Trichinella papuae and *Trichinella zimbabwensis* induce infection in experimentally infected varans, caimans, pythons and turtles

E. POZIO[1]*, G. MARUCCI[1], A. CASULLI[1], L. SACCHI[2], S. MUKARATIRWA[3], C. M. FOGGIN[4] *and* G. LA ROSA[1]

[1] *Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, viale Regina Elena 299, 0061 Rome, Italy*
[2] *Department of Animal Biology, University of Pavia, Piazza Botta 9, 27100 Pavia, Italy*
[3] *Faculty of Veterinary Science, Department of Paraclinical Veterinary Studies, P.O. Box MP167 Mount Pleasant, Harare, Zimbabwe*
[4] *Central Veterinary Research Laboratory, P.O. Box CY 551 Causeway, Harare, Zimbabwe*

(*Received 12 August 2003; revised 12 September 2003; accepted 15 September 2003*)

SUMMARY

The discovery of *Trichinella zimbabwensis* in farm crocodiles of Zimbabwe has opened up a new frontier in the epidemiology of the *Trichinella* genus. The objective of the present study was to investigate the infectivity of encapsulated species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni*) and non-encapsulated species (*T. pseudospiralis*, *T. papuae* and *T. zimbabwensis*) in caimans (*Caiman crocodilus*), varans (*Varanus exanthematicus*), pythons (*Python molurus bivittatus*) and turtles (*Pelomedusa subrufa*) raised at their natural temperature range (26–32 °C). Mice and chickens were used as controls. At 6 days post-infection (p.i.), adult worms were detected in the small intestine of reptiles infected with *T. papuae* and *T. zimbabwensis*, of chickens infected with *T. pseudospiralis* and of mice infected with all encapsulated and non-encapsulated species. At 60 days p.i., *T. papuae* and *T. zimbabwensis* adult worms were collected from the intestine of varans and caimans and larvae from muscles of the four reptile species, *T. pseudospiralis* larvae from muscles of chickens, and larvae of all *Trichinella* species from mouse muscles. The highest reproductive capacity index of both *T. papuae* and *T. zimbabwensis* was observed in varans. The results show that *T. papuae* and *T. zimbabwensis* are able to complete their entire life-cycle in both poikilothermic and homoiothermic animals.

Key words: *Trichinella papuae*, *Trichinella zimbabwensis*, reptile, caiman, varan, python, turtle, body temperature, experimental infection.

INTRODUCTION

For 145 years following their discovery (Owen, 1835), nematodes of the *Trichinella* genus were considered to infect only mammals (Campbell, 1988). In 1980, a non-encapsulated species, *Trichinella pseudospiralis*, was found to also infect birds (Shaikenov, 1980). In 1995, non-encapsulated larvae belonging to a new species, *Trichinella zimbabwensis*, were discovered in farm crocodiles (*Crocodylus niloticus*) of Zimbabwe (Pozio *et al.* 2002), representing the first report of naturally infected reptiles. Although attempts have been made to infect reptiles with all 5 encapsulated *Trichinella* species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni*) and with *T. pseudospiralis*, all of these attempts failed when the animals were bred at their natural temperature range (i.e. 29–30 °C) (Kapel *et al.* 1998), whereas in snakes, lizards and turtles, the life-cycle of *T. spiralis* was successfully reproduced when the animals were bred at 37 °C (i.e. the body temperature of mammals) (Pozio, 2001).

The objectives of the present study were to evaluate the infectivity of all *Trichinella* species, the development of the nurse cell and the critical point of development in experimentally infected reptiles belonging to the orders Loricata, Squamata and Chelonide.

MATERIALS AND METHODS

Experimental animals

Tropical carnivorous reptiles were used, specifically: 15 spectacled caimans (*Caiman crocodilus*, synonymous of *Caiman sclerops* L., 1758), a species originating from Central and South America, age 6–8 months, weight 350–550 g; 15 savannah monitors (referred to as 'varans') (*Varanus exanthematicus* Bosc, 1792), a species originating from equatorial Africa, age: 2–3 years, weight 300–450 g; 40 Burmese pythons (*Python molurus bivittatus* Kuhl, 1820), a species originating from Southeast Asia, age 6–8 months, weight

* Corresponding author: Department of Infectious, Parasitic and Immunomediated Diseases, Istituto Superiore di Sanità, viale Regina Elena 299, 00161 Rome, Italy. Tel: +39 06 4990 2304. Fax: +39 06 4938 7065. E-mail: pozio@iss.it

350–850 g; and 40 African helmeted turtles (*Pelomedusa subrufa* Lacépède, 1788), a species originating from equatorial Africa, age about 2 years, weight 50–100 g. All animals were born and raised in captivity. Caimans and pythons were kept 1 to a terrarium, whereas varans and turtles were kept 2 to a terrarium. The terrariums for caimans and turtles consisted of 2/3 water and 1/3 dry substratum. The terrariums for pythons and varans had a water tank and a substratum consisting of small pieces of cluster-pine bark. All animals were kept under a day/night rhythm of 12/12 h. Pythons and turtles were kept at 28–30 °C during the day and at 26–28 °C at night; caimans and varans were kept at 30–32 °C during the day and at 28–30 °C at night (the temperature refers to the air). During the study, the body temperature of reptiles was measured every 2 weeks. Since the source of heat was not inside the terrarium, the body temperature was, in all cases, quite similar to the air temperature of the terrarium. Pythons and varans were fed laboratory mice; turtles were fed fish or meat; and caimans were fed fish. The frequency of feeding depended on the specific species. Forty Swiss CD1 female mice weighing 25 g and 32 two-week-old chickens weighing 80 g were used as controls. Animals were housed and treated according to the European directive 8/609 EEC.

