First report of a mixed infection of *Trichinella nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from the Greater Kruger National Park, South Africa

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At least three *Trichinella* species, namely *Trichinella nelsoni*, *Trichinella britovi* and *Trichinella zimbabwensis*, and one genotype (*Trichinella* T8), have been isolated from sylvatic carnivores on the African continent. With the exception of *T. britovi*, the other species are known to circulate in wildlife of the Kruger National Park (KNP), South Africa, and KNP neighbouring game reserves (collectively known as the greater KNP area). Lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) appear to be the most important reservoirs of *T. nelsoni* and *Trichinella* T8 in the KNP and surrounding areas. Interspecies predation between lions and hyenas has been implicated as a primary mode of maintaining the life cycles of these two *Trichinella* species. This is the first report of a mixed natural infection of *T. nelsoni* and *Trichinella* T8 in a leopard (*Panthera pardus*) from South Africa. *Trichinella* muscle larvae were identified to species level by multiplex polymerase chain reaction (PCR). Probable sources of infection, based on the known dietary preference and prey species' range of leopards, are also discussed. The described occurrence of *Trichinella* species in a leopard from the greater KNP area raises the question of possible sources of infection for this predator species.

Introduction

The genus *Trichinella* is comprised of the species *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella pseudospiralis*, *Trichinella murrelli*, *Trichinella nelsoni*, *Trichinella papuae*, *Trichinella zimbabwensis* and three genotypes (*Trichinella* T6, T8, T9) (Pozio & Zarlenga 2005; Pozio *et al.* 2009), and a newly described species, *Trichinella patagoniensis* from Patagonia, Argentina (Krivokapich *et al.* 2008, 2012). Of these, at least three species (*T. nelsoni*, *T. britovi* and *T. zimbabwensis*) and one genotype (*Trichinella* T8) have been isolated from sylvatic carnivores in Africa (Pozio *et al.* 1994, 2002, 2005, 2007). With the exception of *T. britovi*, these species have been reported in wild carnivores from the Kruger National Park (KNP) and neighbouring game reserves of South Africa (La Grange, Marucci & Pozio 2010; La Grange *et al.* 2013; Marucci, La Grange & Pozio 2009).

Lions (*Panthera leo*) and spotted hyenas (*Crocuta crocuta*) appear to be important reservoirs of *T. nelsoni* and *Trichinella* T8 in the greater KNP area of South Africa. Interspecies predation between lions and hyenas has been hypothesised to play a role in the maintenance of the life cycles of these two parasite species (Mukaratirwa *et al.* 2013). *Trichinella* spp. infections in humans have been reported due to the consumption of undercooked meat derived from bush pigs (*Potamochoerus larvatus*) in Kenya (Forrester 1964; Forrester, Nelson & Sander 1961; Hutcheon & Pamba 1972; Nelson 1970; Okelo & Bhatt 1987) and warthogs (*Phacocoerus africanus*) in Tanzania (Bura & Willett 1977). Results from previous studies suggest that these species of sylvatic Suidae may play an additional, yet less pronounced, role in the epidemiology of *T. nelsoni* and *Trichinella* T8, and have shown that warthogs and bush pigs represent only a small percentage of naturally infected animal reservoirs in Africa (Mukaratirwa *et al.* 2013). Limited passive surveillance based on convenient samples from 17 warthogs culled in the KNP and adjacent nature reserves have, to date, not yielded any positive samples (La Grange & Mukaratirwa unpublished data; Marucci *et al.* 2009). *Trichinella nelsoni* infection in leopards (*Panthera pardus*) has been reported from Tanzania (Pozio *et al.* 1997) and Kenya (Pozio 2007), whereas *T. britovi* has been described in a leopard from Iran (Mowlavi *et al.* 2009). In addition to one leopard tested in 1974 in KNP (Marucci *et al.* 2009), three more, originating from the nature reserves adjacent to the western KNP, have been tested since 2012, with negative results (La Grange & Mukaratirwa unpublished data).

