**Title:** *Trichinella spiralis* Owen, 1835 in American minks (*Neovison vison* Schreber, 1777) and wild rodents (Muridae and Cricetidae) in Chile.

**Running title:** *Trichinella* in minks and rodents of Chile

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**Summary:**

Trichinellosis is a worldwide disease that is considered emerging and neglected. Several hosts have been recognized around the world, however, there is a lack of knowledge of the role of free-range mammals in Chile. Herein we examined 555 individuals among American minks (*Neovison vison* Schreber, 1777. n = 100) and several myomorph rodent species (Muridae and Cricetidae. n = 455) from southern Chile with artificial digestion and molecular analyses. Rodents were captured in agricultural and wild protected areas, while minks were captured in mixed agricultural and unprotected wild areas. One rat (0.24%) in the Ñuble Administrative Region and seven minks (8.2%) in the Los Ríos Region were infected with *Trichinella spiralis* Owen, 1835. Our results suggest that native rodents are of low or null importance in the reservoir of *T. spiralis* in Chile. Conversely, our results suggest that *T. spiralis* is circulating in minks, but with low prevalence. Further studies are needed to assess whether minks are maintaining *T. spiralis* life cycle or are a dead-end host of this parasite in Chile. This study represents the first record of *T. spiralis* in a mustelid mammal in South America, increasing the number of free-range species that could participate in the reservoir.

**Keywords**

Chile, mammals, mink, rats, Trichinella, zoonoses

## Introduction

Invasive populations are not only an important issue from the conservationist point of view (Bellard, Cassey, & Blackburn, 2016; Wilcove & Master, 2005), but they can also be a source of infection for humans and domestic animals (Hulme, 2014; Lemming et al., 2020), in such a way that emerging infectious diseases are frequently considered as invasive species (Ogden et al., 2019). Trichinellosis is a disease of worldwide distribution (Korhonen et al., 2016) with several outbreaks reported yearly (Ribicich et al., 2020). It is also considered neglected (Bruschi, 2012) and emerging (Boutsini et al., 2014; Dupouy-Camet, 1999; Murrell & Pozio, 2000). This disease is mainly associated with cultural factors, especially the household slaughter of pigs without veterinary inspection (Pozio, 2014), a situation that is entrenched and difficult to eradicate. Thus, the knowledge of the cycle can give information about the epidemiological behavior of this disease and may allow us to suggest measures for reducing the incidence.

*Trichinella spiralis* Owen, 1835 is the most widespread species of the genus and presents both domestic and sylvatic cycles. The domestic cycle includes pigs, rats, dogs, cats and humans, and the sylvatic cycle includes wild and feral carnivore and omnivore mammals. Cougars, wild boars, foxes, opossums, sea lions, armadillos and a cricetid rodent, *Graomys centralis* Thomas, 1902, are the described wild hosts of *Trichinella* in South America (Dick & Pozio, 2000; Minoprio, Hipólito, & Dantis, 1967; Ribicich et al., 2020).

In South America, *Trichinella* (i.e. larvae or antibody) has been reported in Bolivia, Argentina, Brazil, Ecuador and Chile (Ribicich et al., 2020). In Chile, *T. spiralis* is the sole species reported, but with few larvae identified to the species level (Hidalgo et al., 2019; Landaeta-Aqueveque et al., 2015; Schenone et al., 2002). Conversely, in Argentina there are four reported *Trichinella* species: *T. spiralis*, *T. patagoniensis* Krivokapich et al. 2012, *T. britovi* Pozio et al. 1992 and *T. pseudospiralis* Garkavi, 1972 (Krivokapich et al., 2019; Krivokapich, Gonzalez Prous, Gatti, & Saldia, 2015; Krivokapich, Molina, Bergagna, & Guarnera, 2006; Krivokapich et al., 2012), which suggest the need for further studies in Chile. The domestic cycle is fairly well studied around the world (Alcaíno & Arenas, 1981; Pozio, 2000, 2014), but the sylvatic cycle is less known, and Chile is not the exception given that the reports of infected wild/feral mammals correspond to agricultural localities and that there is only one study of the prevalence in a feral species (Hidalgo et al., 2019). It is for this reason that the research pertaining to this parasite in wild environments, as well as to assess the role of free-range mammals, is neglected.