Experimental infections

Eight *Trichinella* reference strains were used to infect the animals. Of these 5 were encapsulated species: *T. spiralis* (code ISS3), *T. nativa* (code ISS10), *T. britovi* (code ISS2), *T. murrelli* (code ISS35) and *T. nelsoni* (code ISS29); and 3 were non-encapsulated species: *T. pseudospiralis* (code ISS13), *T. papuae* (code ISS572) and *T. zimbabwensis* (code ISS1029) (Pozio *et al.* 1999, 2002).

Larvae were collected from skinned and eviscerated mouse carcasses by artificial digestion, following a protocol slightly modified from Pozio (1987). The digestive fluid consisted of 0·5% hydrochloric acid, 0·5% pepsin 1 : 10 000, and PBS at 40 °C. Mouse carcasses were cut into small pieces and minced for 30 s in a blender with the digestive fluid at a concentration of 1 : 40 (w/vol). The mixture was then incubated at 39–40 °C, stirring constantly, for either 15 min (for non-encapsulated species) or for 30 min (for encapsulated species). Muscle larvae were then allowed to sediment at 39–40 °C for 15 min, and the supernatant was discarded. The muscle larvae were washed 4 times with warm PBS and counted 3 times under a dissection microscope at a magnification of 20–40×.

The reptiles and chickens were inoculated *per os* with 3000 larvae/animal. A 2 ml syringe connected to a silicone stomach tube was used for varans, pythons, and caimans, whereas, for turtles and chickens, the syringe was connected to a gavage needle. For varans, pythons, and caimans, a small aluminium bar with a hole in the middle was used to keep the animal's jaws open and prevent the stomach tube from being crushed. The control mice were inoculated *per os* with 500 larvae/animal using a 2 ml syringe connected to a gavage needle. To ensure that all of the larvae entered the stomach, the stomach tube was flushed with water following larva inoculation. The animals of each species were divided into groups of 5. The turtles, pythons, chickens, and mice were inoculated *per os* with the 8 *Trichinella* species; the varans and caimans were inoculated *per os* with *T. papuae*, *T. zimbabwensis* and *T. nelsoni*. For each group of 5 animals, 2 specimens were killed 6 days post-infection (p.i.); the other 3 specimens were killed 60 days p.i.

Worm collection from the intestine

The reptiles were killed by hypothermia, whereas the mice and chickens were killed by CO_2 inhalation to collect intestinal worms at 6 and 60 days p.i. To facilitate the recovery of adult worms from the intestine, the reptiles were not fed during the week prior to their killing and the chickens and mice were not fed for 1 day prior to sacrifice. The intestine was collected, opened longitudinally, and incubated in PBS at 37 °C for 2 h, following a previously published protocol (Blair, 1983). Worms were searched for in the sediment under a microscope at a magnification of 20–40×; they were counted 3 times and then stored in 70% ethyl alcohol.

Muscle larvae collection

At 60 days p.i., the reptiles, chickens and mice were skinned and eviscerated. For the turtles, pythons and mice, the entire carcass was digested separately for each animal, whereas for the chickens, varans and caimans, muscles were collected from the bones, which were also scraped with a scalpel to collect the largest possible amount of muscle to allow for the identification of predilection muscles. For these latter animals, to determine the preferential muscles, digestion was conducted separately for single muscles or muscle groups, according to the protocol reported above. For all animals, the larvae were collected, counted 3 times, and stored in 70% ethyl alcohol for the morphological and molecular studies. The reproductive capacity index (RCI) (i.e. the number of larvae collected from muscles divided by the number of larvae given *per os*) was evaluated for each animal. In total, 100 larvae of each of the *Trichinella* species which had succeeded in developing in varans and caimans were given *per os* to Swiss CD1 mice immunosuppressed with cyclophosphamide (4 mg/mouse) at 0 and 4 days p.i., to evaluate their infectivity. At 40 days p.i., these mice were killed, skinned, and eviscerated; the entire carcass was digested and the larvae were counted.

Table 1. Number of adult worms of *Trichinella* collected from the small intestine of two specimens for each host species 6 days post-infection

Trichinella species	Turtles		Pythons		Varans		Caimans		Chickens		Mice	
T. spiralis	L_1*	Neg†	L_1	Neg	N.D.	N.D.	N.D.	N.D.	Neg	Neg	234	198
T. nativa	Neg	Neg	Neg	Neg	N.D.	N.D.	N.D.	N.D.	Neg	Neg	98	74
T. britovi	Neg	Neg	Neg	Neg	N.D.	N.D.	N.D.	N.D.	Neg	Neg	131	109
T. pseudospiralis	Neg	Neg	Neg	Neg	N.D.	N.D.	N.D.	N.D.	115	86	184	161
T. murrelli	Neg	Neg	L_1	Neg	N.D.	N.D.	N.D.	N.D.	Neg	Neg	86	65
T. nelsoni	Neg	Neg	L_1	Neg	Neg	Neg	Neg	Neg	Neg	Neg	201	178
T. papuae	68	53	86	61	1825	1517	937	779	Neg	Neg	127	96
T. zimbabwensis	59	36	61	45	1234	1058	647	328	Neg	Neg	143	123

* A few dead L_1 larvae.
† Neg, no larva.
N.D., Not determined.

Worm morphology and the ultrastructure of the nurse-cell larva complex

To assess the influence of the host on worm morphology, the total length and width at the midbody was measured for 20 adult worms (10 females and 10 males) collected from the small intestine of varans, caimans and mice at 6 days p.i. and for 20 muscle larvae collected from varans, caimans and mice at 60 days p.i. A descriptive univariate statistical analysis (mean and standard deviation) and Student's *t*-test (two-tailed) were used to evaluate the statistical differences in the size of muscle larvae and adult worms.

