Journal of Helminthology

cambridge.org/jhl

Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga provinces of South Africa

S. Mukaratirwa[1], L.J. La Grange[1,2], M.P. Malatji[1], B. Reininghaus[2] and J. Lamb[1]

[1]School of Life Sciences, University of KwaZulu-Natal, Westville Campus, Durban 4000, South Africa and [2]Mpumalanga, Department of Agriculture, Rural Development, Land and Environmental Affairs, South Africa

Research Paper

Cite this article: Mukaratirwa S, La Grange LJ, Malatji MP, Reininghaus B, Lamb J (2019). Prevalence and molecular identification of *Trichinella* species isolated from wildlife originating from Limpopo and Mpumalanga provinces of South Africa. *Journal of Helminthology* **93**, 50–56. https://doi.org/10.1017/S0022149X17001079

Received: 29 August 2017
Accepted: 18 October 2017
First published online: 23 November 2017

Author for correspondence:
S. Mukaratirwa, E-mail: mukaratirwa@ukzn.ac.za

Abstract

Trichinella species are widely distributed on all continents with the exception of Antarctica, although the full spectrum of *Trichinella* species found in sub-Saharan African countries, and their hosts, has not been fully documented. This study was conducted to determine the prevalence of *Trichinella* in wildlife from the Greater Kruger National Park (GKNP) and adjacent areas located in the Limpopo and Mpumalanga provinces of South Africa, and to identify the species and/or genotypes of *Trichinella* larvae isolated from muscle tissues, using molecular techniques. A review of *Trichinella* spp. and their wildlife hosts reported during 1964–2011 was also conducted and the results were compared with our current study. Ninety samples representing 15 mammalian, two bird and three reptile species were screened for *Trichinella* infection during 2012–2016, using artificial digestion. Isolates detected were identified using a multiplex polymerase chain reaction (PCR) amplification of the internal transcriber spacers ITS1 and ITS2, and expansion segment V (ESV) regions of ribosomal DNA, followed by molecular analysis of the sequences. Twenty samples from seven wildlife species were positive for *Trichinella* spp. larvae, with an overall prevalence of 21.1% (20/90). The prevalence was higher in carnivores (18.9%, 18/90) than in omnivores (2.2%, 2/90). Analysis of sequences showed that eight of the isolates – two from spotted hyaena (*Crocuta crocuta*) (2/8), three from lion (*Panthera leo*) (3/13), one from leopard (*Panthera pardus*) (1/6), one from small spotted genet (*Genetta genetta*) (1/2) and one Nile monitor lizard (*Varanus niloticus*) (1/2) – conformed to *Trichinella zimbabwensis*. One isolate from a hyaena was grouped under the encapsulated species clade comprising *T. nelsoni* and genotype *Trichinella* T8 reported to be present in South Africa. This is the first report confirming natural infection by *T. zimbabwensis* in hyaena, leopard, genet and Nile monitor lizard, adding to the body of knowledge on the epidemiology of *Trichinella* infections in the Greater Kruger National Park of South Africa. Ten *Trichinella*-like larval isolates recovered after digestion from four wildlife species in this study (2012–2016) revealed inconclusive results due to DNA degradation resulting from poor storage or too few larvae for analysis, in comparison to 20 unidentified isolates from five wildlife species during the 1964–2011 period.

Introduction

Trichinellosis is an important zoonotic disease caused by the infectious nematodes of the genus *Trichinella* (Fu *et al.,* 2009; Gottstein *et al.,* 2009; Pozio *et al.,* 2009; Krivokapich *et al.,* 2012). The parasite belongs to the family Trichinellidae, phylum Nematoda, and there are currently nine encapsulated species and genotypes, namely *Trichinella britovi, T. murrelli, T. nativa, T. spiralis, T. nelsoni, T. patagoniensis, Trichinella* T6, *Trichinella* T8 and *Trichinella* T9, with three additional non-encapsulated species: *T. pseudospiralis*, *T. zimbabwensis* and *T. papuae* (Pozio & Zarlenga, 2013).

Trichinella spp. have a direct life cycle characterized by completing both intermediary and definitive stages in a single host (Pozio, 2007; Gottstein *et al.,* 2009; Pozio *et al.,* 2009). Unlike other nematodes, *Trichinella* spp. are also characterized by an infective first larval stage (L1) in contrast to the typical infective third-stage larvae (L3) found in most nematode genera (Gajadhar *et al.,* 2009; Pozio *et al.,* 2009). Vertebrates, including humans, are infected through the ingestion of raw or undercooked meat infected with *Trichinella* larvae in the muscle tissue (Gottstein *et al.,* 2009). Newborn larvae (NBL) are transported passively to the striated muscles (Dupouy-Camet, 2000; Gottstein *et al.,* 2009) via the host lymphatic and blood vessels (Gottstein *et al.,* 2009). Based on the findings by Gottstein *et al.* (2009), *Trichinella* larvae may remain viable in the nurse muscle for many years after encysting. This however, may depend on the *Trichinella* species/genotype and the host's immune response.

