

The occurrence of *Trichinella zimbabwensis* in naturally infected wild crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa

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Abstract

Trichinella zimbabwensis has been found naturally infecting crocodiles (*Crocodylus niloticus*) in Zimbabwe, Mozambique, Ethiopia and South Africa, as well as monitor lizards (*Varanus niloticus*) in Zimbabwe. The reports on natural infections were mostly accidental rather than structured surveys and involved very few animals. Previous surveillance studies in South Africa reported a 38.5% prevalence of *T. zimbabwensis* among wild crocodiles tested from the Mpumalanga province and Kruger National Park (KNP). No studies have been conducted to date on the geographical distribution and occurrence of *T. zimbabwensis* in wild crocodiles and varans in countries in southern Africa. Recent outbreaks of pansteatitis in crocodile populations of the KNP, South Africa, provided an opportunity to conduct a more structured survey aimed at elucidating the occurrence and distribution of *T. zimbabwensis* in culled wild crocodile populations within the KNP. Results from this study showed that *T. zimbabwensis* occurred in 10 out of 12 culled crocodiles from the KNP. The results also showed that the natural distribution of *T. zimbabwensis* in crocodiles includes all the major river systems in the KNP. The predilection sites of larvae in muscles followed a different pattern in naturally infected crocodiles compared to observations in experimentally infected mammalian hosts.

Introduction

The discovery of the non-encapsulated *Trichinella zimbabwensis* in crocodiles from commercial farms in Zimbabwe in 1995 and the descriptions thereof (Foggin *et al.*, 1997; Mukaratirwa & Foggin, 1999; Pozio *et al.*, 2002)

has led to several studies that include the phylogeny, ecological characteristics and geographic history of the species (Pozio & Murrell, 2006), but also specifically the host range and extent of geographical occurrence in Africa. These studies have shown that *T. zimbabwensis* naturally infects crocodiles in Zimbabwe, Mozambique, Ethiopia and South Africa (Pozio *et al.*, 2007; La Grange *et al.*, 2009), as well as Nile monitor lizards (*Varanus niloticus*) in Zimbabwe (Pozio *et al.*, 2007). South African surveillance studies reported a 38.5% prevalence of

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T. zimbabweensis among wild crocodiles from the Mpumalanga province and Kruger National Park (KNP) (La Grange *et al.*, 2010). Despite experimental studies demonstrating the ability of *T. zimbabweensis* to infect mammalian hosts including rodents, pigs, carnivores and primates (Mukaratirwa & Foggin, 1999; Pozio *et al.*, 2004; Mukaratirwa *et al.*, 2008) there has only been a single report of a naturally infected mammal to date (La Grange *et al.*, 2010) and the continued surveillance of other potential natural host species is vital in order to fully unravel the epidemiology of this parasite.

A recent survey in KNP to determine the extent of pansteatitis in crocodiles additionally provided an opportunity to establish the occurrence and distribution of *T. zimbabweensis* in wild Nile crocodiles representing the whole of KNP and to evaluate muscle predilection patterns in naturally infected crocodiles. Studies on different *Trichinella* species in various mammalian hosts have shown muscle predilection to be influenced by host and parasite species characteristics as well as infection intensity and age (Serrano *et al.*, 1999; Kapel *et al.*, 2005). Host characteristics appear to be the most important determinant for predilection (Kapel *et al.*, 2005). Predilection patterns of *T. zimbabweensis* in red foxes (*Vulpes vulpes*) and other species revealed little deviation from that of other *Trichinella* species in the same host (Mukaratirwa & Foggin, 1999; Hurníková *et al.*, 2004) and similarly insignificant deviations were observed between *T. spiralis*, *T. britovi*, *T. nativa* and *T. pseudospiralis* infections in pigs, wild boars and horses (Kapel *et al.*, 2005). However, *T. zimbabweensis* has a proven propensity in nature towards infecting reptilian hosts and, more specifically, Nile crocodiles (*Crocodylus niloticus*) and Nile monitor lizards (*V. niloticus*) (Pozio *et al.*, 2007; La Grange *et al.*, 2009), whereas the encapsulated taxa reported in Africa, *T. britovi*, *T. nelsoni* and *Trichinella* T8, only infect mammalian hosts (Pozio *et al.*, 1997, 2005; Marucci *et al.*, 2009). The importance of host characteristics, natural infection patterns of *T. zimbabweensis* and the physiological and anatomical differences between mammals and reptiles provides the incentive for studies aimed at elucidating predilection patterns of this species to reptilian hosts. The aim of this study was to determine the occurrence, distribution and predilection patterns of *T. zimbabweensis* muscle larvae in naturally infected Nile crocodiles from the KNP, South Africa.

