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Infectivity and temperature tolerance of non-encapsulating *Trichinella zimbabwensis* in experimentally infected red foxes (*Vulpes vulpes*)

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Summary

The non-encapsulating *Trichinella zimbabwensis* was evaluated for infectivity in red foxes (*Vulpes vulpes)*, the larval distribution and cold tolerance in fox muscle tissue. Six red foxes were experimentally infected with *T. zimbabwensis* larvae. Five weeks after inoculation, muscle larvae were recovered from 9 different muscle types using artificial digestion method. The establishment of infection in all infected red foxes demonstrated the ability of *T. zimbabwensis* to complete its life cycle in a carnivore mammal host. The larvae recovered from fox muscle tissue were infective to mice, they have a moderate tolerance to freezing and they survived for 4 weeks in decaying tissue at room temperature. This is the first study to describe these biological characteristics of *T. zimbabwensis*.

Key words: *Trichinella zimbabwensis*; red fox; infectivity; temperature resistance; decaying tissue

Introduction

The detection of *Trichinella* muscle larvae in farmed crocodiles (*Crocodylus niloticus*) from Zimbabwe (Foggin *et al.*, 1997) represented the first observation of a reptile naturally infected with *Trichinella*, which has enlarged the host spectrum of the genus *Trichinella* on poikilothermic animals. Experimental infections showed that these larvae were able to infect domestic pigs, baboon, laboratory rats (Mukaratirwa and Foggin, 1999) and mice, but not birds (Pozio *et al.*, 2002), and additional analysis justified the erection of a new non-encapsulated species, *Trichinella zimbabwensis* (Pozio *et al.*, 2002). Prior, Kapel *et al.* (1998) demonstrated that all other species in the genus failed to infect reptiles. As the normal reservoirs of species in the genus are mammalian predators and scavengers, the present study evaluates the infectivity of *T. zimbabwensis* in a carnivore host and the survival of larvae at different temperatures in carnivore tissue.

Material and Methods

Trichinella zimbabwensis isolate (Code ISS 1029 at the *Trichinella* Reference Centre in Rome) was propagated by serial passage in female outbred NMRI mice at the Royal Veterinary and Agricultural University of Denmark.

Six adult farm-bred red foxes were experimentally infected with *T. zimbabwensis* larvae at two dose-levels: 10 000 larvae/animal (fox # 1 – 3) and 15 000 larvae/animal (fox # 4 – 6). Prior to inoculation, animals were sedated by intra muscular injection of mixture of zolazepam, tiletamin and medetomidin and orally inoculated using stomach tube. Foxes were kept in a containment level 3 facility, separately in elevated cages, and fed daily with a fish meal diet and water *ad libitum*. During the study period all foxes were observed for clinical symptoms (in-appetence, fever, reduced activity, apathy, etc.). Five weeks after inoculation foxes were euthanized by intravenous injection of pentobarbital.

For determination of muscle larvae burdens, the tissue from 9 different muscle groups (Tab. 1) was recovered from individual foxes. Samples of 10 g muscle tissue were minced and artificially digested for 2 h on magnetic stirrers according to Kapel and Gamble (2000) and followed by two rounds of sedimentation and washing. Larvae were counted under a dissecting microscope (40 x) and the value of larvae per gram (lpg) was calculated.

To evaluate the temperature resistance of *T. zimbabwensis* larvae in fox tissue, all tissue remaining after sampling for the above digestion was collected and minced to a homogeneous mass (max. diameter 3 mm). From this 100 g samples were packed into plastic bags, pressed flat (max. 5

mm high) after which they were exposed to temperatures of +20, +5, -5 and -18°C for periods of 1, 2 and 4 weeks. After storage, larvae were recovered the individual samples by digestion counted and inoculated into 6-wk-old female outbreed NMRI mice. The infective dose in individual groups was determined according to number of recovered larvae, but mice were never given more than 500 larvae. In the control group, 5 mice were administered by the dose of 500 infective muscle larvae immediately after recovery from fox tissue. Mice were sacrificed 5 weeks post infection and the muscle larvae burden as well as the reproductive capacity index (RCI – mean number of larvae recovered/number of larvae inoculated) was determined.

The status of larvae concerning their motility and shape was evaluated during counting to estimate the percentage of active larvae. The number of motile, C-shaped and coiled larvae was calculated in the batch of 100 larvae for each group and expressed as the percentage.

Results

Infection established in all foxes, and none of the foxes showed any clinical signs. The experimental dose of 10 000 larvae per fox resulted in higher larval burdens (mean 13.7 ± 4.1 lpg) and in more uniform infection than infective dose of 15 000 larvae per fox with mean 7.3 ± 2.5 lpg (Table 1) but the difference was not significant.

The distribution of larvae in respective muscles, or muscle groups showed diaphragm, tongue and masseter to be most infected muscles and thus likely predilection sites for *T. zimbabwensis* in this host.