For the histological and ultrastructural analyses of the nurse cell-larva complex, small pieces of intercostal muscle were collected from varans, caimans, and mice at 60 days p.i. The muscle samples were fixed in 0·1 M cacodylate buffer (pH 7·2) containing 2·5% glutaraldehyde for 4 h at 4 °C. The samples were then washed in the same buffer and post-fixed in 1% OsO_4 in cacodylate buffer for 1·5 h at 4 °C. All samples were dehydrated in ethanol and embedded in Epon 812 for sectioning. For light-microscopy, semi-thin sections (0·5 μm) were stained with 0·5% toluidine blue and examined under a Zeiss photomicroscope III. Thin sections (80 μm), stained with uranyl acetate and lead citrate, were examined under a Zeiss EM900 transmission electron microscope (TEM).

Molecular identification of adult worms and larvae

To confirm that the adult worms and larvae that were collected from the animals belonged to the same species used for experimental infection, a multiplex PCR analysis (Zarlenga *et al*. 1999) was used, according to a previously published protocol (Pozio & La Rosa, 2003).

RESULTS

Detection of intestinal worms

At 6 days p.i., in mice, we detected live adult worms (i.e. both male worms and females with developed embryos in the uterus) belonging to all 8 *Trichinella* species, whereas in chickens, only *T. pseudospiralis* worms were found (Table 1). For all reptile species, only live worms of *T. papuae* and *T. zimbabwensis*, probably at the adult stage (i.e. no well-developed embryo detected in the uterus), were detected. In turtles and pythons, a few dead L_1 larvae belonging to *T. spiralis*, *T. murrelli* and *T. nelsoni* were detected (Table 1).

At 60 days p.i., live adult worms (including females with embryos in the uterus) were detected only in varans and caimans. A mean of 138 *T. papuae* worms (range 98–187) and 127 *T. zimbabwensis* worms (range 62–165) were detected in varans; a mean of 85 *T. papuae* worms (range 39–124) and 79 *T. zimbabwensis* worms (range 18–131) were detected in caimans.

Detection of larvae in muscles

At 60 days p.i., larvae of all 8 *Trichinella* species were detected in mice, with an RCI ranging from 1·2 for *T. papuae* to 80·5 for *T. spiralis*; larvae were also detected in chickens infected with *T. pseudospiralis* and in all reptile species infected with *T. papuae* and *T. zimbabwensis* (Table 2). In reptiles, the RCI was higher for *T. papuae* than for *T. zimbabwensis*. The RCI was highest for varans (41·4 for *T. papuae*) and lowest for turtles (0·05 for *T. zimbabwensis*) (Table 2). In none of the reptile species were larvae of any encapsulated species or of the non-encapsulated species *T. pseudospiralis* detected.

Preferential muscles were studied in varans and caimans. In both animals, the tongue was the most infected muscle, followed by muscles of the anterior legs, of the posterior legs, muscles of the tail, intercostal muscles, and muscles of the spinal column (Table 3).

For neither *T. papuae* nor *T. zimbabwensis* were significant differences in the size of the intestinal worms observed when comparing varans, caimans, and mice 6 days p.i. The muscle larvae of *T. papuae*

Table 2. Average larvae per g (lpg), standard deviation (S.D.) and reproductive capacity index (RCI) in the muscles of three specimens of each host species (for chickens only two specimens were examined) 60 days post-infection

(For turtles, pythons and mice, lpg refers to the weight of the entire skinned and eviscerated carcass; for varans, caimans and chickens, lpg refers to the weight of most of the striated muscles.)

Trichinella species	Turtles			Pythons			Varans			Caimans			Chickens			Mice		
	lpg	S.D.	RCI	Lpg	S.D.	RCI	lpg	S.D.	RCI	lpg	S.D.	RCI	lpg	S.D.	RCI	lpg	S.D.	RCI
T. spiralis	Neg	—	—	Neg	—	—	N.D.	—	—	N.D.	—	—	Neg	—	—	2236	135·5	80·5
T. nativa	Neg	—	—	Neg	—	—	N.D.	—	—	N.D.	—	—	Neg	—	—	369	42·5	13·3
T. britovi	Neg	—	—	Neg	—	—	N.D.	—	—	N.D.	—	—	Neg	—	—	409	87·5	15·6
T. pseudospiralis	Neg	—	—	Neg	—	—	N.D.	—	—	N.D.	—	—	215·1	59·4	8·1	526	81·5	20·4
T. murrelli	Neg	—	—	Neg	—	—	N.D.	—	—	N.D.	—	—	Neg	—	—	298	115·5	11·1
T. nelsoni	Neg	—	—	Neg	—	—	Neg	—	—	Neg	—	—	Neg	—	—	399	57·5	15·2
T. papuae	8	2·0	0·05	0·5	0·4	0·05	1074	680·2	41·4	29·8	23·9	1·5	Neg	—	—	33	29·19	1·2
T. zimbabwensis	7	2·0	0·05	1·0	1·4	0·1	589	366·1	17·1	15·3	9·4	0·6	Neg	—	—	62	9·6	2·3

and *T. zimbabwensis* detected in mice 60 days p.i. were significantly smaller than those detected in varans and caimans at 60 days p.i. (Table 4).