CAMBRIDGE UNIVERSITY PRESS

Cases of trichinellosis have been reported worldwide with the exception of Antarctica (Pozio *et al.,* 2009; Mukaratirwa *et al.,* 2013). *Trichinella zimbabwensis, T. britovi, T. nelsoni* and genotype T8 have been reported in sub-Saharan Africa and occur mainly in carnivorous and omnivorous sylvatics with *T. zimbabwensis* being the most prevalent species (Mukaratirwa *et al.,* 2013). According to Blaga *et al.* (2009), 10,000 cases of human trichinellosis have been reported globally, with an annual mortality rate as low as 0.2% (Pozio, 2007). Generally, animals remain asymptomatic and, in the absence of clinical disease manifestation, infection with *Trichinella* spp. is referred to as *Trichinella* infection rather than trichinellosis (Gottstein *et al.,* 2009).

Trichinella zimbabwensis, *T. nelsoni* and *Trichinella* T8 have been reported in wildlife from South Africa during epidemiological investigations in the Greater Kruger National Park (GKNP) and adjacent areas of the Limpopo and Mpumalanga provinces (Marucci *et al.,* 2009; La Grange *et al.,* 2010; Mukaratirwa *et al.,* 2013; La Grange *et al.,* 2014). The main reservoirs for *Trichinella* spp. in the Kruger National Park are carnivorous wildlife with scavenging and cannibalistic behaviour (Mukaratirwa *et al.,* 2013). The spotted hyaena (*Crocuta crocuta*) and the lion (*Panthera leo*) appear to be the major reservoirs for *Trichinella* infections as they currently have the highest documented prevalence in South Africa (Marucci *et al.,* 2009; Mukaratirwa *et al.,* 2013). Mixed infections of *T. nelsoni* and *Trichinella* T8 have been reported in both a lion and a leopard (Marucci *et al.,* 2009; La Grange *et al.,* 2014) and *T. zimbabwensis* has also been reported in a lion (La Grange *et al.,* 2010). To date, no report of human infections or cases involving domestic animals have been documented in South Africa, despite this country having the highest documented prevalence of *Trichinella* infections in wildlife in sub-Saharan Africa (Mukaratirwa *et al.,* 2013).

There is paucity of information on *Trichinella* infections in humans, domestic and wild animals in most of sub-Saharan Africa (Dupouy-Camet, 2000; Pozio, 2007) including South Africa. Hence, the aim of this study was to close the gap by determining the prevalence of *Trichinella* infection in convenience samples collected from wildlife species from the GKNP and identifying the *Trichinella* spp. larvae isolates using molecular techniques.

Materials and methods

Sample collection and processing

Muscle samples were conveniently collected from carcasses of wildlife, either culled from nature reserves, killed by hunters or animals that died of natural causes or as a result of vehicular accidents and/or poisoning in the GKNP, private nature reserves and towns neighbouring the GKNP in Limpopo and Mpumalanga provinces. Ninety samples representing 15 mammal, three reptile and two bird species were collected and tested during the period 2012–2016 (tables 1 and 2). Samples were digested as described by Nöckler & Kapel (2007) as a preliminary screening for the presence of *Trichinella* larvae in the muscle sample. From the positive samples, *Trichinella* larvae were collected in small vials containing 70% ethanol and later used for DNA extraction. Due to logistic constraints, some of the muscle samples were either frozen and kept for extended periods of time prior to testing or, in other instances, sample collection was delayed, resulting in larvae being degraded and not suitable for molecular analysis.

DNA extraction from Trichinella *larvae*

Genomic DNA was extracted from at least five larvae of each positive sample whenever possible, using the genomic DNA™ tissue mini-prep kit (Zymo Research Corporation, Irvine, California, USA) according to the manufacturer's instructions.

Polymerase chain reaction and sequencing

DNA was subjected to multiplex polymerase chain reaction (PCR) using primers ESVIF + ESVIR, ITS1AF + ITS1AR, ITS1BF + ITS1BR, ITS2AF + ITS2AR and ITS2BF + ITS2BR as described by Zarlenga *et al.* (1999) (table 3). The amplifications were performed in reactions of 50 µl volume containing 20 µl TopTaq master mix, 2 µl of each primer (forward and reverse) and 10 µl of DNA. Thermal cycling was carried out at 94°C for 3 min; followed by 40 cycles of 94°C for 1 min, 60°C for 1 min and 72°C for 2 min; followed by a final extension at 72°C for 7 min. Amplicons were separated by electrophoresis in 2% agarose gels, and visualized by staining with ethidium bromide. A laboratory-maintained reference strain of *T. zimbabwensis* was used as control. PCR amplification products of the expansion segment V (ESV) region were sent for sequencing by the Sanger dideoxy method at Inqaba Biotechnical Industries (Pty) Ltd, South Africa.