Cricetid rodents are the most abundant group of native rodents in Chile, with many species inhabiting large part of the country. Their diet, in addition to seeds and vegetables, encompasses animal food including insects and wild and domestic birds (Iriarte, 2008), suggesting that they also eat carrion. Murid rodents are the most abundant group of introduced mammals in Chile and they are largely associated to the maintenance of *Trichinella* sp., most likely because of their cannibal behaviors (Bilska-Zajac et al., 2018). On the other hand, the American mink, *Neovison vison* Schreber, 1777, is an important invasive species around the world (Brzeziński, Żmihorski, Nieoczym, Wilniewczyc, &

Zalewski, 2020; Failla & Fasola, 2019), with the fur industry being the main cause of its introduction. In Chile, the American mink is distributed from the nation's southernmost limit in the Magallanes Region to the Araucanía Region (Vergara & Valenzuela, 2015), but there were no findings of *Trichinella* in a previous study (Ramirez-Pizarro et al., 2019); and animals are currently being trapped by a control program in Los Ríos Region (SAG, 2020).

Thus, given the lack of studies of *Trichinella* in free-range mammals in Chile, the aim of this study was to assess the role of rodents in agricultural and protected areas of southern Chile, as well as, of *N. vison* in several localities in Los Ríos Region, in the maintenance of *Trichinella* sp.

**Materials and method**

Captures of rodents: four public and one private protected wild area, encompassing four administrative regions, were studied searching evidences of a sylvatic cycle. Those areas were in administrative regions (R.) with human cases of trichinellosis. These were: Ñuble National Reserve in the Ñuble R., Nahuelbuta National Park (NP) and Conguillío NP in the Araucanía R., Alerce Costero NP in the Los Ríos R. and Sendero Bosque Piedra (private park) in the Los Lagos R. Several localities between the O'Higgins and Aysén R. were chosen for studying the domestic cycle (Fig. 1). These localities encompass farms varying from those maintaining a couple of pigs with meadow access to a small breeding operation of pigs confined in a barn with two adult males and eight adult females. Some farms presented infected pigs some weeks before sampling, others presented infected pigs some months before sampling and others did not previously present infected pigs. These localities encompass climate that vary from Mediterranean (Csb, after Köpen classification. Chilean scrub eco-region), in the Ñuble NR., to Temperate Oceanic (Cfb. Valdivian forest eco-region), in the rest of the localities, with increasing humidity and decreasing temperatures as we head further south. Rodents were trapped with 200 live traps (Sherman: 240x80x90 mm, and Rodentraps: 300x100x110 mm) for five consecutive nights in each locality. In the protected areas, traps were put in transects besides the paths (at least 2 m distanced) or grids through the habitat. Likewise, in farms, traps were put in transects following the fences with shrubbery or within the barn. Rodents were morphologically identified following Iriarte (2010). According to the AVMA Guidelines for the Euthanasia of Animals 2020 Edition, once trapped, rodents were killed with anesthesia overdose putting them within a bag containing isoflurane (USP, Baxter) on a piece of cotton placed inside a tea infuser to avoid contact with liquid isoflurane. Then, the complete diaphragm, masseters, tongue, intercostal and peri-femoral muscles were removed and put in 0,1% thimerosal solution until laboratory examination.

Captures of minks: The Los Ríos Region (R.), between 39° 17' 14" and 40° 40' 55" S, has a Temperate Oceanic climate (Cfb, after Köpen classification), belonging to the Valdivian forest eco-region. The region is characterized by a complex river system with many lakes, most of them flowing into the Valdivia River Basin. These hydrologic characteristics are an ideal territory for the dissemination of American minks. Minks were captured with Tomahawk-like traps in most communes of the Los Ríos Region during a mink eradication plan of the 'Servicio Agrícola y Ganadero' (SAG. Governmental institution), between August 2018 and February 2020. See Figure 2 for the detail of the communes included in the study. According to the AVMA Guidelines for the Euthanasia of Animals 2020 Edition, animals were euthanized with Xilazine-Ketamine overdoses, frozen at -20°C and transported to the Departamento de Patología y Medicina Preventiva of the Facultad de Ciencias Veterinarias, Universidad de Concepción. After defrosting, about 10 g of a mixture of peri-femoral, masseter, tongue, diaphragm and intercostal muscles of each mink were submitted to parasitological examination.