The degenerative patterns observed in the muscles of caimans and varans were very similar when comparing *T. papuae* with *T. zimbabwensis*. At 60 days p.i., in caimans, larvae were enclosed in muscle fibres, which were disarranged in transversal sections, as revealed by light microscopy. Neither a nurse cell-like structure nor a fibrous envelope was observed around the larva-muscle complex (Fig. 1A). The TEM micrographs revealed that the structure of the muscle was altered: the typical fibrous structure had disappeared and several vacuoles (i.e. degenerate mitochondria) were present (Fig. 1B and C). As a consequence of the sarcomer dissociation, clusters of highly degenerated mitochondria were coalesced around the larva (Fig. 1D). The degenerative events in the muscle cells of infected varans were quite similar to those observed in caimans. In varans, light microscopy revealed larvae enclosed in a disarranged fibrous structure (Fig. 2A). Neither a nurse cell-like structure nor inflammatory cell infiltrates were observed. The TEM micrographs showed an interruption in the continuity of the fibrous architecture: clusters of dissociated muscle fibres and vacuoles (i.e. degenerate mitochondria) were present (Fig. 2B and C). In the muscle, where sarcomers were totally desegregated, a large number of mitochondria were coalesced (Fig. 2D). In some mitochondria, the matrix was partially conserved, whereas in others it was totally vacuolated (Fig. 2D).

In mice infected with *T. papuae* and *T. zimbabwensis*, radical changes had occurred in the micro-architecture of the muscle fibre around the muscle larvae at 60 days p.i. Specifically, the muscle larvae appeared to be enclosed in a fibrous structure delimited by an irregular coat. As expected for non-encapsulated species, the collagen capsule that is typical to encapsulated species was not present, and no infiltration of inflammatory cells was observed around the infected muscle fibres (Fig. 3A). The TEM micrographs showed a nurse-cell in which myofilaments were totally disarranged and interspersed with clusters of vacuolated mitochondria, small vesicles, tubules containing electron-dense material and lamellar bodies (Fig. 3B). In some samples, the texture of the muscle cell around the intracellular larvae appeared to be partially conserved (Fig. 3C). In the outer zone of the nurse-cell, a thin layer of collagen fibres was observed (Fig. 3C). In some degenerate muscle cells, clusters of collagen fibres were scattered in the nurse-cell (Fig. 3D).

The multiplex PCR analysis of adult worms and muscle larvae collected from the different host species confirmed that the parasites belonged to the *Trichinella* species used to infect them. At 40 days p.i., CD1 mice inoculated with larvae of *T. papuae* and *T. zimbabwensis* collected from the muscles of the four

Table 3. Average number of *Trichinella* larvae per g of muscle in three varans and three caimans infected with *T. papuae* or *T. zimbabwensis*

	T. papuae						*T. zimbabwensis*					
Muscles	Varan			Caiman			Varan			Caiman		
Tongue	158	2529	3361	7	17	51	234	1028	1189	4	14	18
Anterior legs	291	1526	2500	3	88	143	357	631	893	9	39	75
Intercostal	288	1459	1700	4	23	45	251	495	649	7	9	23
Posterior legs	243	827	1300	4	14	42	89	337	507	2	12	19
Spinal column	238	891	1076	4	9	11	76	332	426	2	6	10
Tail	253	1465	1466	2	7	12	113	546	712	3	5	7

Table 4. Morphological features and standard deviation (S.D.) of *Trichinella papuae* and *Trichinella zimbabwensis* larvae (L_1) collected from muscles of mice, varans and caimans (all measurements are in μm) 60 days post-infection

	T. papuae		*T. zimbabwensis*	
Host	Total length ±S.D.	Total width* ±S.D.	Total length ±S.D.	Total width* ±S.D.
Mice				
L_1 male	961±43†	32±6†	911±52†	26±7†
L_1 female	999±61†	33±8†	934±47†	28±6†
Varans				
L_1 male	986±32	33±5	1057±38	34±5
L_1 female	1119±23	34±7	1101±67	35±8
Caimans				
L_1 male	1093±52	35±2	1002±46	35±3
L_1 female	1120±91	36±7	1129±55	36±4

* The width was measured at the midbody.
† These measurements are statistically different from those of larvae collected from varans and caimans at the same time post-infection.

reptile species were positive (RCI = 8·4 for *T. papuae* and RCI = 25·3 for *T. zimbabwensis* in immunosuppressed mice), indicating that the larvae present in reptile muscles at 60 days p.i. were infective.

Clinical signs

In reptiles, it is very difficult to evaluate the clinical signs of disease, although it is known that the trophic activity and the moulting process are good indicators of health. In our study, the varans stopped eating for about 2 weeks, beginning at around 2 weeks after infection, which could correspond to the invasive phase of the larvae in muscles. None of the other reptiles showed any changes in trophic activity. In none of the animals were decreases in agility observed, not even in varans, which harboured up to 3000 larvae per g of muscle.

DISCUSSION

The present study shows that both *T. papuae* and *T. zimbabwensis* establish as infective muscle larvae in reptiles. This biological characteristic is consistent with the biochemical and molecular findings of these two *Trichinella* species and with their capacity to cross between them and to produce a viable but not reproductive F1 generation in a murine model (Pozio *et al.* 2002).

In a previous study, a nurse cell-like structure with a thin envelope of collagen fibres at the periphery of the muscle-larva complex had been observed in the muscles of a crocodile experimentally infected with *T. zimbabwensis* 18 months earlier (Pozio *et al.* 2002). In the present study, a period of 60 days appeared to be insufficient for the complete development of the nurse cell-larva complex in caimans and varans, whereas this time-period was sufficient in mice. These results suggest that the infective larvae of *T. papuae* and *T. zimbabwensis* produce the same degenerative patterns in the muscle cells of both cold-blooded and warm-blooded animals yet that the rapidity at which the changes in the muscle-fibre architecture occur is strongly influenced by the physiology of the vertebrate group.

