



## Short communication

*Trichinella britovi* in a leopard (*Panthera pardus saxicolor*) in IranGholamreza Mowlavi<sup>a,\*</sup>, Gianluca Marucci<sup>b</sup>, Iraj Mobedi<sup>a</sup>, Farzaneh Zahabiioon<sup>a</sup>, Hamed Mirjalali<sup>a</sup>, Edoardo Pozio<sup>b</sup><sup>a</sup> Department of Medical Parasitology & Mycology, School of Public Health & Institute of Public Health Research, Tehran University of Medical Sciences, P.O. Box 6446, Tehran 14155, Iran<sup>b</sup> Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, Rome, Italy

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## ABSTRACT

Nematodes of the genus *Trichinella* are zoonotic parasites with a cosmopolitan distribution. In Iran, these parasites have mainly been detected in carnivorous mammals, yet information on the *Trichinella* taxa circulating in this country date back to a time when biochemical and molecular tests were not available. We describe the first detection of *Trichinella* larvae in a leopard (*Panthera pardus saxicolor*) in Asia and its identification at the species level. The larvae recovered from the leopard muscles were identified as *Trichinella britovi* using multiplex PCR. The detection of *Trichinella* infection in a leopard confirms literature data on the high prevalence of infection in carnivorous mammals in Iran.

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## 1. Introduction

Parasite worms of the genus *Trichinella* are zoonotic nematodes with a cosmopolitan distribution (Pozio and Murrell, 2006). There are eight currently recognised *Trichinella* species and four genotypes, each of which is characterised by their host pattern and distribution (Pozio et al., 2009a). Human trichinellosis, formerly known as “trichinosis” or “trichiniasis”, has been documented in 55 countries worldwide, with a yearly occurrence of about 10,000 clinical cases (Pozio, 2007).

The circulation of *Trichinella* in wild and/or domestic animals does not always mean that humans will become infected, in that eating habits play an important role in transmission (Pozio, 2007). In Muslim countries, human trichinellosis was believed to be practically non-existent because of the almost nil consumption of pork, which is strictly prohibited for religious reasons. However, the

recent occurrence of outbreaks and single cases in Algeria, Israel, Lebanon and Turkey (Pozio, 2007) strongly suggests that the risk of acquiring trichinellosis from domestic or sylvatic animals also exists in these countries and that it is thus important to monitor the circulation of these zoonotic parasites.

In Iran in the past, only one case of human infection has been reported and was due to the consumption of pork from a wild boar (*Sus scrofa*), although there was insufficient evidence for a definitive diagnosis (Moin, 1966). More recently, an outbreak of trichinellosis involving six persons occurred for the consumption of pork from a wild boar hunted in the Javaher–Dasht forest (Siahkal, Gilan province) (Kia et al., 2008). *Trichinella* spp. were first detected in wild boars (Afshar and Jahfarzadeh, 1967) and later in seven carnivorous mammal species and one rodent species (*Meriones persicus*) in several of the country's regions (Mobedi et al., 1973; Sadighian et al., 1973; Massoud, 1978; Hamidi, 1979; Mowlavi et al., 2000). In the present work, we describe the detection of *Trichinella* sp. larvae in a leopard (*Panthera pardus saxicolor*) in Iran, which is the first report of an infected leopard in Asia.

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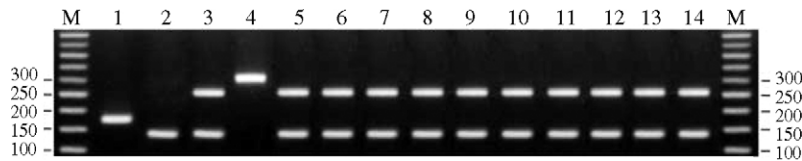


Fig. 1. Electrophoretic patterns of amplicons from multiplex PCR of single larvae of *Trichinella* collected from the leopard (*Panthera pardus saxicolor*) muscles and from reference strains. Lane 1, *Trichinella spiralis* reference larva (code ISS003); lane 2, *Trichinella nativa* reference larva (code ISS010); lane 3, *Trichinella britovi* reference larva (code ISS002); lane 4, *Trichinella pseudospiralis* reference larva (code ISS013); lanes 5–14, single larvae collected from the leopard (code ISS2044); M, molecular weight marker; Pharmacia, 50 bp ladder.

