

TRICHINELLA ZIMBABWENSIS – THE FIRST KNOWN SPECIES OF THE GENUS TRICHINELLA INFECTING COLD-BLOODED ANIMALS: A REVIEW

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ABSTRACT

Trichinellosis is a meat-borne zoonotic disease caused by nematodes belonging to the genus *Trichinella*. These nematodes are among the least host-specific helminths. To date, 10 different *Trichinella* species have been described, including the encapsulating species *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella nelsoni*, *Trichinella murrelli*, *Trichinella chanchalensis*, *Trichinella patagoniensis* and three genotypes whose taxonomic status remains unstipulated - *Trichinella* T6, T8 and T9 exclusive to mammals, and three non-encapsulating species *Trichinella papuae*, *Trichinella pseudospiralis* - infecting mammals and birds, and *Trichinella zimbabwensis* – infecting both mammals and reptiles. Muscle larvae of *T. zimbabwensis*, like those of *T. papuae*, do not infect birds. The morphology of adults and larvae is very similar to that of *Trichinella papuae*. These two species can cross between each other in both directions (i.e. female of *T. zimbabwensis* per male of *T. papuae* and female of *T. papuae* per male of *T. zimbabwensis*) producing F1 offspring which produce very few and less viable F2 larvae. These two nematodes cause similar degenerative patterns in the muscles. To summarize, the presence of different species which infect reptiles, mammals or birds in the genus *Trichinella* strongly suggests the possibility of this nematode group being ancient and it have evolved with the evolution of reptiles into mammals and birds (i.e. from poikilothermic to homoiothermic vertebrates).

Key words: *Trichinella zimbabwensis*, cold-blooded animals, meat-borne zoonosis, crocodile farms, Zimbabwe.

Introduction

Trichinellosis is a meat-borne zoonotic disease caused by nematodes belonging to the genus *Trichinella*. These nematodes are among the least host-specific helminths (Kapel, 2000). Currently, 10 *Trichinella* species have been described (Sharma *et al.*, 2020), including seven encapsulating species - *Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella nelsoni*, *Trichinella murrelli*, *Trichinella patagoniensis*, *Trichinella chanchalensis* and three genotypes whose taxonomic status remains unstipulated - *Trichinella* T6, T8 and T9 exclusive to mammals, and three non-encapsulating species *Trichinella papuae*, *Trichinella pseudospiralis* - infecting mammals and birds, and *Trichinella zimbabwensis* – infecting both mammals and reptiles (Kapel, 2000; Pozio *et al.*, 2002).

Until recently parasites of the genus *Trichinella* had only been detected in warm-blooded animals. However, in 1995, *Trichinella* larvae were detected in the muscles of crocodiles (*Crocodylus niloticus*) on crocodile farms in Zimbabwe (Pozio *et al.*, 2002; Pozio *et al.*, 2007; La Grange *et al.*, 2009) representing the first observation of a reptile naturally infected with *Trichinella*. The mode of transmission is the consumption of the meat of slaughtered crocodiles, used as feed (Pozio *et al.*, 2007). This recent discovery in Zimbabwe, Mozambique and Ethiopia have had a significant impact

on the commercial crocodile trade industry in South Africa (La Grange *et al.*, 2009; Magnino *et al.*, 2009).

Muscle larvae of *T. zimbabwensis*, like those of *T. papuae*, do not infect birds. The morphology of adults and larvae is very similar to that of *Trichinella papuae*. These two species can cross between each other in both directions (i.e. female of *T. zimbabwensis* per male of *T. papuae* and female of *T. papuae* per male of *T. zimbabwensis*) producing F1 offspring which produce very few and less viable F2 larvae. These two nematodes cause similar degenerative patterns in the muscles (Kapel, 2000; Pozio *et al.*, 2004).

