Experimental infections of baboons (*Papio* spp.) and vervet monkeys (*Cercopithecus aethiops*) with *Trichinella zimbabwensis* and successful treatment with ivermectin

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ABSTRACT

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Experimental *Trichinella zimbabwensis* infections were established in three baboons (*Papio* sp.) and four vervet monkeys (*Cercopithecus aethiops*) and the clinical-pathological manifestations assessed. The infected animals showed clinical signs ranging from fever, diarrhoea, periorbital oedema and muscular pain in varying degrees. One baboon became blind due to the infection. Levels of creatinine phosphokinase and lactate dehydrogenase increased to reach a peak on Day 42 post-infection (pi) for both baboons and monkeys. Blood parameters such as packed cell volume, levels of red blood cells and white blood cells did not change significantly from the normal ranges except for the levels of eosinophils which peaked above the normal ranges at Day 28 and 56 pi in baboons and at Day 56 pi in monkeys.

Two baboons and two monkeys died during the course of the experiment. They were emaciated and showed lesions such as ascites, hydropericardium, congested liver and enlarged gall bladder. Histopathological findings of various muscles included a basophilic transformation of muscle cells, the disappearance of sarcomere myofibrils and basophilic sarcoplasm with the presence of *Trichinella* larvae in the sarcoplasm. These changes were mainly in the massetter and were of various intensities in the tail, gastrocnemius and biceps muscles. Five consecutive treatments with an oxfendazole-levamisole combination on surviving animals failed to clear the infection whereas ivermectin cleared the infection after one treatment in two monkeys and after two treatments in a baboon.

Keywords: Baboons, *Cercopithecus aethiops,* ivermectin, levamisole, monkeys, oxfendazole, *Papio* spp., pathology, *Trichinella zimbabwensis*

INTRODUCTION

Trichinellosis is a parasitic zoonosis manifesting in humans as a syndrome with specific clinical signs and symptoms of variable intensity (Kocieka 2000). The infection is contracted by eating raw or undercooked infected meat resulting in various symptoms that range from mild subclinical to classic forms depending on the extent of invasion, the parasite load, the species of *Trichinella* involved and the immune response of the host (Clausen, Meyer, Krantz, Moser, Gomme, Kayser, Albrectsen, Cui, Wang, Wu & Jin 1997; Taratuto & Venturiello 1997; Kocieka 2000).

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The description of a new non-encapsulated species, *Trichinella zimbabwensis*, by Mukaratirwa & Foggin (1999), Pozio Foggin, Marucci, La Rosa, Sacchi, Corona, Rossi & Mukaratirwa (2002), which affects both mammals and reptiles is a new addition to the already existing five encapsulated species infecting only mammals (*Trichinella spiralis*, *Trichinella nativa*, *Trichinella britovi*, *Trichinella murrelli* and *Trichinella nelsoni*) and the two non-encapsulated species *Trichinella pseudospiralis* occurring in both mammals and birds and *Trichinella papuae* occurring in both mammals and reptiles (Pozio, Owen, Marucci & La Rosa 2004a).

The only report of a natural infection of *T. zimbabwensis* is in farmed crocodiles (*Crocodylus niloticus*) in Zimbabwe (Foggin, Vassilev & Widdowson 1997), where animals are reared for their meat and skin.

Experimental infections have shown that *T. zimbabwensis* readily infects domestic pigs and laboratory mice and rats (Mukaratirwa & Foggin 1999; Mukaratirwa, Nkulungo, Matenga & Bhebhe 2003) confirming its chance to complete the entire life cycle irrespective of whether the host is homoiothermic or poikilothermic (Pozio, Marucci, Casulli, Sacchi, Mukaratirwa, Foggin & La Rosa 2004b). However, the epidemiology and the public health significance of this parasite are still obscure.

Non-human primates by virtue of their close relationship to humans are good models for the study of the clinical-pathological manifestations of trichinellosis and its treatment. The objective of this study was to evaluate the clinical-pathological manifestations of baboons (*Papio* spp.) and vervet monkeys (*Cercopithecus aethiops*) experimentally infected with *T. zimbabwensis* and their response to treatment.

MATERIALS AND METHODS

Experimental animals

Two adult female (codes SFB and BFB) and one male (code SMB) baboons and two adult female (codes BFM and SFM) and two male (codes SMM and BMM) vervet monkeys were randomly selected for the study. These animals were born and bred at the animal house unit of the Faculty of Veterinary Science, University of Zimbabwe. They were reared on a commercial primate diet and water was available *ad libitum*. A week before the infection, biopsies were collected from the animals while they were under anaesthesia with a combination of xylazine (2 mg/kg) and ketamine (10 mg/kg) administered intramuscularly, from the massetter, biceps and gastrocnemius muscles to ensure that the animals were free from *Trichinella* spp. infection.

Restraint and experimental infection of animals

The *T. zimbabwensis* used was an isolate derived from a naturally infected crocodile. It was maintained in the laboratory by serial passages in rats. First-stage larvae were collected from five skinned carcasses of infected rats following methods described by Pozio *et al*. (2002).