Larvae and DNA extraction: Once in the laboratory, a mixture of muscles per individual, either rodent or mink, was examined by mean of artificial digestion (Gajadhar et al., 2019), and larvae were conserved in 96% ethanol. The DNA was extracted with DNeasy Blood & Tissue Kit (Qiagen®). The molecular identification, was performed following Pozio and Zarlenga (2019).

Statistical methods: A minimum sample size of 540 animals was calculated following Vallejo et al (2013), considering an unknown population size, a confidence of 95%, an expected proportion of 33% (Krivokapich et al., 2006), and a maximum error of 4%. The prevalence is reported with 95% confidence interval (CI), which was calculated using Stata 11 SE (Stata Corp).

Bioethical considerations: All animal procedures were approved by the Bioethics Committee of the Faculty of Veterinary Sciences of the University of Concepción (code CBE 47-2017; CBE-51-2019) and by the Scientific Ethics Committee for the Care of Animals and the Environment, Pontificia Universidad Católica de Chile (N˚160816007, 07-Nov-2017). Captures were approved by the National Forest Corporation (CONAF. #045-2017 and #005-2019) and Agricultural and Livestock Service (permit number: #7034-2017, #7684/2017, #3731/2018, #8517/2018, #774/2019 and #1829/2020).

**Results and Discussion**

A total of 455 rodents and 100 minks were captured and examined. Five species, encompassing 164 rodents were captured in protected areas: *Rattus rattus* Linnaeus, 1758, *Abrothrix olivacea* Waterhouse, 1837, *Abrothix hirta* Thomas, 1895, *Oligoryzomys longicaudatus* Bennett, 1832 and *Abrothrix sanborni* Osgood, 1943. Their distribution by species and parks is presented in Table 1. A total of 291 rodents were collected from agricultural environments, which belonged to the following species: *Mus musculus* Linnaeus, 1758, *Rattus norvegicus* Berkenhout, 1769, *R. rattus*, *A. olivacea*, *A. hirta*, *O. longicaudatus*, *Phyllotis darwini* Waterhouse, 1837, *Geoxus valdivianus* Philippi, 1858 and *Loxodontomys micropus* Waterhouse, 1837 (Table 2). Only one rodent (0.22%, CI: 0.00% – 0.65%), *R. rattus*, from Pemuco was infected with *Trichinella* larvae. This infected rat was captured at 760 m distant from the location where an infected pig was reported by sanitary governmental department two weeks prior. *Trichinella* larvae were isolated from 7 minks (7%, CI: 2–12). The geographic distribution of captured and infected minks is presented in Figure 2.

The molecular analysis of the larvae output a PCR product of 173 bp, corresponding to the species *T. spiralis*.

Prevalence herein is similar to that reported in wild boars in rural areas of the endemic Araucanía R. and Los Ríos R. (1.8%. Hidalgo et al., 2019) and higher than 0% reported in American minks in Southern Chile (Ramirez-Pizarro et al., 2019); however, most of the minks examined in that study were caught in non-endemic region of the country. Another study did not find *Trichinella* in 2.603 terrestrial wild/feral mammals, encompassing 311 carnivore mammal specimens belonging to *Lycalopex culpaeus* Molina, 1782, *Lycalopex griseus* Gray, 1837, *Galictis cuja* Molina, 1782, *Puma concolor* Linnaeus, 1771, *Leopardus guigna* Molina, 1782 and *Conepatus chinga* Molina, 1782; and 12 cricetid rodents belonging to *P. darwini* (Alvarez, Rivera, Neghme, & Schenone, 1970). It is not possible to adequately compare those results with our findings because that study did not mention the diagnostic technique, which likely was trichinoscopy. Another study that did not find *Trichinella* in the native felid *L. guigna*, also did not report the technique for muscle examination (González-Acuña et al., 2010). Thus, the prevalence reported herein in American minks is the highest prevalence of *T. spiralis* reported in a free-range mammal population in Chile.

