Research Note

*Trichinella spiralis* parasitizing *Puma concolor*: first record in wildlife in Chile

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Summary

The genus *Trichinella* is widespread in all continents but Antarctica. The only way to identify the species/genotypes is through molecular analyses. In Chile, only one study has reported *Trichinella* larvae in a cougar, but the species of *Trichinella* was not identified. In this work, the finding of *Trichinella* larvae in a cougar, together with their genotypic identification, is the first documentation of such in Chile. The cougar was found run over by a vehicle in the Biobío Region. Larvae were isolated following artificial digestion of the diaphragm and analyzed by means of multiplex polymerase chain reaction (PCR). A PCR product of 173 base pairs allowed for the classification of the larvae as *T. spiralis*. It is the first record of the species in Chilean wildlife. This finding in Chile is interesting in terms of human health, suggesting a possible role of the cougar as a reservoir for this parasite.

Keywords: Trichinellosis; native; wild; mammal; cougar

Introduction

The *Trichinella* species represent one of the most widespread parasites in the world. Several species of the genus *Trichinella* have been identified as infecting both synanthropic and wild vertebrates in all continents but Antarctica (Pozio & Murrell, 2006; Pozio, et al., 2009; Pozio & Zarilenga, 2013). However, in Chile, reports of *Trichinella* parasitization are mostly restricted to synanthropic mammals such as pigs, rats, dogs, and cats (Álvarez, et al., 1970; Schenone, et al., 1999). There is only one finding of this genus in wild animals; however, this finding lacks the specific identification of larvae (Hidalgo, et al., 2013).

Until 1971, *T. spiralis* was the only recognized species. At present, there are nine recognized species, as well as three genotypes that have not yet been demonstrated to be distinct species (Pozio & Zarilenga, 2013). In South America, there are three recognized *Trichinella* species: *T. spiralis* in Argentina and Chile (Fonseca-Salamanca, et al., 2006; Krivokapich, et al., 2006; Ribichich, et al., 2010), *T. patagoniensis* in Argentina (Krivokapich, et al., 2012), and *T. pseudospiralis* in Argentina (Krivokapich, et al., 2015). Bolivia also presents *Trichinella* infection, but the genotypes have not yet been identified (Bjorland, et al., 1993; Brown, et al., 1996).

It is known that the *Trichinella* species, especially *T. spiralis*, present both domestic and sylvatic cycles. The domestic cycle includes pigs, dogs and cats, and the sylvatic cycle includes wild carnivorous and omnivorous species (Minoprio, et al., 1967; Krivokapich, et al., 2006), where synanthropic rats can act as a vector of *Trichinella* from wildlife to domestic animals (Pozio, 2000).

In Chile, *Trichinella* has been found in domestic pigs (Poupin, 1897; Schenone, et al., 1999), dogs, cats (Álvarez, et al., 1970), and synanthropic rats (several reports from Poupin, 1897), but only isolates taken from pigs and brown rats have been identified at species level as *T. spiralis* (Schenone, et al., 2002; Fonseca-Salamanca, et al., 2006; ITRC-SS, 2014). On the other hand, there are only three published articles, those by Álvarez et al. (1970), González-Acuña et al. (2010) and Hidalgo et al. (2013), concerning *Trichinella* in wildlife. The two former studies, which did not find *Trichinella* larvae, included six rodent species, two canid species, two mustelid species (one of them classified as a rodent by Álvarez et al. (1970)), two felid species, two lagomorph species (classified as rodents in by Álvarez et al. (1970)), and three whale species. The latter study reported the presence of *Trichinella* sp. in a cougar, but without genotypic identification. There is also one report of human trichinellosis associated with the consumption of...
wild boar (Sus scrofa) hunted in the sylvatic environment (García, et al., 2005).

In Chile, trichinellosis is considered an endemic disease associated with domestic slaughtering without veterinary supervision (MINSAL, 2011). In addition, although the hunting of wild fauna is regulated by law (SAG, 2012), hunting of wild carnivores is still a reality given that these animals are considered detrimental by livestock owners, and subsequent consumption is to be expected. Given the fact that humans must eat poorly cooked infected meat in order to be parasitized, the most likely cause of this parasitosis lies in people’s culinary customs.

Conversely, the transmission of parasites between native and introduced mammals is a major issue in conservation biology, because introduced species (including parasites) are one of the most significant causes of species loss (Wilcove, et al., 1998; Wilcove & Master, 2005). Given that clinical diseases in animals experimentally infected by Trichinella infection have been documented, especially when infecting dose is high (Rice, et al., 1990; Ribicich, et al., 2007; Bowman, 2009; Ribicich, et al., 2013), it is expected that native carnivores would show clinical signs after a highly loaded prey consumption. From this point of view, the identification of the Trichinella species circulating in wild mammals allows us to establish whether these parasites were or not transmitted from introduced hosts.

Therefore, more information about the presence of the Trichinella species in wild mammals is valuable. In this work, we report the first record of T. spiralis identified via molecular method in the cougar, Puma concolor concolor, and, hence, the first record of T. spiralis in a wild animal in Chile.

Materials and Methods

The cougar was found to have been run over by a vehicle in the Biobío Region in the Quilleco district, locality of Las Canteras (Coordinates UTM: H18, 765622 East, 5863308 South). The date of the death was March 27, 2014. The cougar was transported to the Facultad de Ciencias Veterinarias of the Universidad de Concepción (Chillán, Chile) by Servicio Agrícola y Ganadero of Chile staff members. The cougar was male and weighed 55 kg.