Analysis of the sequence data available in GenBank for the other remaining taxa, *T. zimbabwensis*, *T. papuae*, *T. patagoniensis* and *Trichinella* T8 and T9, the ITS-1 primers also detected the marker fragments of these taxa for their differentiation by the NGS method (Lobanov *et al.*, 2023). If the amplification band is 264 bp, the larva is known to belong to *T. zimbabwensis* (Pozio and Zarlunga, 2019).

The geographic area with the highest level of possibility for *T. zimbabwensis* spread only over the southeastern areas of Africa (Mozambique, South Africa and Zimbabwe). Lower distribution is also found over the central South America, followed by focal point in the western South America (Peru), southwestern Africa (Namibia), the south part of Madagascar, and the northeastern part of Australia (Yayeh *et al.*, 2020).

Experimental infections have shown that this nematode can infect other reptile species, as well as mammals (i.e., mice, rats, pigs, foxes, and primates) (La Grange *et al.*, 2009). Despite the ability of *T. zimbabwensis* to infect mammalian hosts there has only been a single report of a naturally infected mammal to date - *Panthera Leo* of the Kruger National Park (KNP) of South Africa (La Grange *et al.*, 2010).

T. zimbabwensis was previously isolated from wild Nile crocodiles (*Crocodylus niloticus*) in Africa and just beyond its northwestern and southern borders and in a Nile monitor turtle (*Varanus niloticus*) from the town of Nelspruit near the southwestern border of the South African National Park. Furthermore, it has also been detected in farmed crocodiles in South Africa. This species is infectious to mammals and reptiles. Results of passive monitoring indicated that *T. zimbabwensis* had the highest prevalence in crocodiles and carnivores from the three species known to roam South Africa. Vertical transmission of *T. zimbabwensis* via transmammary and transplacental routes has been experimentally demonstrated in rodents (*Rattus norvegicus*), suggesting endemic rodent populations in the studied area may play an integral role in maintenance and transmission of the three known *Trichinella* taxa circulate in region. It showed that *T. zimbabwensis* is not only the most prevalent, but also infects the widest host range of all *Trichinella* species. This certainly suggests that the general knowledge and perception of interspecies fermentation and cleaning is incomplete (La Grange and Mukaratirwa, 2020).

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, age and many other factors. The host characteristics appear to be the most important determinant for the predilection to specific muscle groups. *T. zimbabwensis* has a proven propensity in nature towards infecting reptilian hosts. More specifically, Nile crocodiles (*Crocodylus niloticus*) and Nile monitor lizards (*V. niloticus*). The importance of host characteristics, especially the physiological and anatomical differences between reptiles and mammals and the natural infection patterns of *T.*

zimbabwensis provides the incentive for studies aimed at clarifying the predilection patterns of this species to reptilian hosts (Pozio *et al.*, 2004; La Grange *et al.*, 2013; Kapel *et al.*, 1998).

Numerous studies have been conducted to characterize the muscle differentiation of *T. zimbabwensis*. Louis J. La Grange and Samson Mukaratirwa have used 15 crocodiles randomly divided into three experimental groups of five animals each, representing high infection rate (642 larvae/kg of average body mass), medium rate (414 larvae/kg) and low rate (134 larvae/kg). In the first group the highest percentage of larvae has been observed in the triceps muscle and hind limb muscle also. In the second group larvae were found in the triceps muscle, followed by sternomastoid and hind leg muscle. In the third group with low rate of infection *Trichinella* larvae were found predominantly in the intercostal muscle.

Another study shows an interesting observation that the dorsal tail muscles appeared to harbor a higher number of larvae than the ventral body muscles. Similarly, the superficial muscles of the dorsal tail containing more trichinella larvae than the deeper body muscles (La Grange *et al.*, 2013; La Grange and Mukaratirwa, 2014).