Although all of the above seems to suggest the presence of a sylvatic cycle, most infected minks were caught in rural areas where they could be infected from the peri-domestic cycle. The American mink is a generalist predator whose diet includes mammals, especially rodents (Ibarra, Fasola, Macdonald, Rozzi, & Bonacic, 2009), in such a way that it could become infected with *Trichinella* larvae after the consumption of synanthropic rodents, especially rats (*Rattus* spp.), which are one of the most important component of the reservoir of *T. spiralis*, particularly in co-existence with domestic pig production farms (Bilska-Zajac et al., 2018; Oksanen et al., 2018; Schenone et al., 2002). This is not the first record of *Trichinella* infection in American minks. A previous study in Poland reports a prevalence of 3.5% in feral populations, encompassing tree species, *T. spiralis*, *T. pseudospiralis* and *T. britovi* (Hurníková, Kołodziej-Sobocińska, Dvorožňáková, Niemczynowicz, & Zalewski, 2016). However, this is the first record of a mustelid species harboring *Trichinella* in South America. The role of minks in the epidemiology of *Trichinella* remains unknown because there is no report of predators for this mustelid species in Chile. Hence, carrion animals such as rodents could be the main group of animals that can become infected by the consumption of this mammal, and this could be how larvae to continue their life cycle, in such a way that those carrion animals could become the source population for either minks or other mammals, including the domestic cycle. Otherwise, minks would be dead-end hosts favoring the decoy effect for this parasite (Johnson & Thieltges, 2010).

Although the likelihood of finding infected cricetids seems to be low given their scarce carnivore behavior, there are previous reports of infection in this family of rodents (Dick & Pozio, 2000; Pozio, 2005; Pozio & La Rosa, 2010). In fact, *T. spiralis* has been found to have infected a typically herbivorous feral mammal, the beaver, *Castor fiber* Linnaeus, 1758, in Europe (Różycki et al., 2020). The low prevalence in rodents in the agricultural localities is unexpected, since several studies have found higher prevalence in Chile (Alcaíno & Arenas, 1981) and elsewhere (Bilska-Zajac et al., 2018; Krivokapich et al., 2006). One explanation is the time elapsed between identifying the infected pig and the capture of the rodents, which varied from a week to several months. In a case, particularly in Los Lagos R., all pigs were removed from the farm after the detection of an infected pig, and then they were replaced by uninfected pigs. This agrees with the finding that the prevalence of *Trichinella* infection in free-range mammals decreases after controlling *Trichinella* in domestic pigs (Hill et al., 2010; Oksanen et al., 2018).

Our results, in addition to previous results in wild boar (Hidalgo et al., 2019) and American minks (Ramirez-Pizarro et al., 2019), suggests that *T. spiralis* is circulating in free-range mammals, but at a lower prevalence than elsewhere (Bilska-Zajac et al., 2018; Krivokapich et al., 2006). The low infection rate detected in this study agrees the lower infection rate in Chile, varying between 0.05 and 0.48 cases per $10^5$ inhabitants per year between years 2000 and 2015 (Fuentes, 2016). The low aggregate dispersion of the infected free-range mammals suggests that a domestic outbreak caused by feral mammals is difficult to predict and a pig can be infected in southern Chile anywhere and with no warning, thereby putting people at risk of human trichinellosis.

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**Conflict of interest statement**

Authors declare that there is no conflict of interest.