Leopards are solitary animals; they avoid contact with lions and hyenas and do not normally prey on them (Hayward *et al.* 2006). This suggests the existence of alternate sylvatic species serving as sources of infection, such as small and medium-sized game species, including warthogs, bush pigs and baboons (*Papio* spp.), all of which could potentially serve as sources of *Trichinella* spp.

infection. Although natural infection with *Trichinella* spp. has not been documented in free-ranging baboons, monkeys and bush pigs in South Africa, they could possibly serve as hosts for *Trichinella* spp. in southern Africa because experimental infection of *T. zimbabwensis* in baboons, monkeys (*Cercopithecus aethiopis*) (Mukaratirwa *et al.* 2008) and domestic pigs (Matenga *et al.* 2006; Mukaratirwa & Foggin 1996) has previously been successful. Furthermore, they are known to hunt and feed on other vertebrates in the wild (Butynski 1982). Between 1966 and 1974, five baboons from the greater KNP area have been tested, with negative results (Marucci *et al.* 2009); an additional two have been tested since 2012 (La Grange & Mukaratirwa unpublished data). As in the case with bush pigs, the number of samples tested is limited and this precludes any assumptions that these species do not play a role in the epidemiology of the *Trichinella* spp. in southern Africa. Experimental infections by Mukaratirwa *et al.* (2008) have shown that baboons are highly susceptible to the parasite, with fatal cases, and this might be the case in the wild.

In the present study, a mixed natural infection of *T. nelsoni* and *Trichinella* T8 was confirmed in a leopard from a nature reserve in the west of the greater KNP area of South Africa.

Materials and methods

In July 2012, a fresh carcass of an adult male leopard (about 5 years old) was found in a riverine stretch in a nature reserve in the west of the greater KNP area (24°47′57.1′′S, 31°29′24.0′′E), and this was reported to the local state veterinary services. On post-mortem examination the animal showed signs of severe and generalised haemorrhages, including widespread distinct suggillations in the large muscle groups of the forelimbs and hindlimbs. Secondary rodenticide poisoning was identified as the most likely reason for the observed findings. As part of the post-mortem examination and sampling procedures, muscle specimens were collected for routine surveillance for *Trichinella* infection and submitted for testing to the Parasitology Laboratory, School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Durban. Muscle specimens that were collected consisted of sections of about 40 g, and were collected bilaterally from the *musculus masseter*, *musculus tricipitis brachii*, flexor muscle group of the antebrachium, *musculus intercostales*, *musculus diaphragmaticus*, *musculus quadriceps femoris* and the flexor muscle group of the lower hindlimbs, which were pooled as one source sample. Muscle samples were artificially digested as previously described (Nöckler & Kapel 2007) and examined under a stereo microscope for the detection of *Trichinella* larvae. Larvae observed in the digest were washed and used to infect Sprague-Dawley rats and the remaining muscle tissues were preserved in 2% sodium azide solution and submitted to the International *Trichinella* Reference Centre (ITRC) in Rome, Italy, for harvesting of additional larvae for species identification. A sample of the larvae was deposited in the ITRC in Rome, Italy (Isolate code ISS 4413).

Results

Initial testing by artificial digestion showed an infection intensity of three larvae per gram (lpg) in pooled muscle samples. In subsequent experimental infection of three Sprague-Dawley rats, only one rat established infection with an establishment of 702 lpg of muscle tissues. Three larvae were recovered from the muscle tissue submitted to ITRC and identified by a multiplex polymerase chain reaction (PCR) (Pozio & La Rosa 2003). One larva was identified as *T. nelsoni*, whilst the two remaining larvae were identified as *Trichinella* T8.