The cougar’s diaphragm was removed through a ventral incision of the abdominal muscles. Trichinoscopic examination and pepsin-clorhidric artificial digestion method (Gamble, et al., 2000) were used on a 15 g sample of diaphragm in order to study the presence and density (larvae per gram [lpg]) of Trichinella larvae in the Laboratory of Parasitology Dr. Luis Rubilar of the aforementioned Faculty. Isolated larvae were conserved in 99 % ethanol.

Molecular analyses were performed in the Departamento de Parasitología, Instituto Nacional de Enfermedades Infecciosas, Administración Nacional de Laboratorios e Institutos de Salud “Dr. Carlos Malbrán” (Buenos Aires, Argentina). Extraction of the genomic DNA of muscle larvae of Trichinella was performed individually from four parasites, as previously described (Krivokapich, et al., 2006), and identification at the species level was carried out by multiplex polymerase chain reaction (PCR) (Zarlenga, et al., 1999).

Results

The diaphragm was found to be infected with encapsulated living larvae at a density of 1.5 lpg. Morphologic attributes (larva in a capsule, the presence of stichosome) indicated that they were of the genus Trichinella. The molecular analysis of the larvae from Trichinella isolate generated a PCR product of 173 bp, corresponding to the species T. spiralis (Fig. 1). The cougar did not show external physical signs of acute trichinellosis (Ribicich, et al., 2007; Bowman, 2009; Ribicich, et al., 2013); it only showed facial lesions attributable to the cause of death.

Discussion

The finding and posterior report of T. spiralis in a cougar is uncommon. However, it is expected that since the cougar is at the top of the food chain, this animal species should be one of the main reservoirs of Trichinella in nature. As far as we know, T. spiralis has been reported in cougars only in Argentina (Krivokapich, et al., 2012), and this parasite species has been also reported in the Florida panther (P. concolor coryi) in the United States (ITRC-ISS, 2014). Other Trichinella species have also been found in cougars in the Nearctic Region (Gajadhar & Forbes, 2010).

The density of larvae in the cougar in this report was low in comparison with that of the previous report from Chile (Hidalgo, et al., 2013). Other Trichinella species have also been found in cougars in the Nearctic Region (Gajadhar & Forbes, 2010).
The cougar in this study showed a better overall condition than the one described by Hidalgo et al. (2013). Although the disease has been reported in animals (Rice, et al., 1990; Ribicich, et al., 2007; Bowman, 2009; Ribicich, et al., 2013), the infecting dose necessary for clinical signs is usually high, resulting in higher larvae densities than that observed in this report. Another likely cause for the lack of clinical signs is that the animal is in the chronic phase of the infection.

Although sylvatic cycles are described for Trichinella spp. and can be considered as a source of infection, this finding must be looked at carefully because a sylvatic cycle implies the existence of several host species, which, in turn, supports the hypothesis that the parasite is cycling in the sylvatic environment. There is no report of Trichinella in wild hosts other than in P. concolor in Chile (Álvarez, et al., 1970; González-Acuña, et al., 2010), and rats reported to host Trichinella larvae have been found only in association with human settlements (e.g. Poupin, 1897). Thus, in Chile, the cougar and the wild boar are the only species that represent any evidence of a possibly existing sylvatic cycle of Trichinella (Garcia, et al., 2005; Hidalgo, et al., 2013). In contrast, in Argentina, Trichinella has been documented in wild boar (Cohen, et al., 2010), and it has also been found in the armadillo (Chaeotophractus villosus), the central perecote (Graomys centralis), and in a species of fox (Lycalopex gymnecerus gracilis) (Minoprio, et al., 1967; Krivokapich, et al., 2006). In addition, another species, T. patagoniensis, is also reported circulating among Argentinean cougars (Krivokapich, et al., 2012).

Another possible source of the infection of this cougar is the consumption of domestic or synanthropic animals infected with the parasite, such as rats or pigs. The cougar was found on a road that crosses several agricultural and livestock farms, so it is possible that the cougar had consumed synanthropic animals, as mentioned in Iriarte et al. (1991) and Rau et al. (1991).

A third alternative is that the parasite arrived to the cougar from synanthropic mammals by means of a complex food web. For example, rodents are reported to be prey of the wild boar (Skewes, et al., 2007), and the wild boar is reported to be prey of the cougar (Skewes, et al., 2012). Although other carnivorous and omnivorous mammals are reported as prey of the cougar (Skewes, et al., 2012), the wild boar is the only one documented as a possible host for Trichinella sp. in Chile. This mechanism is supported by the fact that micromammals are a small part of the feeding ecology of the cougar in Chile (Iriarte, et al., 1991; Rau, et al., 1991; Skewes, et al., 2012), but they are an important part of the diet of the wild boar (Skewes, et al., 2007), and the wild boar is reported as an important prey of the cougar.

Ecological and molecular studies could support these or new emerging hypotheses of the current status of T. spiralis circulating in the wild environment.

The infection in wild and feral animals means that these animals, especially those that are carnivorous or omnivorous, can become sources of infection for humans. The hunting of animals in Chile is regulated by the hunting law (SAG, 2012) in order to protect natural resources. However, although the cougar is protected by law (SAG, 2012), livestock owners perceive cougars as a threat, (CONAF, 2013), and this enhances the possibility of them being hunted and consequently eaten by humans. Thus, the cougar can be a source of human infection by T. spiralis in Chile. The importance of wild carnivores must be assessed in order to design new strategies to prevent this transmission.

Thus, this study raises the number of native mammals affected by introduced parasites in Chile (see Landeta-Aqueveque, et al., 2014) reporting a new host-parasite association: T. spiralis parasitizing the cougar. The presence of this zoonotic parasite in a native mammal can have important consequences for the public health underlying the need to design new public health strategies addressed to hunters.

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