Each experimental animal was anaesthetized with a combination of xylazine (2 mg/kg) and ketamine (10 mg/kg) intramuscularly. Two of the baboons were infected with doses ranging from 9 000–16 600 larvae per kg, whereas 17 600–25 000 larvae per kg were used to infect three of the monkeys (Table 1). One baboon and one monkey were kept as uninfected controls.

Sample collection and analysis

Muscle biopsies and blood samples were collected while the animals were under anaesthesia at weekly intervals after infection and at each time of collection the rectal temperatures were recorded. The muscle biopsies were processed by the HCL-pepsin digestion method (Pozio *et al.* 2002) to detect the L-1 stage of *T. zimbabwensis* and the resulting larval counts were expressed as larvae per gram (lpg) of muscle. A portion of the muscle biopsy from each muscle was preserved in 3% glutaraldehyde and processed for electron microscopy. The levels of creatinine kinase (CK) and lactate dehydrogenase (LDH) were determined in serum samples.

The clinical signs were recorded daily until the end of the experiment. The animals were treated for 5 consecutive days with a combination of levamisole (40 mg/kg) and oxfendazole (50 mg/kg) and dexamethasone (5 mg/kg) on Days 35 and 51 post-infection (pi) for the monkeys and baboons, respectively. An intramuscular injection of ivermectin (300 µg/kg) was administered after the levamisole-oxfendazole treatment. The efficacy of treatment was monitored through weekly collection of muscle biopsies from the masseter muscles to detect viable first stage larvae.

Histological and ultrastructure studies of the nurse cell-parasite complex

Muscle biopsies and muscles of animals which died during the study were cut into small sections and in

part fixed in 10 % buffered formalin and in part preserved in glutaraldehyde for ultrastructural studies. For histology, fixed specimens were dehydrated using different grades of alcohol, cleared in xylene embedded in paraffin wax at 58–60 °C and finally, 3 µm sections were stained by haematoxylin and eosin stain (Anderson & Gordon 1996). Sections were examined by light microscopy using 5–40X magnification.

Small pieces of the biopsy from the masseter muscle of a baboon, collected 51 days pi, were fixed for 4 h at 4 °C in 0.1 M cacodylate buffer (pH 7.2) containing 2.5 % glutaraldehyde. The samples were then washed in the same buffer and post-fixed for 1.5 h at 4 °C with 1 % OsO_4 in cacodylate buffer. All samples were dehydrated in ethanol and embedded in Epon 812 for sectioning. For light microscopy, semithin sections (0.5 µm) were stained with 0.5 % toluidine blue. Thin sections (80 nm), stained with uranyl acetate and lead citrate, were examined under a Zeiss EM 900 transmission electron microscope.

RESULTS

Parasitological aspects

The pre-infection muscle biopsies from all experimental animals were negative for *Trichinella* larvae. In the infected baboons that died, muscles with the highest lpg were the diaphragm, psoas, laryngeal, and tongue in this order (Table 1). In monkeys, the diaphragm, tongue and masseter had the highest lpg compared to the other muscles (Table 1). No relationship was observed between the infective dose, the severity of the disease and the lpg in both baboons and monkeys.

Clinical manifestations

The clinical signs observed are summarized in Table 2. The time between infection and onset of the first clinical signs ranged from 9 to 30 days pi. The rectal temperatures ranged between 38.4–40 °C in the baboons and 38.9–39.9 °C in the monkeys for a period of 8 weeks.

TABLE 1 Number of larvae per gram (Lpg) of muscle in baboons and monkeys that died due to *Trichinella zimbabwensis* infection

	Larvae per gram of muscle			
Animal code	SFB	SMB	BMM	BFM
Infective dose/kg	16 600	16 400	17 600	25 000
Day of death pi	50	49	35	30
Diaphragm	621	9 987	255	100
Massetter	260	465	ND	168
Tongue	820	888	215	165
Psoas	965	1 819	ND	ND
Laryngeal muscle	545	1 600	135	ND
Digital flexus	552	287	75	86
Hamstring muscle	910	479	115	ND
Eye	37	144	ND	1

ND = not determined
pi = post infection

TABLE 2 Clinical manifestations of baboons and monkeys infected with *Trichinella zimbabwensis*

Animal code	**SFB**	**SMB**	**BFB**	**BFM**	**SFM**	**SMM**	**BMM**
Infective dose/kg	**16 600**	**16 400**	**7 300**	**25 000**	**24 500**	**24 500**	**17 600**
Clinical signs and symptoms	**Day of the first manifestation pi**						
Fever	9	11	16	14	13	18	10
Diarrhoea	16	16	16	16	19	21	21
Depression	12	12	12	12	12	12	12
Periorbital oedema	30	30	30	30	30	30	30
Muscular pain	30	30	30	30	30	30	30
Alopecia	26	26	26	26	26	26	26
Blindness	38	no	no	no	no	no	no
Death	49 dpi	50 dpi	s	30 dpi	s	36 dpi	s