Materials and methods

Source of crocodiles

Following the deaths of more than 300 crocodiles attributed to pansteatitis in the Olifants and Letaba rivers near the western border of the KNP, South Africa, during 2008–2010, a survey of the disease in crocodiles from KNP perennial rivers was conducted in July 2010. A total of 12 Nile crocodiles from the five perennial rivers that originate outside the KNP, one large seasonal river (Shingwedzi River) and Shiloweni dam on an internal stream, were lethally sampled (fig. 1). Four crocodiles were also obtained in February 2011 from mine tailing dams in the Phalaborwa area, approximately 40 km upstream from where the crocodile deaths were detected

(fig. 1). Larger crocodiles (>2.5 m in length) were targeted for the survey as they were deemed to have a greater chance of developing pansteatitis as a result of their propensity towards cannibalism and the increased amounts of food consumed compared to smaller or younger animals. This criterion also favoured the *Trichinella* surveillance as larger and older animals generally have a greater chance of becoming infected, for the same reasons. All animals were humanely slaughtered by means of firing a free projectile through the brain with a large-calibre rifle before full necropsies were conducted.

Detection of larvae in muscles

During necropsy, muscle samples were collected from the head (base of tongue and caudal pterygoid), neck (sternomastoid), back (longissimus complex), internal intercostals, anterior legs (triceps brachii), posterior legs (external tibial flexor) and tail (longissimus caudalis and ilioischiodalis). Additional samples were collected superficially from the lateral aspects of the tail base. These additional samples were collected bearing in mind that a biopsy sample would primarily consist of superficial musculature and the aim of this was to determine whether the superficial musculature could be deemed suitable for the detection of *T. zimbabweensis*. Fifty grams of muscle tissue were collected from each of the muscle groups. A total amount of 100 g of muscle tissue from the various muscle groups for each animal was pooled together for digestion. The muscle tissue was artificially digested as described by Nöckler & Kapel (2007) to determine the presence of *Trichinella* sp. first-stage larvae. After digestion, larvae from each of the positive animals were harvested, preserved in absolute ethyl alcohol and a proportion of larvae from each animal was referred to the International *Trichinella* Reference Centre in Rome, Italy, for species identification. From the remaining samples, 25 g from each of the muscle groups from positive animals were tested individually to determine intensity of infection in each individual muscle or group of muscles.

Data analysis

The occurrence of infection was calculated as the number of crocodiles positive for *Trichinella* sp. first-stage larvae in muscle divided by the total number of crocodiles tested and expressed as a percentage. Intensity of infection was quantified by the number of first-stage larvae in 1 g (lpg) of muscle, muscle group or tongue. Analysis of variance was used to determine the differences in the mean intensity of infection among the different muscles/muscle groups and the level of significance was set at $P \leq 0.05$.

Results and discussion

Of the 12 crocodiles originating from KNP, ten tested positive for *Trichinella* sp. muscle larvae, and only one of the four animals originating outside KNP, which were donated for the survey, tested positive (table 1). All the