No correlation was observed between biochemical parameters and disease severity. Peak antibody levels were observed at day 49 post-infection in the moderate infestation experimental group and on day 42th in both other (high and low infection rate) groups. The intensity of infection could not be correlated with the magnitude of humoral immunity or the time to seroconversion. The results of this study are consistent with those reported in mammals infected with other *Trichinella* species and show that antibody titers are not detectable indefinitely (La Grange and Mukaratirwa, 2014).

It was concluded that *T. zimbabwensis* infection resulted in an increase in amino acids, pipercolic acid, histidine, urea and upregulation of glucose and meso-erythritol substances. In addition, *T. zimbabwensis* infection resulted in upregulation of fatty acids, retinoic acid, and acetic acid. These findings highlight the potential of metabolomics as a novel approach for basic investigations of host-pathogen interactions as well as disease progression and prognosis (Ndlovu *et al.*, 2023).

Studies on the intestine and muscle of male rats revealed significantly higher levels of *T. zimbabwensis* establishment than females due to sex hormones linked immunological reasons (Hlaka *et al.*, 2015).

Trichinella zimbabwensis represents a unique member of the genus *Trichinella* in its ability to infest and complete development in poikilothermic (cold-blooded) hosts such as crocodiles and other reptiles. This trait distinguishes it from the majority of *Trichinella* species, which are physiologically adapted to warm-blooded mammals. Several evolutionary, metabolic, and cellular factors underlie this specialization. Most encapsulated *Trichinella* species (*T. spiralis*, *T. britovi*, *T. nativa*, etc.) require relatively constant mammalian body temperatures ($\approx 35\text{--}40^\circ\text{C}$) to support: intestinal development and reproduction, intracellular modification of muscle cells, formation of the nurse-cell complex etc. (Pozzio, 2005 and 2007).

In contrast, *T. zimbabwensis* exhibits physiological and metabolic activity optimized for significantly lower temperatures ($\approx 20\text{--}30^\circ\text{C}$), which are characteristic of reptilian hosts. This thermal tolerance enables all parasitic stages to develop in poikilothermic tissues, where fluctuating temperatures would otherwise impair larval survival in typical mammal-adapted species. *T. zimbabwensis* belongs to the non-encapsulated lineage and therefore does not induce the formation of a stable collagen capsule (nurse-cell complex) in host muscle fibers. This biological feature confers

greater resilience to environmental temperature variability. The absence of capsule formation appears to be an adaptive advantage in reptilian hosts, whose muscle tissues undergo wider daily and seasonal thermal fluctuations compared with those of mammals (Yayeh *et al.*, 2020).

Larval infestation, muscle cell modification, and maintenance of intracellular stages require specialized proteolytic and metabolic enzymes. In *T. zimbabwensis*, these enzymes exhibit reduced thermal optima, allowing effective function at temperatures that would inhibit the metabolism of other *Trichinella* species (Pozio & La Rosa, 2005). Such biochemical plasticity likely plays a central role in enabling successful establishment, survival, and development in reptilian hosts (Korhonen *et al.*, 2016).

The geographic distribution of *T. zimbabwensis* - primarily in sub-Saharan Africa corresponds with ecological systems dominated by large reptiles (e.g., crocodiles, varanids) and scavenging mammals that feed upon them. These predator-prey and scavenger cycles have driven host-specific evolutionary adaptation, enabling the parasite to exploit reptilian hosts efficiently. By contrast, other *Trichinella* species have undergone long-term coevolution almost exclusively within mammalian host communities, resulting in narrow thermal and physiological host constraints. *Trichinella zimbabwensis* exhibits a markedly restricted distribution, occurring predominantly in Zimbabwe and neighboring regions of sub-Saharan Africa. Although cold-blooded vertebrates (the primary hosts for this species) are found across many biogeographic zones, several ecological, evolutionary, and epidemiological factors collectively explain the parasite's limited range (Pozzio, 2005 and 2007).