Data and molecular analysis

The number of wildlife species with positive isolates from digestion of muscle were tabulated and compared with the data reported in the period 1964–2011.

Sequence alignments were analysed using the maximum parsimony method in PAUP 4.0b10 (Swofford, 2002) and Bayesian inference as implemented in MrBayes 3.0b4 (Huelsenbeck & Ronquist, 2001). For parsimony analyses, starting trees were obtained by stepwise addition. The addition sequence was random, with one tree held at each step and with ten replicates. Node support was estimated using 1000 bootstrap replicates. Bayesian analyses were run using four Markov chains, sampling every 100 generations, for 500,000 generations, or until the standard deviation of the split frequencies was less than 0.01. The chains were heated with the temperature scaling factor $T = 0.02$. We discarded the first 2000 trees as burn-in, in each case having checked in a preliminary run that this was more than sufficient to achieve stationarity. Bayesian inference trees were presented, with node support indicated as Bayesian posterior probabilities and maximum parsimony bootstrap values.

Analyses included *T. zimbabwensis, T. nativa, T. papuae, T. spiralis, T. britovi, T. pseudospiralis,* genotype 12 and genotype T8 as in-groups and *Paratrichosoma* sp. and *Trichuris arvicolae* as outgroups. Individual pairwise genetic p-distances between the sequences were determined using MEGA6 (Tamura *et al.*, 2013).

Results

Prevalence of Trichinella *spp. from digestion*

Extrapolated data from previously published reports (1964–2011) were combined with the findings of this study (2012–2016) on the screening of *Trichinella* larvae and identification of *Trichinella* spp. in wildlife carnivores (table 1) and omnivores (table 2) from South Africa. Results show that *T. zimbabwensis* was the most prevalent species recorded to date. The species has been reported in five wildlife species (lion, hyaena, leopard (*Panthera*

https://doi.org/10.1017/S0022149X17001079 Published online by Cambridge University Press

Table 1. Wild carnivores from the greater Kruger National Park, South Africa, screened for *Trichinella* spp. larvae and the prevalence of *Trichinella* spp. in each species for the period 2012–2016 and previous studies (1964–2011).

	Current study (2012–2016)						Previous study (1964–2011)						
Animal species	No. positive/tested	Tz	Tn	T8	NID	Total prevalence (%)	No. positive/tested	Tz	Tn	T8	NID	Total prevalence (%)	References
Panthera leo	8/13	3	–	–	5	61.5	14*/85	1	3	4	6	16.5	Young & Kruger (1967), La Rosa & Pozio (2000), Marucci *et al.* (2009), La Grange *et al.* (2010)
Panthera pardus	2*†/6	1	1	1	0	33.3	0/1	–	–	–	–	NC	Young & Whyte (1975), Marucci *et al.* (2009)
Varanus niloticus	1/2	1	–	–	–	NC	0/0	–	–	–	–	NC	
Crocuta crocuta	5/8	2	–	–	3	62.5	12/18	–	–	1	11	66.7	Young & Kruger (1967); Marucci *et al.* (2009)
Lycaon pictus	0/4	–	–	–	–	0	0/0	–	–	–	–	NC	
Necrosyrtes monachus	0/1	–	–	–	–	NC	0/0	–	–	–	–	NC	
Manis temminckii	0/1	–	–	–	–	NC	0/0	–	–	–	–	NC	
Varanus albigularis	0/1	–	–	–	–	NC	0/0	–	–	–	–	NC	
Asio capensis	1/1	–	–	–	1	NC	0/0	–	–	–	–	NC	
Naja annulifera	0/1	–	–	–	–	NC	0/0	–	–	–	–	NC	
Felis silvestris lybica	1/1	–	–	–	1	NC	0/0	–	–	–	–	NC	
Canis mesomelas	0/1	–	–	–	–	NC	0/0	–	–	–	–	NC	
Canis adustus	0/0	–	–	–	–	NC	1/3	–	–	–	1	NC	Marucci *et al.* (2009)
Crocodylus niloticus	0/0	–	–	–	–	NC	16/43	16	–	–	–	37	La Grange *et al.* (2009, 2013)

Tz, *Trichinella zimbabwensis*; Tn, *Trichinella nelsoni*; T8, *Trichinella* genotype T8; NID, not identified; NC, not calculated due to sample size < 4.
*One animal represents a mixed infection of *Trichinella nelsoni* and *Trichinella* T8.
†Four animals reported by La Grange *et al.* (2014) included in present work.

Table 2. Wild omnivores from the greater Kruger National Park, South Africa, screened for *Trichinella* spp. larvae and the prevalence of *Trichinella* spp. in each species for the period 2012–2016 and previous studies (1964–2011).