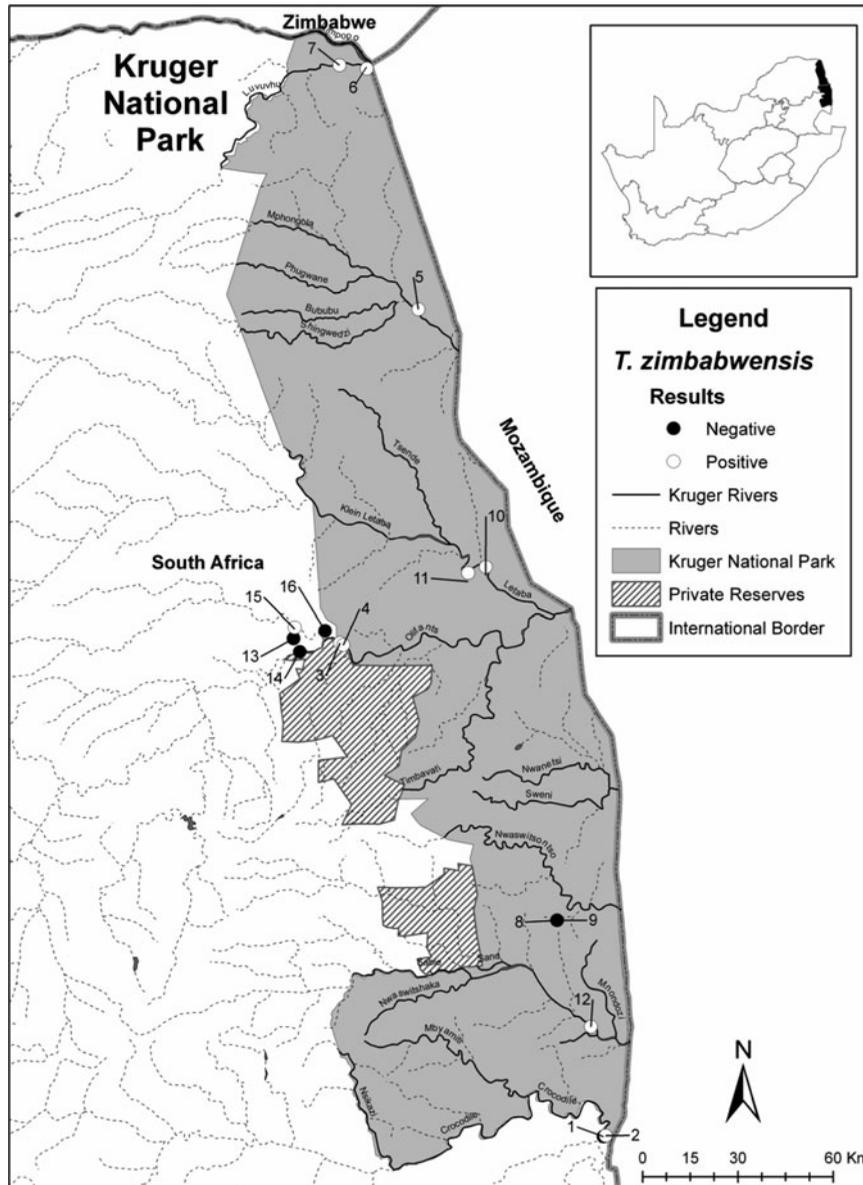


Fig. 1. Map of Kruger National Park (KNP) showing major river systems and the localities where crocodiles (*Crocodylus niloticus*) were culled, and their status with regard to *Trichinella zimbabwensis* infection. 1 and 2, Ressano Garcia weir, Crocodile River; 3 and 4, Mamba weir, Olifants River; 5, Kanidood dam, Shingwedzi River; 6, Old Pafuri picnic spot, Luvuvhu River; 7, bridge, Luvuvhu River; 8 and 9, internal catchment, Shiloweni dam; 10, Engelhardt dam, Letaba River; 11, Hlanganini mouth, Letaba River; 12, Lower Sabie weir, Sabie River; 13, Selati dam, Phalaborwa; 14, Phalaborwa barrage; 15, Van Ryssen dam, Phalaborwa; 16, Tailings dam, Phalaborwa Mining Company.

specimens of *Trichinella* sp. muscle larvae were sent to the International *Trichinella* Reference Centre for species identification and were confirmed as *T. zimbabwensis*. Larval burdens in pooled samples from each crocodile ranged from 0.1lpg of muscle to 36lpg, with a mean larval burden of 4.3lpg. The intensities of infection for individual muscles/group of muscles are shown in table 1.