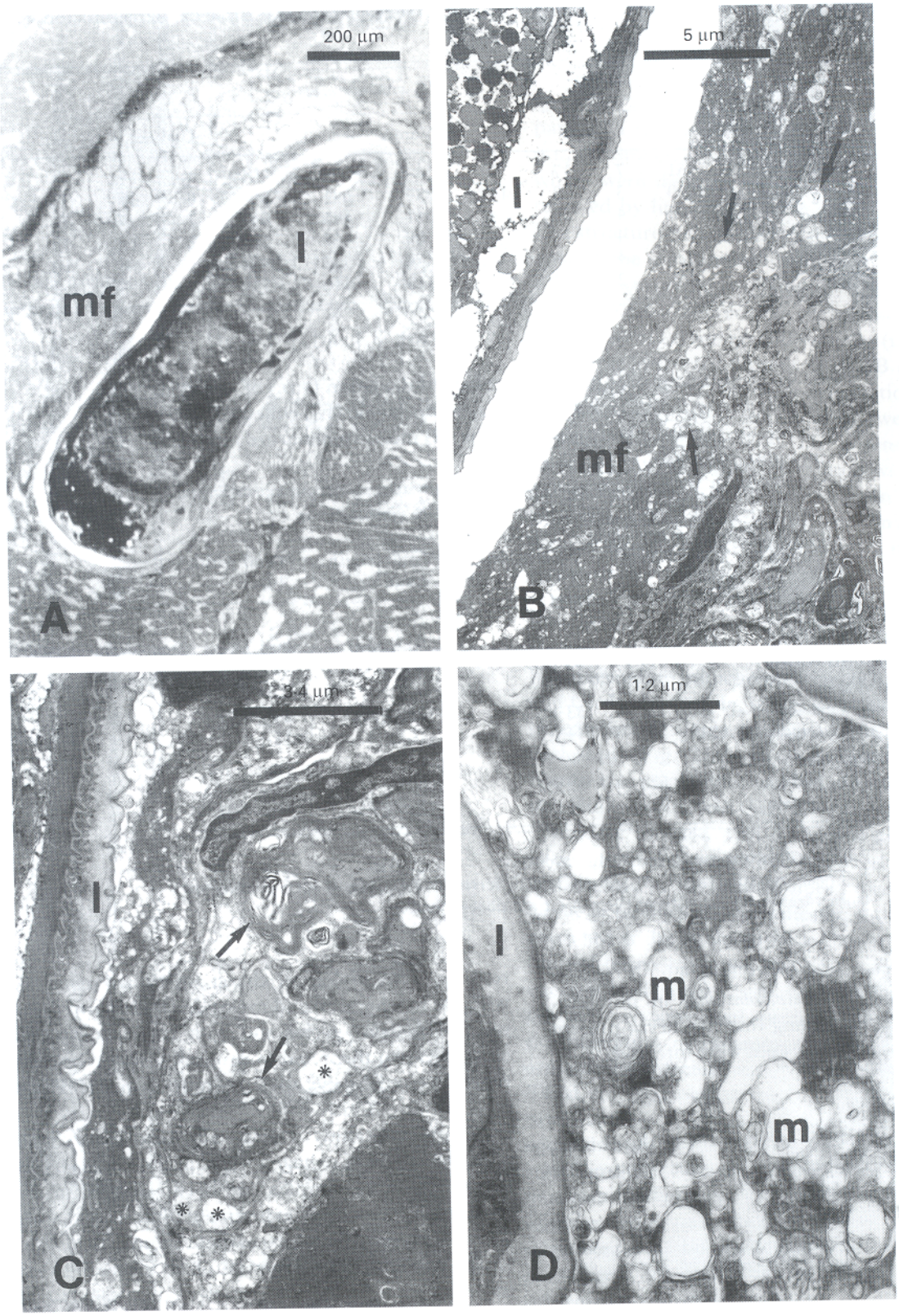

Fig. 1. Larvae of *Trichinella zimbabwensis* in muscles of a caiman 60 days post-infection. (A) Longitudinal semi-thin section showing an intracellular larva (l) enclosed in a disarranged muscle fibre (mf). Neither a nurse-cell-like structure nor fibrous bundles surrounding the larva-muscle complex are present. (B) TEM micrograph showing the loss of the contractile texture of the muscle fibre (mf) and the presence of vacuolated mitochondria (arrows). l, Larva. (C) Higher magnification of the infected muscle cell. Vacuolated mitochondria (asterisks) and residual bodies (arrows) are present. l, Larva. (D) Clusters of highly degenerated mitochondria (m) surrounding the larva (l).