	Current study (2012–2016)						Previous study (1964–2011)					
Animal species	No. positive/ tested	Tz	Tn	T8	NID	Total prevalence (%)	No positive/ tested	Tz	Tn	T8	NID	Total prevalence (%)	References
Civettictis civetta	0/1	–	–	–	NC	0	1/1	–	–	–	1	100	Young & Whyte (1975), Marucci *et al.* (2009)
Genetta genetta	1/2	1	–	–	NC	50	–	–	–	–	–	–	
Phacocoerus africanus	0/35*	–	–	–	0	0	0/12	–	–	–	–	0	Young & Kruger (1967); Marucci *et al.* (2009)
Mellivora capensis	0/2	–	–	–	NC	0	0/0	–	–	–	–	NC	
Papio ursinus	1/6	–	–	–	1	16.7	0/0	–	–	–	–	NC	
Chlorocebus pygerythrus	0/1	–	–	–	NC	0	0/0	–	–	–	–	NC	
Potamochoerus larvatus	0/2	–	–	–	NC	0	0/0	–	–	–	–	NC	
Mungos mungo	0/1	–	–	–	NC	0	0/2	–	–	–	–	NC	Young & Whyte (1975), Marucci *et al.* (2009)
Praomys natalensis	0/0	–	–	–	NC	0	1/44	–	–	–	1	0.02	Young & Kruger (1967); Marucci *et al.* (2009)

Tz, *Trichinella zimbabwensis*; Tn, *Trichinella nelsoni*; T8, *Trichinella* genotype T8; NC, not calculated due to sample size < 4; NID, not identified.
*17 Warthogs reported by La Grange *et al.* (2014) included in present work.

Table 3. Nucleotide sequences amplified during multiplex PCR with respective forward and reverse oligonucleotide sequences.

Amplified sequence	Oligonucleotide sequence
ESVF	5′-GTTCCATGRGAACAGCAGT-3′
ESVR	5′-CGAAAACATAGCACAACTGC-3′
ITS1AF	5′-GCTACATCCTTTTGATCTGTT-3′
ITS1AR	5′-AGACACAATATCAACCACAGTACA-3′
ITS1BF	5′-GCGGAAGGATCATTATCGTGTA-3′
ITS1BR	5′-TGGATTACAAAGAAAACCATCACT-3′
ITS2AF	5′-GTGAGCGTAATAAAGGTGCAG-3′
ITS2AR	5′-TTCATCACACATCTTCCACTA-3′
ITS2BF	5′-CAATTGAAAACCGCTTAGCGTGTTT-3′
ITS2BR	5′-TGATCTGAGGTCGACATTTCC-3′

pardus), genet (*Genetta genetta*) and Nile monitor lizard (*Varanus niloticus*)) (tables 1 and 2). *Trichinella nelsoni* was reported in a leopard and *Trichinella* T8 was reported once in a leopard as a mixed infection with *T. nelsoni* (table 1). A similar mixed infection was also reported previously in a lion (Marucci *et al.*, 2009) (table 1).

Ninety muscle samples from 20 wildlife species (15 mammals, 3 reptiles and 2 birds) were screened. Twenty samples from seven wildlife species were positive for *Trichinella* spp. through digestion (six mammals and one reptile) (tables 1 and 2) and *Trichinella* prevalence was higher in carnivores (18.9%, 18/90) than in omnivores (2.2%, 2/90). In the period from 1964–2011, 45 samples from nine wildlife species were positive for *Trichinella* spp. through digestion (seven mammals and one reptile) (table 1) with a prevalence of 21.4% (45/210). The prevalence of *Trichinella* was 20.5% (43/210) in carnivores and 0.9% (2/210) in omnivores.

Multiplex PCR analysis and phylogenetic analysis

Electrophoresis of multiplex PCR amplicons of putative *Trichinella* isolates produced two general types of amplification pattern. One of these contained two main bands, with sizes of approximately 270 and 350 bp, and was shared by isolates from hyaena, lion, monitor lizard and a *T. zimbabwensis* laboratory reference strain. This is generally consistent with the identification of these isolates as *T. zimbabwensis*. A contrasting amplification pattern, comprising two smaller main bands of approximately 150 and 250 nucleotides, was shared by a genet, lion and leopard. These isolates remain unidentified. It is interesting that a similar main banding pattern (bands of 127 and 253 nucleotides) was exhibited by *Trichinella* isolate T3 (Zarlenga *et al.*, 1999). Isolates from the African wildcat (*Felis silvestris lybica*), Chacma baboon (*Papio ursinus*) and Marsh owl (*Asio capensis*) did not produce amplification patterns.

Alignment of ESV DNA sequences was created, based on 105 nucleotides (fig. 1) as the sequences contained areas of microsatellite repeats, and did not all yield good-quality sequence for the entire length. The alignment resolved phylogenetic relationships with support values as shown in fig. 1.