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**Table 1.** Number of examined rodents by protected area and species in central and southern Chile.

| **Rodent Species †** | *R. r.* | *A. o.* | *A. h.* | *O. l.* | *A. s.* | Totals |
|---|---|---|---|---|---|---|
| **Locality †** | | | | | | 164 |
| Ñuble NR | | 18 | 3 | 7 | | 28 |
| Nahuelbuta NP | | 8 | 9 | 1 | | 18 |
| Conguillío NP | | 10 | 20 | 18 | | 48 |
| Sendero Bosque Piedra | | 14 | | | 5 | 19 |
| Alerce Costero NP | 10 | 15 | 17 | 9 | | 51 |

**R. r.*: *Rattus rattus*; *A. o.*: *Abrothrix olivacea*; *A. h.*: *Abrothrix hirta*; *O. l.*: *Oligoryzomys longicaudatus*; *A. l.*: *Abrothrix longipilis*; *A. s.*: *Abrothrix sanborni*. NR: National reserve; NP: National Park

**Table 2:** Number of examined rodents by rural localities and species in central and southern Chile.

| **Rodent Species†** | *R. r.* | *R. n.* | *M. m.* | *A. o.* | *A. h.* | *A. l.* | *O. l.* | *P. d.* | *G. v.* | *L. m.* | Total |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Locality** | | | | | | | | | | | 291 |
| Doñihue | 5 | | | 1 | | | | | | | 6 |
| Pimpinela | | 1 | | | | | | | | | 1 |
| Chillán | 16 | | | | | | | | | | 16 |
| Pinto | 14 | 1 | | | | | | | | | 15 |
| El Carmen | 6 | | | | | | | | | | 6 |
| Pemuco | 19 | 4 | | 2 | | | 6 | 1 | | | 32 |
| Virgüín | 13 | | 3 | | | | 3 | | | | 19 |
| Los maitenes | 2 | | 10 | 3 | | | 9 | | | | 24 |
| Carahue | 11 | 1 | 5 | | | | 1 | | | | 18 |
| Puerto Saavedra | 2 | | | 1 | | | | | | | 3 |
| Collico | 8 | 1 | | | | | | | | | 9 |
| Ralinco | 1 | | | 1 | | | | | | | 2 |
| Nehuentue | 9 | | | | | | | | | | 9 |
| Ancud | | | | 31 | | | 1 | | 6 | 1 | 39 |
| Castro | 14 | 1 | | 27 | | 1 | 1 | | | | 44 |
| Puerto Aysén | 3 | | | | | | | | | | 3 |
| Puerto Chacabuco | 2 | | | | | | 1 | | | | 3 |
| Melipeuco | 16 | | | 6 | | | 8 | | | | 30 |
| Coñaripe | 1 | | | 1 | 10 | | | | | | 12 |

†*R. r.*: *Rattus rattus*; *R. n.*: *Rattus norvegicus*; *M. m.*: *Mus musculus*; *A. o.*: *Abrothrix olivacea*; *A. h.*: *Abrothrix hirta*; *A. l.*: *Abrothrix longipilis*; *O. l.*: *Oligoryzomys longicaudatus*; *P. d.*.: *Phyllotis darwini*; *G. v.*: *Geoxus valdivianus*; *L. m.*: *Loxodontomys micropusk.*

## Figure legends

**Figure 1:** Map of Chile (left) with administrative regions (continuous limits) and provinces (non-contiguous limits) and a detail of the localities of captures (right). Italicized text indicates wild protected areas and roman text indicates agricultural areas. 1: Doñihue, 2: Pinpinela, 3: Chillán, 4: Pinto, 5: El Carmen, 6: Pemuco, 7: Virguin, 8: Los Maitenes, 9: Carahue, 10: Puerto Saavedra, 11: Collico, 12: Ralinco, 13: Nehuentue, 14: Melipeuco, 15: Coñaripe, 16: Ancud, 17: Castro, 18: Puerto Aysén, 19: Puerto Chacabuco, 20: Ñuble National Reserve, 21: Nahuelbuta National Park (NP), 22: Conguillío NP, 23: Alerce Costero NP, 24: Bosque Piedra Private Park.

**Figure 2:** Communes (delimitated by black lines) and hydrographic systems (colored in blue) of Los Ríos Region. Numbers correspond to captured minks and red crosses are the localities of positive minks.