The pterygoid muscle harboured the highest mean number of larvae (2.68 lpg) followed by the triceps muscle

(1.99 lpg). An interesting observation was that the dorsal tail musculature appeared to harbour a higher mean number of larvae (1.04 lpg) than ventral muscles (0.56 lpg). Similarly, the superficial musculature of the dorsal tail on average harboured more larvae (2.92 lpg) than the deeper muscles (2.25 lpg) and this trend was consistently observed in all but three of the positive animals. The results from the analysis of variance revealed no significant differences in the intensity of infection among the muscles sampled ($F = 0.45$,

Table 1. Intensity of infection of *Trichinella zimbabwensis* larvae in muscles/muscle groups of naturally infected crocodiles (*Crocodylus niloticus*) from the Kruger National Park, South Africa.

| Location | Sex | Length (m) | Intensity of infection (lpg) | | | | | | | | | | | |
|--------------------|--------|------------|------------------------------|--------|-------------|----------|-------------|-------------|----------|-------------|-------------|--------|--------------|-------------|
| | | | Pooled | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Crocodile River | Female | 2.92 | 4.5 | 1.4 | 3.6 | 1.6 | 5.6* | 0.5 | 0.3 | 1.8 | 1.6 | 0.6 | 2.2 | 3.5 |
| Olifants River | Male | 3.73 | 1.5 | 0.4 | 0.4 | 0.4 | 0.6 | 1.3 | 0.6 | 1.8* | 0.5 | 0.4 | 0.9 | 0.2 |
| Olifants River | Male | 3.23 | 1.2 | 0.2 | 1.3 | 0.4 | 1.2 | 0.5 | 0.08 | 1.1 | 0.8 | 0.3 | 1.4* | 0.4 |
| Shingwedzi River | Male | 3.13 | 0.6 | 0.2 | 0.3 | 0.4 | 0.5 | 0.4 | 0.2 | 0.2 | 0.5 | 0.2 | 0.6* | 0.2 |
| Levuvhu River | Male | 3.23 | 0.7 | 0.1 | 0.6 | 0.4 | 0.8* | 0.4 | 0.2 | 0.6 | 0.2 | 0.2 | 0 | 0.4 |
| Levuvhu River | Male | 3.85 | 0.8 | 0.2 | 0.2 | 0.08 | 0.08 | 0.8 | 0.2 | 0.3 | 0.3 | 0.2 | 0.1 | 0.9* |
| Internal catchment | Female | 3.4 | 36 | 6.2 | 21.9 | 6.2 | 11.0 | 12.4 | 7.1 | 10.7 | 6.0 | 3.3 | 24.8* | 18.1 |
| Letaba River | Female | 2.8 | 0.1 | 0.04 | 0 | 0.04 | 0 | 0 | 0.04 | 0 | 0 | 0 | 0.3* | 0 |
| Letaba River | Female | 2.8 | 0.3 | 0.2 | 0.3 | 0.2 | 0.3 | 0.6* | 0.2 | 0.2 | 0.6* | 0.2 | 0.3 | 0 |
| Sabie River | Male | 4.6 | 1.3 | 0.4 | 0.5 | 0.7 | 1.6* | 1.4 | 0.2 | 0.3 | 0.5 | 0.6 | 1.3 | 0.8 |
| Van Ryissen dam | Female | 1.90 | 0.1 | 0 | 0.4* | 0.1 | 0.2 | 0.3 | 0.2 | 0 | 0.4* | 0.2 | 0.2 | 0.2 |
| N = 11 | | | | | | | | | | | | | | |
| Mean ± SE | | | | 0.85 ± | 2.68 ± | 0.96 ± | 1.99 ± | 1.69 ± | 0.85 ± | 1.55 ± | 1.04 ± | 0.56 ± | 2.92 ± | 2.25 ± |
| | | | | 0.55 | 1.95 | 0.54 | 1.02 | 1.08 | 0.63 | 0.94 | 0.51 | 0.28 | 2.20 | 1.61 |
| Median | | | | 0.2 | 0.4 | 0.4 | 0.6 | 0.5 | 0.2 | 0.3 | 0.5 | 0.2 | 0.6 | 0.4 |
| Range | | | | 0–6.2 | 0–21.9 | 0.04–6.2 | 0–11 | 0–12.4 | 0.04–7.1 | 0–10.7 | 0–6.0 | 0–3.3 | 0–24.8 | 0–18.1 |

1, Tongue; 2, pterygoid; 3, sternomastoid; 4, tricep; 5, intercostal; 6, longissimus complex; 7, external tibial flexor; 8, tail – longissimus caudalis; 9, tail – ilioischiodalis; 10, tail – dorsal biopsy; 11, tail – ventral biopsy.

lpg, larvae per gram of muscle.