## 2. Materials and methods

The leopard, a young male, was shot unwillingly in a frighteningly close encounter with villagers in Germin County (48°5'8"E; 39°0'48"N, Ardabil Province, north western Iran). Three days after the leopard was killed, the carcass was frozen and transferred to the Department of Medical Parasitology and Mycology of the Tehran University of Medical Sciences, Tehran. Muscle samples from shoulders, neck and abdomen, were examined for *Trichinella* sp. larvae by trichinostomy. Muscle samples were then digested according to the standard protocol (Nöckler and Kapel, 2007) and preserved in 90% ethyl alcohol. They were then sent to the International Trichinella Reference Centre in Rome, Italy, where they were identified at the species level by an accredited multiplex PCR, as previously described (Pozio and La Rosa, 2003). Single larvae from four reference strains belonging to taxa circulating in Asia were used for comparison: *Trichinella spiralis* (ISS003), *Trichinella britovi* (ISS002), *Trichinella nativa* (ISS010), and *Trichinella pseudospiralis* (ISS013).

## 3. Results and discussion

*Trichinella* larvae were observed by trichinostomy in muscle samples from the shoulders, neck and abdomen. Given that all of the larvae recovered after digestion were dead, experimental infection with mice was not performed. All 40 larvae were identified at the species level. After multiplex-PCR amplification, the larvae displayed a band at 127 bp derived from the expansion segment V of the IsrDNA and a second band at 253 bp from the internal transcribed spacer 1 of the rDNA (Fig. 1). Although this pattern is shared by *T. britovi*, *Trichinella* T8 and *Trichinella* T9, based on the geographical distribution we can reasonably exclude *Trichinella* T8 and *Trichinella* T9, since T8 is restricted to Namibia and South Africa and T9 is restricted to Japan (Pozio and Murrell, 2006).

This is the first report of *T. britovi* in a leopard. Although the leopard is generally not a scavenger, as are most of the main *Trichinella* reservoirs (Pozio, 2005), the detection of an infected leopard is not surprising, given that this animal is at the top of the food chain. The only other report of a *Trichinella* isolate identified at the species level from a

Table 1  
*Trichinella* sp. in wild and synanthropic mammals of Iran.

Host	Province of origin	Positive/tested (%)	References
<i>Sus scrofa</i>	Mazanderan	2/4,950 (0.04)	Afshar and Jahfarzadeh, 1967
<i>Sus scrofa</i>	Golestan	5/21,143 (0.02)	Mobedi et al., 1973 <sup>a</sup>
<i>Sus scrofa</i>	Khuzestan	1/4	Massoud, 1978
<i>Sus scrofa</i>	Gilan	1/1	Kia et al., 2008 <sup>b</sup>
<i>Ursus arctos</i>	Mazanderan (Noor forest)	1/16 (6.25)	Mobedi et al., 1973 <sup>a</sup>
<i>Canis aureus</i>	Mazanderan, Golestan and Gilan	38/63 (60.3)	Mobedi et al., 1973 <sup>a</sup>
<i>Canis aureus</i>	Isfahan	10/18 (55.55)	Sadighian et al., 1973 <sup>b</sup>
<i>Canis aureus</i>	Khuzestan	11/25 (44.0)	Massoud, 1978
<i>Canis aureus</i>	Golestan	105/125 (84.0)	Hamidi, 1979 <sup>c</sup>
<i>Canis familiaris</i> <sup>e</sup>	Isfahan	2/10 (20.0)	Sadighian et al., 1973 <sup>d</sup>
<i>Canis familiaris</i> <sup>e</sup>	Golestan	9/100 (9.0)	Hamidi, 1979 <sup>c</sup>
<i>Vulpes vulpes</i>	Isfahan	2/18 (11.11)	Sadighian et al., 1973 <sup>d</sup>
<i>Vulpes vulpes</i>	Golestan	3/10	Hamidi, 1979 <sup>c</sup>
<i>Hyena hyena</i>	Isfahan	1/1	Sadighian et al., 1973 <sup>d</sup>
<i>Hyena hyena</i>	Khuzestan	1/1	Massoud, 1978
<i>Herpestes auropunctatus</i>	Khuzestan	3/10	Mowlavi et al., 2000
<i>Meriones persicus</i>	Isfahan	1/29 (3.44)	Sadighian et al., 1973 <sup>d</sup>
<i>Panthera pardus</i>	Ardabil	1/1	Present work