Muscle larvae of *T. zimbabwensis* survived freezing at -5°C for 1, 2 and 4 weeks, although with very low infectivity (RCI 0.1, 0.1 and 0.07, respectively), and were not infective after freezing at -18°C. Larvae remained infective after 1 and 2 weeks of exposure to room temperature (RCI 15.6 and 9.6). The study showed good survival and infec-

Table 1. Larval burden (number of larvae/1g of muscle) of different muscles or muscle groups in red foxes (*Vulpes vulpes*) experimentally inoculated with *Trichinella zimbabwensis*

Muscle group	Inoculation dose					
	10 000 larvae			15 000 larvae		
	Fox 1	Fox 2	Fox 3	Fox 4	Fox 5	Fox 6
Tongue	8.8	20.3	36.3	15.9	8.4	16.8
Lower jaw	23.9	24.2	12.8	8.7	13.7	5.9
Neck	5.4	16.2	5.7	6.4	6.4	14.5
Upper front leg	6.8	15.1	7	1.8	0.4	8.5
Lower front leg	12.4	2.2	5.8	3.6	2.9	13.9
Back	5.8	22.9	12.7	6.1	2.4	1.9
Diaphragm	19.7	33	17	11.4	5.6	26.3
Upper hind leg	3.3	6.4	5.6	6.1	1.8	8
Lower hind leg	3.8	22.7	16	1	4.3	1.7
Mean	9.9	18.1	13.2	6.8	5.1	10.8
Group mean			13.7 ± 4.1			7.3 ± 2.5

Table 2. Evaluation of the infectivity of *Trichinella zimbabwensis* larvae present in fox muscle tissues to laboratory mice, after storage at different temperatures for one to four weeks

Storage time	Storage temperature (°C)	Condition of larvae after recovery by digestion			Larvae inoculated per mouse / nr. of mice	RCI
		motile (%)	C-shaped (%)	coiled (%)		
1 week	+20	100	0	0	500/2	15.6
	+5	100	0	0	500/2	19.9
	-5	0	0	100	400/2	0.1
	-18	0	60	40	250/2	0
2 weeks	+20	60	0	40	500/2	9.6
	+5	100	0	0	500/2	13.9
	-5	0	50	50	350/2	0.1
	-18	0	0	100	150/2	0
4 weeks	+20	-	-	-	-	-
	+5	90	0	10	350/2	4.7
	-5	0	50	50	500/2	0.07
	-18	0	100	0	350/2	0

tivity after exposure to +5°C, which even after 4 weeks infection in mice established (RCI 4.7); in the control group, the mean RCI was 50. In general there was a good correlation between the number of motile larvae observed after storage and the following establishment in mice (Tab 2).

Discussion

The present findings establish for the first time that *T. zimbabwensis* is able to complete its life cycle in a carnivore mammal. This adds new information to the previous observations that *T. zimbabwensis* infects reptiles (Pozio *et al.*, 2004), but not freshwater fish (Pozio and La Rosa, 2004) and chickens (Pozio *et al.*, 2002).

Although the number of foxes in the present study is limited, the relative uniform infection of the foxes (mean larval burden 10.65 ± 4.5 lpg) it is interesting to observe that the diaphragm, the tongue and the masseter are the muscles with the highest larval intensity. These findings are consistent with other experimental fox infection, where *T. papuae* and *T. pseudospiralis* are found in diaphragm, neck and masseter (Webster *et al.*, 2002; Kapel *et al.,* in press), as opposed to the encapsulating species which had predilection site also in the tongue, extremities and diaphragm (Kapel *et al.*, in press). Any additional information on predilection sites of *T. zimbabwensis* is scarce; Mukaratirwa and Foggin (1999) found tongue, masseter and intercostals to be the most infected muscles in domestic Mukota pigs, and Pozio *et al.* (2002) identified predilection sites as intercostals and tail muscles in crocodiles.

The relatively wide temperate tolerance of *T. zimbabwensis* in fox tissue is also interesting. Thus, the ability to tolerate freezing at -5°C for 4 weeks, although with low infectivity, contrasts previous observations on non-encapsulating species. In fox muscle tissue, *T. pseudospiralis* tolerated freezing at -5°C for only 1 week (Kapel *et al.*, 2004) and *T. papuae* was unable to infect mice after 1 week of freezing (Webster *et al.*, 2002). In crocodile tissue and mouse carcasses, infected with *T. zimbabwensis*, and frozen at -10°C for 1, 2, 3, and 7 days, none of the muscle larvae collected from previously frozen crocodile or mouse muscles was infectious for laboratory mice (Pozio *et al.*, 2002).

Also the tolerance of *T. zimbabwensis* to the decaying processes are comparable to characteristics for *T. papuae* observed by Webster *et al.* (2002), who demonstrated infective larvae in fox tissue after respectively 9 days at room temperature and 4 weeks at +5°C. On the contrary, *T. pseudospiralis* in fox tissue was found to lose its infectivity after 1 week at room temperature (von Kőller *et al.*, 2001) although the encapsulating species, in the same study were infective after two week of storage. The natural survival of different *Trichinella* species in decaying host muscle tissue exposed to environment after predation, hunting a.o. has previously been suggested as an important factor in transmission among wildlife (von Köller *et al.*, 2001). As, the body temperature range for reptiles (26 – 32°C) is lower than for warm blooded mammals and since *T. zimbabwensis* and *T. papuae* infect both group of animals, the temperature range in which their muscle larvae persist infective is theoretically wider (26°C in reptiles - 40.5°C in mammals) than for *Trichinella* species, infecting only mammals, or birds. This may explain, why *T. zimbabwensis* and *T. papuae* is more resistant also in dead tissue in spite of the lack of capsule in comparison with *T. pseudospiralis.*

In conclusion, our findings demonstrated that *T. zimbabwensis* can complete its life cycle in a carnivore mammal, that the muscle larvae show some tolerance to cold and decaying processes.

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