N = 11, depicts the number of muscles/muscle groups tested for each animal.

*Depicts the muscle/muscle group with the highest intensity of infection for each animal sampled.

$P = 0.92$). However, the results from the dorsal tail, which mimic biopsies in live animals, indicate the potential that biopsies have for surveillance of *T. zimbabwensis* in crocodiles, since in 10 of the 11 positive animals (91%), even with relatively low levels of infection, larvae were successfully detected with biopsy samples of only 10 g.

The prevalence of *T. zimbabwensis* in wild crocodiles tested previously from South Africa, particularly the Mpumalanga province, was reported to be 38.5% (La Grange *et al.*, 2009). The prevalence within KNP was much higher at 80%, but these previous results from KNP represented only a small sample of five animals that all originated from the Olifants Gorge area (La Grange *et al.*, 2009). At that time it was thought that the high prevalence could have resulted from a possible increase in the frequency of cannibalism following the large number of crocodile deaths attributed to pansteatitis. The results of the present survey show the occurrence among the tested animals to be as high as 83.3%. Results also show the high number of infected crocodiles not to be limited to any specific area or the result of unusual circumstances, but suggest a natural phenomenon within the general crocodile population of KNP. In contrast with the findings of experimental studies conducted on encapsulated *Trichinella* sp. in mammals (Serrano *et al.*, 1999; Kapel *et al.*, 2005) and non-encapsulated *Trichinella* sp. in varans (*Varanus exanthematicus*) (Pozio *et al.*, 2004), where the tongue was the most important predilection site, in this study the tongue did not harbour the highest number of lpg. In studies with non-encapsulated *Trichinella* sp. in caimans (*Caiman crocodylus*), however, the anterior legs harboured, on average, more larvae than the tongue, and the intercostal muscles and posterior legs were also shown to be important predilection sites (Pozio *et al.*, 2004). The results from the present study support these findings. The lower larval burdens observed in the tongue musculature when compared to mammals may be explained in part by the fact that in crocodiles, the tongue is fixed to the floor of the oral cavity, making it less mobile than that of mammals and thus less dependent on a high vascular supply associated with higher cellular metabolic requirements of moveable musculature (Huchzermeyer, pers. comm., 2010). This phenomenon was also seen in experimental studies involving *T. pseudospiralis* in monkeys (*Macaca fascicularis*) (Kociecka *et al.*, 1980), and does not seem to support the aforementioned. Additionally, the results of the present study did not support the findings of experimental studies conducted with non-encapsulated species in mammals where the diaphragm appeared to be the most important predilection site (Kapel *et al.*, 2005; Mukaratirwa *et al.*, 2008). These inconsistencies do, however, support the important role of host characteristics in muscle predilection and the pattern of infection of the host in nature, as alluded to in previous studies (Serrano *et al.*, 1999; Kapel *et al.*, 2005). It is also important to note that the results from the present study are based on natural infections where the initial infection doses are not known.

More structured surveys involving the primary prey items of crocodiles are needed to elucidate the existence of other natural hosts that may play a role in the epidemiology of the parasites. Additional research with experimental infection of animals of uniform size and

age, where the initial level of infection is controlled, is also required to remove any bias in predilection that may result from differences in age, immunological status and initial infection levels. The results of this study confirm the importance of the KNP as a natural refuge for *T. zimbabwensis* circulating among its wildlife (La Grange *et al.*, 2010). These results also support the importance of host characteristics in muscle predilection by the larvae and show that muscle predilection of non-encapsulated *T. zimbabwensis* in reptilian hosts follows distinctly different patterns from those observed in mammals.

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