to the increasing temperature, decreasing humidity and snow cover, and increasing exposure to heat stress. We can expect a reduction of the distribution area of T. nativa and Trichinella T6, and an advance toward the north of the distribution area of T. britovi.

References

Boev, S.N., Britov, V.A., Orlov, I.V., 1979. Species composition of Trichinellae. Wiad. Parazytol. 25, 495–503.
Boutisini, S., Papatsiros, V.G., Stougou, D., Marucci, G., Laindris, E., Athanasiou, L.V., Papadoudis, A., Karagiogopoulos, E., Bisias, A., Pozio, E., 2014. Emerging Trichinella britovi infection in free-ranging pigs of Greece. Vet. Parasitol. 190, 278–282.
Brandy, P.J., Rausch, R., 1950. A preliminary note on trichinosis investigations in Alaska. Arctic 3, 105–107.
Brown, R.D., Robinson, D.A., 2011. Northern Hemisphere spring snow cover variability and change over 1922–2010 including an assessment of uncertainty. Cryosphere 5, 219–229.
Bukina, L.A., Odoevskaya, I.M., 2013. The epidemiology of trichinosis on the Arctic shores of Chukotka during traditional nature management. Med. Parasitol. (Mosk) 2, 133–137.
Chadee, K.C., Dick, T.A., 1982. Biological characteristics and host influence on a geographical isolate of Trichinella (Wolverine: 55 00 N, 100 00 W, 1979). J. Parasitol. 68, 451–456.
Chmuryżska, E., Różycki, M., Bilska-Zajęc, E., Nöckler, K., Mayer-Scholl, A., Pozio, E., et al., 2013. Trichinella nativa in red foxes (Vulpes vulpes) of Germany and Poland: possible different origins. Vet. Parasitol. 198, 254–257.
Christensen, J.H., Whitworth, B., Busiuoc, A., Chen, A., Gao, X., Held, I., et al., 2007. Regional climate projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averett, K.B., et al. (Eds.), Climate Change: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom, New York, pp. 847–940.
Clark, P.S., Brownsberger, K.M., Saslow, A.R., Kagan, I.G., Noble, G.R., Maynard, J.E., 1972. Bear meat trichinosis. Epidemiologic, serologic, and clinical observations from two Alaskan outbreaks. Ann. Intern. Med. 76, 955–961.
Davidson, R.K., Handeland, K., Kapel, C.M., 2008. High risk to repeated cycles of freezing and thawing in different Trichinella nativa isolates. Parasitol. Res. 103, 1005–1010.
De Bruyne, A., Ancelle, T., Vallee, I., Boireau, P., Dupouy-Camet, J., 2006. Human trichinosis acquired from wild boar meat: a continuing parasitic risk in France. Euro Surveill. 11, E060914.5.
Dekesne, G., Seglió, J., Jahundovič, I., Esite, Z., Bakasejevs, E., Bagrade, G., et al., 2016. High prevalence of Trichinella spp. in carnivore mammals of Latvia. Vet. Parasitol. (in press).
Desponmier, D.D., 1990. Trichinella spiralis: The worm that could be virus. Parasitol. Today 6, 193–196.
Dick, T.A., 1983. Species, and Intraspecific Variation. In: Campbell, W.C. (Ed.), Trichinella and Trichinosis. Plenum Press, New York and London, pp. 31–33.
Dick, T.A., Belosevic, M., 1978. Observations on a Trichinella spiralis isolate from a polar bear. J. Parasitol. 64, 1143–1145.
Dick, T.A., Chadee, K., 1981. Biological characterization of same North American isolates of Trichinella spiralis. In: Kim, C.W., Ruitenbeek, E.J., Teppema, T.S. (Eds.), Trichinellosis. Readbooks, Surrey England, pp. 23–27.
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, Iowa, pp. 380–396.
Díez, K., 1980. Survival of Trichinella spiralis larvae in deep-frozen wolf tissue. Can. Vet. J. 21, 38.
Dubinski, P., Stefančíková, A., Kincerek, J., Ondriska, F., Reiterová, K., Medvedová, M., 2001. Trichinellosis in the Slovak Republic. Parasite 8(2 S), S100-S102.
Dupouy-Camet, J., Yera, H., Dahane, N., Boutry, E., Kapel, C.M.O., 2016. A cluster of three cases of trichinellosis linked to bear meat consumption in the Arctic. J. Travel Med. 24, 1–3.
Dworkin, M.S., Gamble, H.R., Zarlanga, D.S., Tennican, P.O., 1996. Outbreak of trichinelllosis associated with eating cougar jerky. J. Infect. Dis. 174, 663–666.
Eaton, R.D., 1979. Trichinosis in the Arctic. Can. Med. Assoc. J. 120, 22.
Fichi, G., Stefanelli, S., Pagani, A., Luchi, S., De Gennaro, M., Gómez-Morales, M.A., Selmi, M., Rovai, D., Mari, M., Fischetti, R., Pozio, E., 2015. Trichinelllosis outbreak caused by meat from a wild boar hunted in an Italian region considered to be at negligible risk for Trichinella. Zoonoses Public Health 62, 285–291.
Forbes, L.B., 2000. The occurrence and ecology of Trichinella in marine mammals. Vet. Parasitol. 93, 321–334.
Forbes, L.B., Measures, L., Gajadhar, A., Kapel, C., 2003. Infection of Trichinella nativa in traditional northern (country) foods prepared with meat from experimentally infected seals. J. Food Prot. 66, 1857–1863.
Fountain, A.G., Campbell, J.L., Schuur, E.A.G., Stammerjohn, S.E., Williams, M.W., Ducklow, H.M., 2012. The disappearing cryosphere: Impacts and ecosystem responses to rapid cryosphere loss. Bioscience 62, 405–415.
Gajadhar, A.A., Pozio, E., de Borchgrave, J., Mortelmans, J., De Meurichy, W., 1990. Characterization of a Trichinella isolate from polar bear. Ann. Soc. Belg. Med. Trop. 70, 131–135.
Gottstein, B., Pozio, E., de Borchgrave, J., Mortelmans, J., De Meiurichy, W., 1990. Characterization of a Trichinella isolate from polar bear. Ann. Soc. Belg. Med. Trop. 70, 131–135.

E. Pozio / Food and Waterborne Parasitology 4 (2016) 4–12

11
