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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 & Zarlenga, 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 & Zarlenga, 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; Ribicich*, 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-ISS, 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.

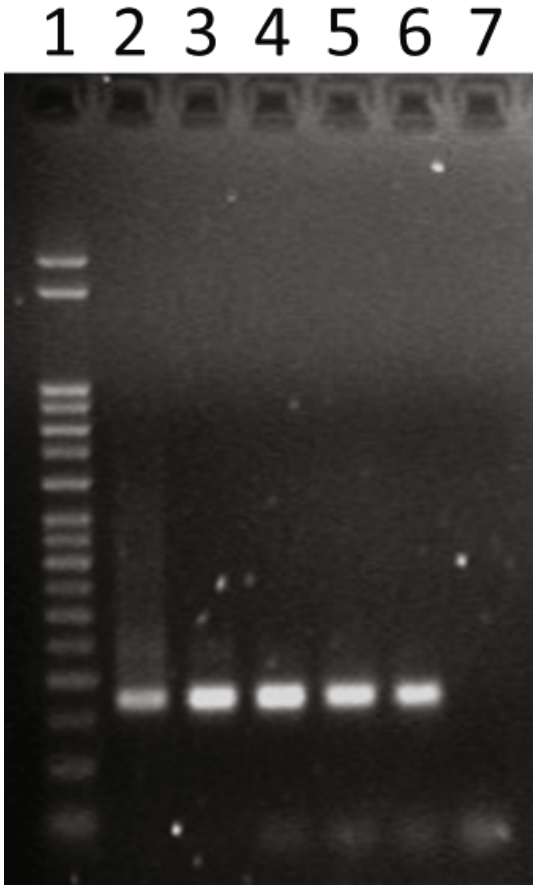

Fig. 1. Agarose gel separation of multiplex PCR products from four *Trichinella spiralis* larvae isolated from a cougar in Chile. Lane 1: Marker of 50 bp.; ane 2 – 5: isolates 173 bp; lane 6: positive control; lane 7: negative control

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, 5 lpg.), and similar to that of a report of *T. spiralis* in cougars in Argentina (Ribicich*, et al.*, 2010, 1 lpg). The cougar in this work 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* (García*, 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 (*Chaetophractus villosus*), the central pericote (*Graomys centralis*), and in a species of fox (*Lycalopex gymnocerus 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 Landaeta-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 underlining the need to design new public health strategies addressed to hunters.

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