Trichinella isolates formed a monophyletic clade (A) with reference to the outgroups (fig. 1). There was strong support for

https://doi.org/10.1017/S0022149X17001079 Published online by Cambridge University Press

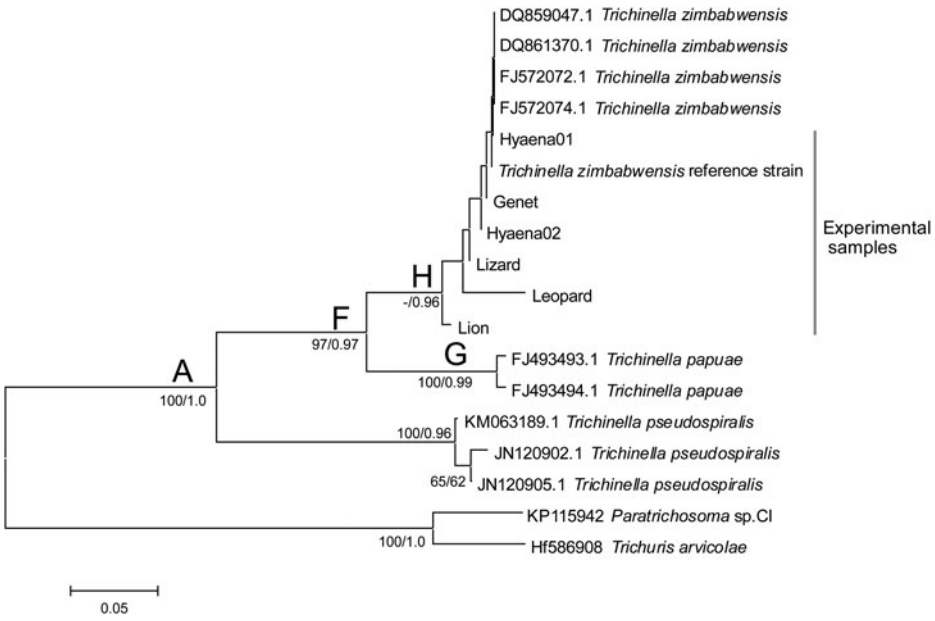

Fig. 1. Bayesian inference tree based on 105 nucleotides of the ESV DNA region depicting relationships between experimental samples and sequences downloaded from GenBank (National Center for Biotechnology Information). Nodal support from maximum parsimony and Bayesian analyses is shown in that order.

clade (F), which included *T. papuae* and *T. zimbabwensis* from GenBank and experimental isolates from our study. The *T. papuae* clade (G) was strongly supported and sister to *T. zimbabwensis* (fig. 1), a well-supported clade (H) comprising GenBank *T. zimbabwensis* samples and experimental *Trichinella* isolates from two hyaenas, lion, leopard, monitor lizard, genet and *T. zimbabwensis* reference isolate. Based on the phylogenetic species concept (Cracraft, 1983), these experimental isolates are *T. zimbabwensis.* Further, genetic distances separating these isolates from the *T. zimbabwensis* reference sample are small (0.00–0.02), and consistent with those separating other GenBank samples of *T. zimbabwensis* from the reference strain (also 0.00–0.02). This is consistent with the identification of the experimental isolates as *T. zimbabwensis* based on the genetic species concept (Baker & Bradley, 2006). In contrast, and as would be expected, genetic distances between *T. zimbabwensis* isolates and other species of *Trichinella* are considerably higher, consistent with a greater level of taxonomic separation from *T. papuae* (0.11–0.16), *T. pseudospiralis* (0.26–0.31), *T. nativa* (0.36–0.39), *T. britovi* (0.38–0.41) and *T. spiralis* (0.43–0.47).

A third hyaena isolate was present in a strongly supported unresolved clade (D) containing GenBank samples of the encapsulated species *T. spiralis*, *T. britovi* and *T. nativa* (figure not shown), although it could not be identified to species level.

Distribution and prevalence of Trichinella *spp. in wildlife species*

Trichinella spp. larvae were isolated from eight lions during the course of this study (8/13, prevalence 61.5%). Of these, three were *T. zimbabwensis* (37.5%) and five isolates were unidentified to species level (table 1). Previous studies (1964–2011) reported isolates from 14 lions (14/85, prevalence 16.5%). Of these, one was *T. zimbabwensis*, three were *T. nelsoni*, four were *Trichinella* T8 and six were unidentified to species level (table 1).

Of eight hyaenas tested in this study, five were found to harbour *Trichinella* spp. larvae (5/8, prevalence 62.5%) (table 1). Based on ESV sequence analysis, two were *T. zimbabwensis* (40%) (fig. 1), and a third fell under the encapsulated clade, although it was not possible to identify this isolate to species level. The other two isolates were also unidentified to species level. Twelve isolates from hyaena have been reported from 18 screened during the period from 1964 to 2011 (12/18, prevalence 66.7%). Of these, one was *Trichinella* T8 and the remaining 11 were unidentified.