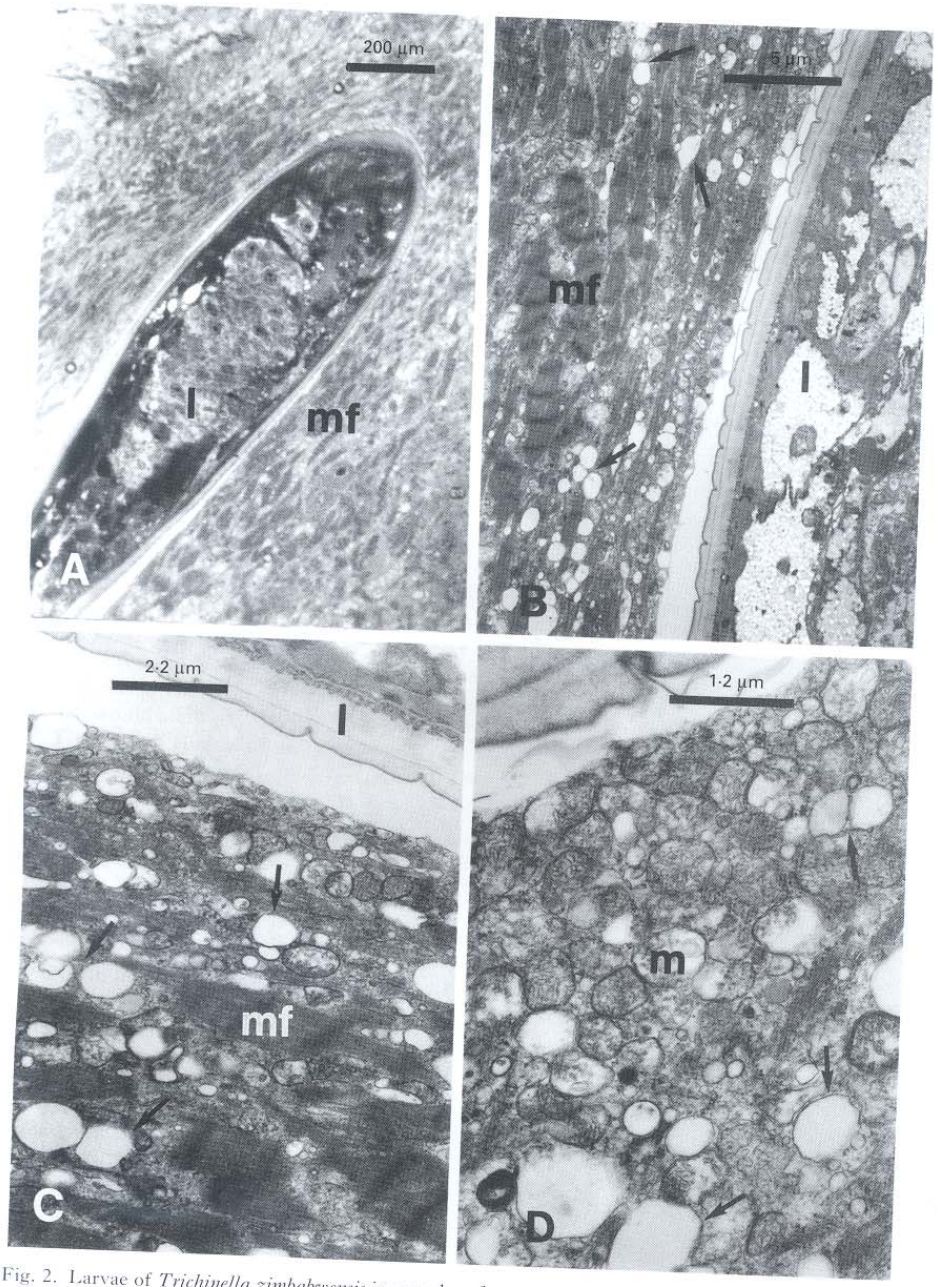

Fig. 2. Larvae of *Trichinella zimbabwensis* in muscles of a varan 60 days post-infection. (A) In the semi-thin section the larva (l) is enclosed in the disarranged muscle fibre (mf). (B) TEM micrograph showing the discontinuity of the muscle fibre (mf) architecture. Several vacuolated mitochondria (arrows) are present. l, Larva. (C) Higher magnification showing remnants of the muscle fibre (mf) and clusters of degenerate and vacuolated mitochondria (arrows). l, Larva. (D) In the region where the sarcomers are totally lacking, clusters of mitochondria (m) surround the larva. Note that the matrix of some mitochondria is beginning to become vacuolated (arrows).

In caimans and varans, at 60 days p.i., infective L_1 larvae of *T. papuae* and *T. zimbabwensis* were detected in muscle cells, yet the TEM micrographs showed that these cells had not completed their development into a nurse cell. These findings suggest that the development of newborn larvae into infective L_1 larvae occurs independently of the changes in the structure of the muscle cell and that this