Discussion

According to Hayward *et al.* (2006), wild pigs and baboons are not frequently preyed upon by leopards, whilst other carnivorous species such as jackals, cheetahs, civets and genets are preyed on more frequently. Prey species composition of leopards reported by Le Roux and Skinner (1989) in the western greater KNP area included the common warthog, making up 4%, the chacma baboon (*Papio ursinus*) 1%, vervet monkeys 6%, small mammalian carnivores (African civets and genets) 2% and reptiles (leopard tortoise, Mozambique spitting cobra and rock monitor) 4%. Other authors have found various carnivorous animals of mammalian and reptile origin to represent 12% of all kills by leopards in north-eastern Namibia (Stander 1997), and large carnivores to represent 4% in the KNP (Bailey 1993). The predation of other carnivorous species by leopards, like the jackals that scavenge on the carcasses of other wild carnivores, suggests a possibly important source of *Trichinella* spp. infection.

The confirmation of a mixed infection in a leopard from South Africa may suggest that wild pigs play a lesser role in the natural epidemiology of *Trichinella* spp. in southern Africa, when compared to lions and hyenas. Other mammals that are more frequently preyed upon by leopards may act as better reservoirs for these *Trichinella* taxa. The absence of human infections from consumption of undercooked meat from wild pigs or products derived from wild pigs in southern Africa may support this.

The report of a mixed infection of two species of *Trichinella* in a leopard confirms the importance of the greater KNP area as a habitat of reservoirs for *Trichinella* species circulating in sylvatic carnivores of this region (La Grange *et al.* 2010). The confirmation of a mixed infection of *T. nelsoni* and *Trichinella* T8 in a leopard further confirms the sympatric status of these parasites, and a mixed infection of the same species has been reported in a lion in the KNP area (Marucci *et al.* 2009). Despite the low number of wild pigs tested from the KNP and adjacent reserves, studies have revealed a 2.5% infection rate in bush pigs from Kenya (Nelson *et al.* 1963), 4.0% from warthogs in Senegal (Gretillat & Chevallier 1970) and 4.3% in warthogs from Kenya, South Africa, Senegal and Tanzania (Sachs 1970). These studies all preceded the elucidation of the *Trichinella* taxon (Pozio *et al.* 1997) but later studies have implicated *T. britovi* and *T. nelsoni* in 23.0% and 7.0% of all infections of swine, respectively (Pozio 2005). Infections of swine from Kenya and Tanzania have most probably involved *T. nelsoni*, whilst those reported from Senegal are considered to be *T. britovi* (Mukaratirwa *et al.* 2013; Pozio 2007). In South Africa, however, warthogs and bush pigs may very well act as reservoirs for both *T. nelsoni* and *Trichinella* T8, since both have been previously found in other mammals from this region (Marucci *et al.* 2009; Mukaratirwa *et al.* 2013), despite the fact that the latter has never been isolated from wild pigs. The preliminary prevalence amongst leopards in KNP and surrounding areas, as documented in the present report, is much higher at 25% and this possibly suggests alternative or additional sources of infection for these predators, although the number of animals tested is limited. The fact that leopards have a tendency to avoid preying on wild pigs (Hayward *et al.* 2006) may support the aforementioned theory, but should be interpreted with caution since the limited data on *Trichinella* spp. infection in leopards and wild pigs in southern Africa do not provide conclusive evidence. Furthermore, the fact that leopards prefer to avoid wild pigs does not imply that leopards never prey on these species at all. Future research should include screening of other smaller carnivorous animals frequently preyed upon by leopards, and surveillance efforts should be intensified amongst wild Suidae to elucidate the role of each in the natural epidemiology of the various *Trichinella* species circulating in the KNP area.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contributions

L.J.L.G. (Department of Agriculture, Rural Development, Land and Environmental Affairs) conducted the initial acquisition and testing of samples, and is the main author of the manuscript. B.R. (Department of Agriculture, Rural Development, Land and Environmental Affairs) performed the post-mortem examination, collected the muscle samples and is co-author of the manuscript. S.M. (University of KwaZulu-Natal) conducted the experimental infection, dissection and testing of muscle samples of rats, submitted the sample to the ITRC and is the secondary author of the manuscript.

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