Two *Trichinella* spp. isolates were recovered from six screened leopards (2/6, prevalence 33.3%). One leopard had a mixed infection of *T. nelsoni* and T8, the results of which were previously published (see La Grange *et al.*, 2014). The remaining isolate from this study was *T. zimbabwensis.* This is the first report of a natural infection with *T. zimbabwensis* in this host. From the previous studies (1964–2011) only one leopard was screened and was found to be negative (table 1).

Two Nile monitor lizards were screened in our study and one was positive for *T. zimbabwensis* (1/2, prevalence 50%). Isolates from a single African wildcat in this study and from a side-striped jackal (*Canis adustus*) in the period (1964–2011) were unidentified.

Of the two small spotted genets tested, only one (1/2, prevalence 50%) tested positive. The isolate was identified as *T. zimbabwensis* and this is the first report of a natural infection with *T. zimbabwensis* in this host.

Six Chacma baboons were screened during the course of this study; one was positive (1/6, prevalence 16.7%) and a single larva recovered. The sample was in an advanced state of autolysis and the identification to species level was unresolved.

Samples from one African wildcat and one Marsh owl were positive from screening. Only a few larvae were detected and, similar to that of baboon, the samples were in an advanced state of autolysis and no species identification was possible.

Discussion

Results from this study are consistent with the commonly accepted postulate (Mukaratirwa *et al.*, 2013) that the most important route for *Trichinella* transmission in wild animals

https://doi.org/10.1017/S0022149X17001079 Published online by Cambridge University Press

appears to be via predation, cannibalism and scavenging. In a review by Mukaratirwa *et al.* (2013), *Trichinella* spp. prevalence was reported to be high in wild carnivores, which is consistent with results of the present study, which revealed a high prevalence in carnivores with predatory and scavenging behaviour compared to omnivorous animals.

The ability of *T. zimbabwensis* to infect mammalian hosts has been demonstrated in several experimental studies (Mukaratirwa & Foggin, 1999; Pozio *et al.,* 2004; Mukaratirwa *et al.,* 2008) and proven to occur in nature (La Grange *et al*., 2010). This species has been documented in Nile crocodiles of Zimbabwe, Mozambique, Ethiopia and South Africa, in Nile monitor lizards of Zimbabwe and in a lion from the Kruger National Park, South Africa (Mukaratirwa & Foggin, 1999; Pozio *et al.,* 2002; La Grange *et al.,* 2009, 2010, 2013). Our results represent the second report of natural infection with *T. zimbabwensis* in a lion and this confirms the previous report by La Grange *et al.* (2010) that the lion is an exceptional host for all three *Trichinella* taxa (*Trichinella* T8, *T. nelsoni* and *T. zimbabwensis*) circulating in South Africa (La Grange *et al.,* 2010).

Results from this study confirm that hyaenas are equally important hosts for at least two *Trichinella* taxa known to circulate in South Africa. Mukaratirwa *et al.* (2013) suggested the existence of a maintenance cycle for *T. nelsoni* and *Trichinella* T8 between lions and hyaenas. Results from this study suggest a similar maintenance cycle for *T. zimbabwensis* between these two carnivorous species. One isolate formed a well-supported association with a clade of encapsulated species and was unidentified to species level. Most likely this isolate was *T. nelsoni* or *Trichinella* T8, since these are the only two encapsulated species to have been reported in lions and hyaenas in South Africa to date (Marucci *et al.,* 2009; Mukaratirwa *et al.,* 2013).

An important finding from this study is that of *T. zimbabwensis* infection in the small spotted genet. Lariviere & Calzada (2001) reported the diet of the small spotted genet to be euryphagous. The diet of this opportunist omnivore consists of small mammals, amphibians, reptiles, fruits and birds (Lariviere & Calzada, 2001) and the genet may have acquired infection from feeding on infected small mammals, such as rodents or reptiles.

Mukaratirwa *et al.* (2013) postulated that carnivorous reptiles from the families Crocodylidae and Varanidae are likely to be the main reservoir hosts for *T. zimbabwensis*. Natural *T. zimbabwensis* infection has been reported in Nile crocodiles of South Africa (La Grange *et al.,* 2009, 2013) and this study reports for the first time a natural infection of *T. zimbabwensis* in a Nile monitor lizard from South Africa. Results from reports to date show a high prevalence of *Trichinella* spp. in carnivores with cannibalistic and scavenging behaviour (Mukaratirwa *et al.,* 2013). This study also reports the occurrence of *T. zimbabwensis* in a leopard for the first time.