T. zimbabwensis belongs to the non-encapsulated clade of *Trichinella*, a lineage that appears to have undergone evolutionary divergence within southern African ecosystems. Its emergence through localized adaptive radiation resulted in a parasite finely tuned to the ecological and physiological characteristics of African reptilian hosts (Murrell & Pozio, 2011). Other continents harbor their own independent non-encapsulated *Trichinella* lineages (e.g., *T. papuae* in Southeast Asia and Oceania), indicating geographically isolated evolutionary trajectories. The maintenance of *T. zimbabwensis* relies on complex trophic networks characteristic of African ecosystems, including: Nile crocodiles (*Crocodylus niloticus*), large varanid lizards, facultative carnivores and scavengers (e.g., hyenas, lions), and pronounced cannibalism within crocodile populations (Diaz *et al.*, 2020). These interactions generate a stable transmission cycle in which reptiles serve as both predators and prey. Comparable reptile-based scavenger networks are far less developed or ecologically stable in most other regions, limiting the parasite's ability to establish persistent sylvatic cycles outside Africa (Bruschi, 2021).

The key reptilian hosts associated with *T. zimbabwensis* exhibit limited natural movement between continents. In contrast, globally distributed synanthropic mammals (e.g., pigs and rats) have facilitated the spread of species such as *T. spiralis* and *T. britovi*. The absence of a migratory or anthropogenically mobile host effectively confines *T. zimbabwensis* to its original biogeographic zone (Zarlenga *et al.*, 2020).

Non-encapsulated *Trichinella* species thrive in environments characterized by high ambient temperatures, high humidity, and rapid carcass degradation—conditions typical of tropical and subtropical Africa. These factors enhance larval survival within decomposing reptile tissues. Cooler climates, seasonal temperature fluctuations, and lower humidity in many other regions would inhibit long-term environmental persistence and transmission. Outside Africa, well-established *Trichinella* species dominate local transmission cycles. Ecological competition, particularly in

mammalian carnivore guilds may prevent the successful establishment of *T. zimbabwensis* even if accidental introduction were to occur. Niche saturation by endemic species thus acts as an additional biogeographical barrier (Crisostomo-Jorquera & Landaeta-Aqueveque, 2022).

Unlike domestic or synanthropic *Trichinella* species, *T. zimbabwensis* is not associated with livestock production, meat trade, or commensal rodents. Its reliance on wildlife-based transmission precludes passive human-mediated spread. Even in crocodile farming operations outside Africa, infections remain isolated and have not resulted in broader ecological dissemination (Bruschi, 2021).

Conclusion

To summarize, results of passive monitoring indicate that *T. zimbabwensis* has the highest prevalence in crocodiles and carnivores from the three species known to roam South Africa. *T. zimbabwensis* is not only the most prevalent, but also infects the widest host range of all *Trichinella* species. Due to the consumption of alligator meat (from alligator industrial farms) in some countries, this disease is potential zoonosis.

The presence of different species which infect reptiles, mammals or birds in the genus *Trichinella* strongly suggests the possibility of this nematode group being ancient and it have evolved with the evolution of reptiles into mammals and birds (i.e. from poikilothermic to homoiothermic vertebrates).

Trichinella zimbabwensis can successfully parasitize cold-blooded animals due to a combination of: physiological tolerance to low and fluctuating temperatures, non-encapsulating muscle larval development, enzymatic systems adapted to lower thermal optima, and long-term ecological specialization toward reptilian host cycles. These adaptations distinguish *T. zimbabwensis* from the mammal-adapted, encapsulated species of the genus *Trichinella*, which are unable to sustain effective development in poikilothermic hosts.

The restricted distribution of *T. zimbabwensis* results from a combination of evolutionary history, host specificity, ecological dependencies, and limited dispersal potential. These factors – coupled with the absence of suitable transmission networks outside sub-Saharan Africa – explain why the species remains endemic to this region despite the global presence of cold-blooded vertebrates.

Conflict of interest

The authors have not declared any conflict of interests.

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