<sup>a</sup> Additional animals tested negative for *Trichinella* sp.: 30 rodents belonging to *Mus musculus*, *Apodemus sylvaticus*, and *Rattus rattus*; 20 *Crocidura russula*; 1 *Meles meles*; 1 *Felis silvestris catus* (domestic cat); and 1 *Canis familiaris* (domestic dog).

<sup>b</sup> The wild boar was the source of trichinellosis in six persons of Teheran in 2007.

<sup>c</sup> Additional animals tested negative for *Trichinella* sp.: 13 *Erinaceus europaeus*; 13 *Crocidura leucodon*; 7 *Lepus capensis*; 21 *Ochotona rufescens*; 56 *Allactaga elater*; 4 *Glis glis*; 160 *Mus musculus*; 206 *Apodemus sylvaticus*; 10 *Nesokia indica*; 7 *Rattus rattus*; 108 *Cricetulus migratorius*; 69 *Microtus* spp.; and 130 *Meriones* spp. or *Rhombomys opimus*.

<sup>d</sup> Additional animals tested negative for *Trichinella* sp.: 43 *Mus musculus*; 9 *Cricetulus migratorius*; 15 *Nesokia indica*; 13 *Meriones crassus*; 2 *Apodemus sylvaticus*; and 11 *Rhombomys opimus*.

<sup>e</sup> Stray dogs.

leopard is that of a *T. nelsoni* s.str. isolate in a leopard of the Serengeti (Tanzania) (Pozio et al., 1997). However, it must be considered that leopards are a highly protected species in most parts of the world and that only specimens that have been accidentally killed or found dead in nature can be examined. In Iran, the leopard inhabits mountainous areas and although its distribution area covers the entire country (except for a small part in the south–west), the number of specimens is very low (Farhadinia, 2004; Khorozyan, 2008).

Before 1983, most of the *Trichinella* larvae detected in Iran were considered to be *T. spiralis* (Mobedi et al., 1973; Sadighian et al., 1973; Hamidi, 1979) because at the time the taxonomy of the *Trichinella* genus was controversial (Pozio et al., 1992). In 1983, Shaikenov and Boev performed cross-breeding experiments and were the first to identify the species of *Trichinella* isolates in Iran. In particular, they identified four isolates from jackals (*Canis aureus*): three isolates were identified as *T. nelsoni* s.l. (i.e., the former name of *T. britovi*, which is today used only to refer to isolates from tropical eastern Africa; Pozio et al., 1992), and one isolate was identified as *T. spiralis* (Shaikenov and Boev, 1983). As shown in Table 1, the prevalence of *Trichinella* sp. infection in the wildlife of Iran is quite high in carnivorous mammals, particularly the golden jackal (71%). The high prevalence of *Trichinella* infection in carnivores and the very low prevalence in wild boars (Table 1) suggest that *T. britovi* may be the prevalent species in Iran and that *T. spiralis*, if still present, is probably restricted to a small area. In fact, *T. britovi* is highly adapted to carnivores and *T. spiralis* to swine (Pozio et al., 2009b). On the basis of the biogeographical data of Iran, we can confidently exclude the presence of *T. nativa* in this country. In fact, the southern distribution boundary has been tentatively identified between the isotherms  $-5^{\circ}$  and  $-4^{\circ}$  C in January (Pozio and Murrell, 2006).

It is important to continue to monitor the circulation of *Trichinella* sp. in the wildlife of Iran, given that wild carnivores and omnivores represent the natural reservoir for these parasites and that humans can acquire trichinellosis when they fail to properly manage domestic animals and wildlife.

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