Trichinella sp. larvae were detected for the first time in a baboon, although with a significantly low number of larvae. Species identification was not possible due to the small number of larvae and degradation of larval DNA. For the first time, a *Trichinella* spp.-like infection was reported in a bird from South Africa. *Trichinella pseudospiralis* is the only *Trichinella* taxon known to infect birds and it has been reported in birds and mammals from Asia, North America, Europe and Tasmania (Pozio & Murrell, 2006). This parasite species has not been reported in Africa and, despite preliminary data from this study suggesting the existence of this parasite in South Africa, the absence of conclusive molecular evidence precludes a definitive report.

Previous reports of natural infections involving *T. zimbabwensis* may suggest a propensity of this parasite species towards infecting reptiles, but results from this study clearly show that *T. zimbabwensis* infects a variety of ecto- and endothermic host species indiscriminately. Additionally, previous reports of a lion (La Grange *et al.,* 2010) and the current report of spotted hyena, leopard, lion and a small spotted genet naturally infected with *T. zimbabwensis* confirm previous suggestions of the significant epidemiological role of mammals in the parasite epidemiology (La Grange *et al.,* 2010; Mukaratirwa *et al.,* 2013).

The infection in a small spotted genet may also suggest the existence of a large biomass of this parasite maintained in a number of smaller rodent and/or reptile species. Similarly, the infection in a leopard suggests that smaller carnivores, such as the genet, are frequently infected in nature and supports the previous hypothesis. Leopards predate small carnivorous mammals (Hayward *et al.,* 2006) and their potential to serve as sources of infection to leopards has previously been alluded to (La Grange *et al.,* 2014). This is a cause of concern from a veterinary public health perspective, since the potential risk of transmission of the parasite from the natural sylvatic cycle to domestic animals can be through rodent infestations.

The presence of *Trichinella* spp. in at least four different omnivorous species in GKNP certainly suggests that they act as maintenance hosts, although results from this study together with reports by Young & Whyte (1975) and Young & Kruger (1967) represent the only four cases of *Trichinella* infections reported in omnivores in the GKNP and adjacent areas.

Epidemiological investigations on *Trichinella* species have been carried out mostly on wild animals from the GKNP and surrounding areas in South Africa. More surveys aimed at elucidating the prevalence and species richness of this parasite genus in the rest of South Africa are required. Such surveys will certainly prove invaluable in adding to the body of knowledge on *Trichinella*. However, more importantly, they will prove crucial in determining the risk of human infection, which will inevitably increase alongside the expansion of the game industry, population growth and the search for alternative food sources to ensure food security for the country's inhabitants.

Acknowledgements. Our sincere appreciation to the Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs for logistical and financial support provided through the Chief Directorate of Veterinary Services; Dr J.P. Raath and staff of Wildlifevets.com and Wildlife Pharmaceuticals for financial, logistical and technical support.

Financial support. Financial support was provided by the National Research Foundation of South Africa from the Incentive for Rated Researchers scheme for S.M. for the laboratory work, and by the Chief Directorate of Veterinary Services, Mpumalanga Department of Agriculture, Rural Development, Land and Environmental Affairs and Wildlife Pharmaceuticals for collection of samples.

Conflict of interest. None.

References

Baker RJ and Bradley RD (2006) Speciation in mammals and the genetic species concept. *Journal of Mammalogy* **87**, 643–662.

Blaga R, Gherman C, Cozma V, Zocevic A, Pozio E and Boireau P (2009) *Trichinella* species circulating among wild and domestic animals in Romania. *Veterinary Parasitology* **159**, 218–221.

Cracraft J (1983) Species concept and speciation analysis. *Current Ornithology* **1**, 159–187.

Dupouy-Camet J (2000) Trichinellosis: a worldwide zoonosis. *Veterinary Parasitology* **93**, 191–200.

https://doi.org/10.1017/S0022149X17001079 Published online by Cambridge University Press

Fu BQ, Liu MY, Yao CY, Li WH, Li YG, Wanga YH, Wub XP, Zhang DL, Cai XP, Blaga R and Boireau P (2009) Species identification of *Trichinella* isolates from China. *Veterinary Parasitology* **159**, 214–217.

Gajadhar AA, Pozio E, Gamble HR, Nöckler K, Maddox-Hyttel C, Forbes LB, Vallee I, Rossi P, Marinculic A and Boireau P (2009) *Trichinella* diagnostics and control: mandatory and best practices for ensuring food safety. *Veterinary Parasitology* **159**, 197–205.

Gottstein B, Pozio E and Nöckler K (2009) Epidemiology, diagnosis, treatment, and control of trichinellosis. *Clinical Microbiology Review* **22**, 127–145.

Hayward MW, Henschel P, O'Brien J, Hofmeyr M, Balme G and Kerley GIH (2006) Prey preferences of the leopard (*Panthera pardus*). *Journal of Zoology* **270**, 298–313.

Huelsenbeck JP and Ronquist F (2001) MrBayes: a Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755.