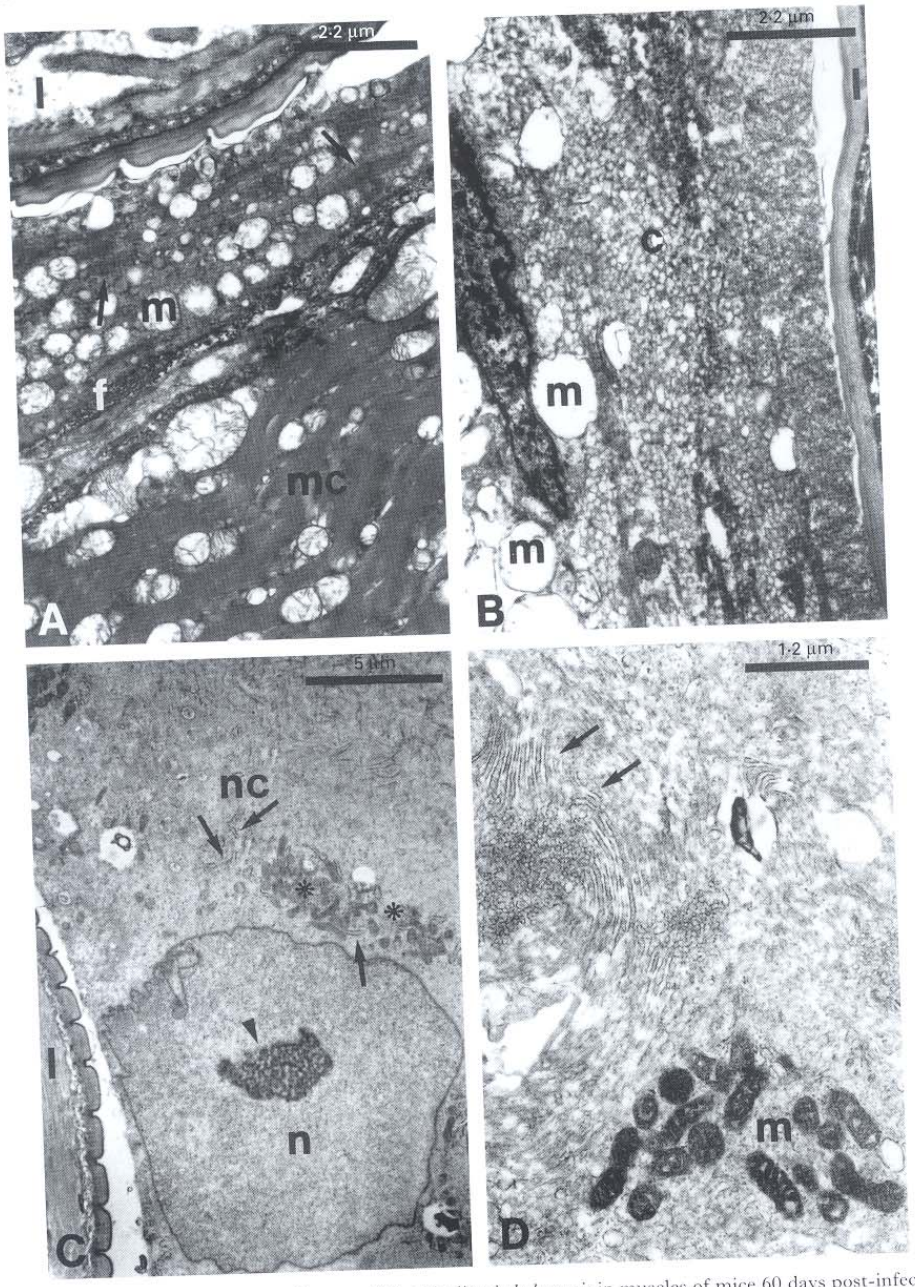

Fig. 3. TEM micrographs of larvae of *Trichinella zimbabwensis* in muscles of mice 60 days post-infection. (A) The muscle fibres that surround the larva (l) appear partially disarranged. Remnants of myofilaments (arrows) are interspersed with clusters of mitochondria (m). At the periphery of the collagen fibres (f), the texture of the muscle cell (mc) appears to be partially conserved. (B) The fibre cells are totally modified and the contractile elements are replaced by cisternae (c) of the endoplasmic reticulum. The mitochondria (m) appear to be swollen and vacuolated and the matrix has totally disappeared. (C) Nurse-cell-like structure (nc) showing a hypertrophic nucleus (n) with the nucleolus (arrow head) in the transcriptional phase. Note the clusters of small mitochondria (asterisks) and the rough endoplasmic reticulum (arrows). l, Larva. (D) Details of the nurse-cell-like structure showing clusters of small mitochondria (m) and the endoplasmic reticulum (arrows).

Table 5. Susceptibility of reptiles, mammals and birds to the eight *Trichinella* species and relationship between *Trichinella* species and the temperature range of the host

Trichinella species	Reptiles 26–32 °C	Mammals 37–40 °C	Birds 40·5–42·5 °C
Non-encapsulated			
T. papuae and *T. zimbabwensis*	Susceptible	Susceptible	Not susceptible
T. pseudospiralis	Not susceptible	Susceptible	Susceptible
Encapsulated			
T. spiralis, *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni*	Not susceptible	Susceptible	Not susceptible

development does not require the formation of the nurse cell. By contrast, these events appear to be closely correlated in homoiothermic animals (Despommier, 1998).

The finding that the muscle larvae of both *T. papuae* and *T. zimbabwensis* were smaller in mice than in reptiles at the same times post-infection suggests that larva size is an adaptive character related to the host species (Table 4). Perhaps the greater size of the larvae in reptiles is related to the slower development of the nurse cell in these poikilothermic animals, which would allow more time for the larvae to grow.

The lack of clinical signs in varans and caimans, together with the high levels of infection, is consistent with the hypothesis that these animals act as potential reservoirs of *T. papuae* and *T. zimbabwensis*. Reptiles belonging to the Varanidae and Crocodilidae families are carnivores with scavenger behaviour, which is the most important biological and ecological character of animals known to act as *Trichinella* reservoirs (e.g. fox, racoon dog, wolf, bear and hyena) (Campbell, 1988). In light of these findings, reptile meat should not be excluded as a potential source of human trichinellosis. The very low number of *T. papuae* and *T. zimbabwensis* larvae detected in pythons and turtles, in spite of a very high infecting dose, seems to suggest that these species do not play an important role in the epidemiology of *Trichinella*. This is consistent with the trophic spectrum of pythons and turtles, which probably does not include *Trichinella* carriers. Nonetheless, that the worm burden in turtles and pythons was low could have been related to the temperature at which these animals were raised (26–30 °C), which is 2 degrees lower than the temperature used for varans and caimans (28–32 °C).

Experimental infections of reptiles with *T. spiralis* have been attempted several times, yet the success of these experiments depended on the temperature at which the animals were raised. Specifically, Texas horned lizards (*Phrynosoma cornutum*) were not successfully infected at their activity temperature (20–28 °C), yet viable larvae were recovered from the muscles of these lizards when the animals had been kept at 37 °C for 1 month (Jordan, 1964). Similarly, the European turtle (*Emys orbicularis*) (Guevara Pozo & Contreras-Pena, 1966), the Caucasian Agama (*Agama caucasica*) (Asatrian, Movsessian & Gevorkian, 2000) and the long-nosed viper (*Vipera ammodytes*) (Cristea & Perian, 1999) were successfully infected with an encapsulated species of *Trichinella* (probably *T. spiralis*) only when kept at 37 °C. Encapsulated species (*T. spiralis*, *T. nativa*, *T. britovi*, *T. murrelli* and *T. nelsoni*) and one non-encapsulated species (*T. pseudospiralis*) have been tested in caimans raised at 29 °C without success (Kapel *et al.* 1998). Among other cold-blooded vertebrates, attempts to infect 5 species of fresh water fish, raised at 18–22 °C, with *T. britovi* failed (Moretti *et al.* 1997).