Krivokapich SJ, Pozio E, Gatti GM, Gonzales Prous CL, Ribicich M, Marucci G, La Rosa C and Confalonieri V (2012) *Trichinella patagoniensis* n. sp. (Nematoda), a new encapsulated species infecting carnivorous mammals in South America. *International Journal for Parasitology* **42**, 903–910.

La Grange LJ, Marucci G and Pozio E (2009) *Trichinella zimbabwensis* in wild Nile crocodiles (*Crocodylus niloticus*) of South Africa. *Veterinary Parasitology* **161**, 88–91.

La Grange LJ, Marucci G and Pozio E (2010) *Trichinella zimbabwensis* in a naturally infected mammal. *Journal of Helminthology* **84**, 35–38.

La Grange LJ, Govender D and Mukaratirwa S (2013) The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa. *Journal of Helminthology* **87**, 91–96.

La Grange LJ, Reininghaus B and Mukaratirwa S (2014) 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. *Onderstepoort Journal of Veterinary Research* **81**, 8–10.

Lariviere S and Calzada J (2001) Mammalian species, *Genetta genetta*. *American Society of Mammalogists* **680**, 1–6.

La Rosa G and Pozio E (2000) Molecular investigation of African isolates for *Trichinella* reveals genetic polymorphism in *Trichinella nelson*. *International Journal for Parasitology* **30**, 663–667.

Marucci G, La Grange LJ, La Rosa G and Pozio E (2009) *Trichinella nelsoni* and *Trichinella* T8 mixed infection in a lion (*Panthera leo*) of the Kruger National Park (South Africa). *Veterinary Parasitology* **159**, 225–228.

Mukaratirwa S and Foggin CM (1999) Infectivity of *Trichinella* sp. isolated from *Crocodylus niloticus* to the indigenous Zimbabwean pig (Mukota). *International Journal for Parasitology* **29**, 1129–1131.

Mukaratirwa S, Dzoma BM, Matenga E, Ruziwa SD, Sacchi L and Pozio E (2008) Experimental infections of baboons (*Papio* spp.) and vervet monkeys (*Cercopithecus aethiops*) with *Trichinella zimbabwensis* and successful treatment with ivermectin. *Onderstepoort Journal of Veterinary Research* **188**, 181–188.

Mukaratirwa S, La Grange L and Pfukenyi DM (2013) *Trichinella* infections in animals and humans in sub-Saharan Africa: a review. *Acta Tropica* **125**, 82–89.

Nöckler K and Kapel CMO (2007) Detection and surveillance for *Trichinella*: meat inspection hygiene, and legislation. pp. 69–97 *in* Dupouy-Camet J and Murrell KD (*Eds*) *FAO/WHO/OIE guidelines for the surveillance, management, prevention and control of trichinellosis*. Paris, World Organisation for Animal Health Press.

Pozio E (2007) World distribution of *Trichinella* spp. infections in animals and humans. *Veterinary Parasitology* **149**, 3–21.

Pozio E and Murrell KD (2006) Systematics and epidemiology of *Trichinella*. *Advances in Parasitology* **63**, 371–445.

Pozio E and Zarlenga DS (2013) Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. *International Journal for Parasitology* **35**, 1191–1204.

Pozio E, Foggin CM, Maruccia G, La Rosa G, Sacchi L, Corona S, Rossi P and Mukaratirwa S (2002) *Trichinella zimbabwensis* n. sp. (Nematoda), a new non-encapsulated species from crocodiles (*Crocodylus niloticus*) in Zimbabwe also infecting mammals. *International Journal for Parasitology* **32**, 1787–1799.

Pozio E, Marucci G and Casulli A (2004) *Trichinella papuae* and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles. *Parasitology* **128**, 333–342.

Pozio E, Hoberg E, La Rosa G and Zarlenga DS (2009) Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. *Infection, Genetics and Evolution* **9**, 606–616.

Swofford DL (2002) *Paup*: phylogenetic analysis using parsimony (*and other methods), version 4.0b10*. Sunderland, Massachusetts, Sinauer Associates.

Tamura K, Stecher G, Peterson D, Filipski A and Kumar S (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology and Evolution* **30**, 2725–2729.

Young E and Kruger SP (1967) *Trichinella spiralis* (Owen, 1935) Railliet 1895, infestation of wild carnivores and rodents in South Africa. *Journal of the South Africa Veterinary Association* **38**, 441–443.

Young E and Whyte IJ (1975) Trichinosis (*Trichinella spiralis* infestations) in wild animals of the Kruger National Park. *Journal of the South Africa Veterinary Association* **6**, 233–234.

Zarlenga DS, Chute MB, Martin A and Kapel CMO (1999) A multiplex PCR for unequivocal differentiation of all encapsulated and non-encapsulated genotypes of *Trichinella*. *International Journal for Parasitology* **29**, 1859–1867.