These data, together with molecular (Zarlenga *et al.* 2004) and biochemical data (La Rosa, Marucci & Pozio, 2003), suggest that temperature plays a role in the speciation mechanism of *Trichinella*. Specifically, the non-encapsulated species *T. papuae* and *T. zimbabwensis* can develop at temperatures ranging from 26 °C to 40 °C, both in cold- and warm-blooded animals (i.e. tropical reptiles and mammals); the non-encapsulated species *T. pseudospiralis* can develop at temperatures from 37 °C to 42·5 °C (i.e., in homoiothermic animals, specifically, mammals and birds); and all of the encapsulated species develop at 37–40 °C (i.e. only in mammals) (Table 5). The presence in the genus *Trichinella* of different species which infect reptiles and mammals or birds and mammals or only mammals strongly suggests that this nematode group is ancient and that it evolved with the evolution of reptiles into mammals and birds (i.e. from poikilothermic to homoiothermic vertebrates).

Finally, to the best of our knowledge, *T. papuae* and *T. zimbabwensis* are the only two parasites known to complete their entire life-cycle independently of whether the host is homoiothermic or poikilothermic. This seems to indicate that these two *Trichinella* species are capable of activating different physiological mechanisms, according to the specific vertebrate class.

We are grateful to Mr Claudio Bagnoli for his assistance in raising the reptiles. This work was in part funded by the project 'TRICHIPORSE' of the European Union, Contract QLK1-CT-2001-01156.

REFERENCES

ASATRIAN, A., MOVSESSIAN, S. & GEVORKIAN, A. (2000). Experimental infection of some reptiles with *T. spiralis* and *T. pseudospiralis*. *Abstract Book, p. 154, The 10th International Conference on Trichinellosis, 20–24 August, Fontainebleau, France.*

BLAIR, L. S. (1983). Laboratory Techniques. In Trichinella *and Trichinosis* (ed. Campbell, W. C.), pp. 563–570. Plenum Press, New York and London.

CAMPBELL, C. W. (1988). Trichinosis revisited, another look at modes of transmission. *Parasitology Today* **4**, 83–86.

CRISTEA, G. & PERIAN, A. (1999). Experimental infestation with *Trichinella* larvae in vipers. *Revista Romana de Parasitologia* **9**, 45.

DESPOMMIER, D. D. (1998). How does *Trichinella spiralis* make itself at home? *Parasitology Today* **14**, 318–323.

GUEVARA POZO, D. & CONTRERAS-PENA, J. A. (1966). *Trichinella spiralis* (Owen 1835), experiencias de infestacion en diversos vertebratos. *Revista Iberica de Parasitologia* **26**, 239–288.

JORDAN, C. A. (1964). Experimentally trichinized Texas horned lizards. *Journal of Parasitology* **50**, 24.

KAPEL, C. M., WEBSTER, P., BJORN, H., MURRELL, K. D. & NANSEN, P. (1998). Evaluation of the infectivity of *Trichinella* spp. for reptiles (*Caiman sclerops*). *International Journal for Parasitology* **28**, 1935–1937.

LA ROSA, G., MARUCCI, G. & POZIO, E. (2003). Biochemical analysis of encapsulated and non-encapsulated species of *Trichinella* (Nematoda, Trichinellidae) from cold- and warm-blooded animals reveals a high genetic divergence in the genus. *Parasitology Research* **91**, 462–466.

MORETTI, A., PIERGILI FIORETTI, D., PASQUALI, P., MECHELLI, L., ROSSODIVITA, M. E. & POLIDORI, G. A. (1997). Experimental infection of fish with *Trichinella britovi*: biological evaluations. In *Trichinellosis* (ed. Ortega Pierres, G., Gamble, R., van Knapen, F. & Wakelin, D.), pp. 135–142. Centro de Investigacion y Estudios Avanzados del Instituto Politecnico Nacional, Mexico, D.F. Mexico.

OWEN, R. (1835). Description of a microscopic entozoon infesting the muscles of the human body. *Transactions of the Zoological Society of London* **1**, 315–323.

POZIO, E. (1987). Isoenzymatic typing of 23 *Trichinella* isolates. *Tropical Medicine and Parasitology* **38**, 111–116.

POZIO, E. (2001). New patterns of *Trichinella* infections. *Veterinary Parasitology* **98**, 133–148.

POZIO, E., FOGGIN, C. M., MARUCCI, G., LA ROSA, G., SACCHI, L., CORONA, S., ROSSI, P. & 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. & LA ROSA, G. (2003). PCR-derived methods for the identification of *Trichinella* parasites from animal and human samples. *Methods in Molecular Biology* **216**, 299–309.

POZIO, E., OWEN, I. L., LA ROSA, G., SACCHI, L., ROSSI, P. & CORONA, S. (1999). *Trichinella papuae* n.sp. (Nematoda), a new non-encapsulated species from domestic and sylvatic swine of Papua New Guinea. *International Journal for Parasitology* **29**, 1825–1839.

SHAIKENOV, B. S. (1980). Spontaneous infection of birds with *Trichinella pseudospiralis* Garkavi, 1972. *Folia Parasitologica* **27**, 227–230.

ZARLENGA, D. S., CHUTE, M. B., MARTIN, A. & KAPEL, M. O. (1999). A single multiplex PCR for unequivocal differentiation of six distinct genotypes of *Trichinella* and three geographical genotypes of *Trichinella pseudospiralis*. *International Journal for Parasitology* **29**, 1859–1867.

ZARLENGA, D. S., LA ROSA, G., POZIO, E. & ROSENTHAL, B. (2004). Identification and classification within the genus *Trichinella*, with special emphasis on non-encapsulated species. *Veterinary Parasitology* (in the Press).