pi = post infection, dpi = days post infection, no = not observed, s = survived

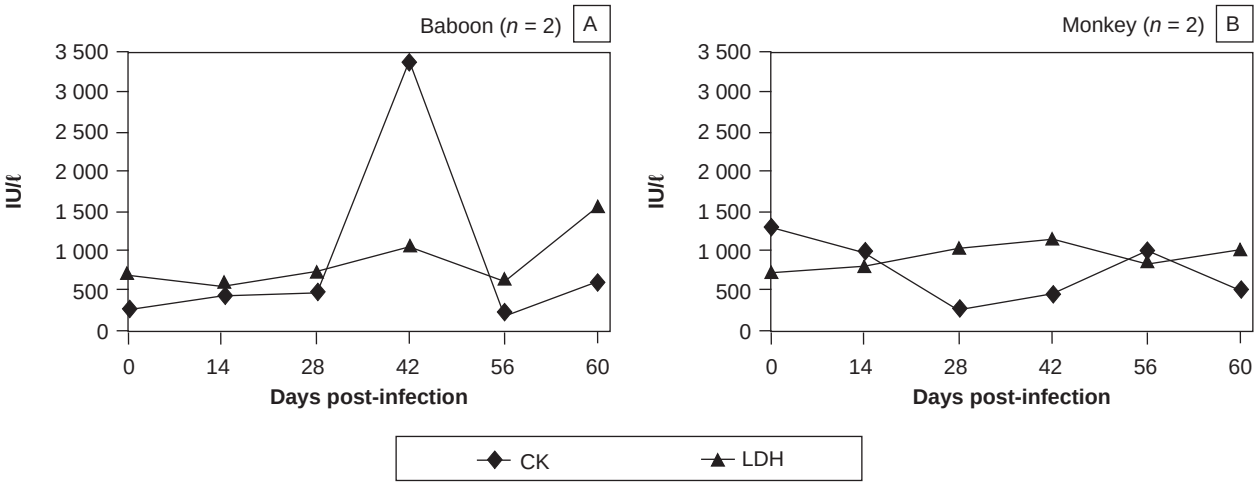

FIG. 1 Kinetics of creatinine phosphokinase (CK) and lactate dehydrogenase (LDH) in a baboons BFB and SMB (A) and monkeys SFM and BMM (B) infected with *Trichinella zimbabwensis*

Diarrhoea was observed in all infected animals from Day 16 pi (Table 2). Gradual depression and deterioration in body condition accompanied by alopecia, muscle pain on palpation and wasting were observed in all infected animals and these became marked from Day 26 pi. Blindness of both eyes was only observed in one baboon (SFB).

Clinical biochemistry and blood parameters

Levels of CK ranged from 197 to 3409 UI/ℓ in baboons and from 78 to 2679 UI/ℓ in the infected monkeys. The levels of CPK in the infected baboons gradually increased from Day 0 to Day 28 pi and then increased tenfold to reach a peak on Day 42 pi. After the peak, there was a sharp drop to reach normal levels from Day 56 pi (Fig. 1A and B). In infected monkeys, the CK levels slightly decreased from Day 0 pi to Day 28 pi before starting to increase with a peak on Day 56 pi.

Levels of LDH ranged from 118 to 1587 UI/ℓ in baboons and from 203 to 1469 UI/ℓ in monkeys. In both monkeys and baboons, LDH reached a peak at day 42 pi.

Packed cell volume (PCV) and white cell counts did not change significantly in the course of the infection. However, the level of eosinophils peaked at Day 28 and 56 pi above the normal ranges in baboons and monkeys, respectively.

Treatment

The 5-day-treatment with oxfendazole-levamisole combination failed to kill *Trichinella* larvae; on the contrary, the single ivermectin treatment successfully destroyed the larvae in all the surviving infected monkeys (BMM and SMM) but in the baboon (BFB) a low level of larvae was still detected in the biopsy (Table 3). In this animal, no living larvae were detected in another muscle biopsy collected after a second treatment with ivermectin.

Histopathology and ultrastructure of muscle tissues

All the animals which died due to the infection (SFB, SMB, BFM and FMM) were emaciated and showed ascites, hydropericardium, congested livers and enlarged gall bladders. Histopathological findings of various muscles included a basophilic transformation of muscle cells, the disappearance of sarcomere myofibrils and a sarcoplasm that was basophilic. The changes also included pale, swollen muscle fibres with the presence of *Trichinella* larvae in the sarcoplasm, and the loss of myofibrils in affected muscles. These changes were mainly in the masseter, and were of various intensities in the tail, gastrocnemius and bicep muscles. Biliary fibrosis, eosinophilic myositis, lymphocytic enteritis and dilatation of lymphatic vessels of the large and small intestines were also observed in SFB.

In muscles infected with *T. zimbabwensis*, clear modifications of the muscle fibre architecture were observed. The myofilaments appeared modified in a nurse-cell-like structure (Fig. 2A). The thick collagen capsule was absent, and no inflammatory reaction was observed around the nurse cell-larva complex. Transmission electron micrographs revealed the ultrastructural changes in the muscle fibres. In the nurse-cell-like structure the contractile elements

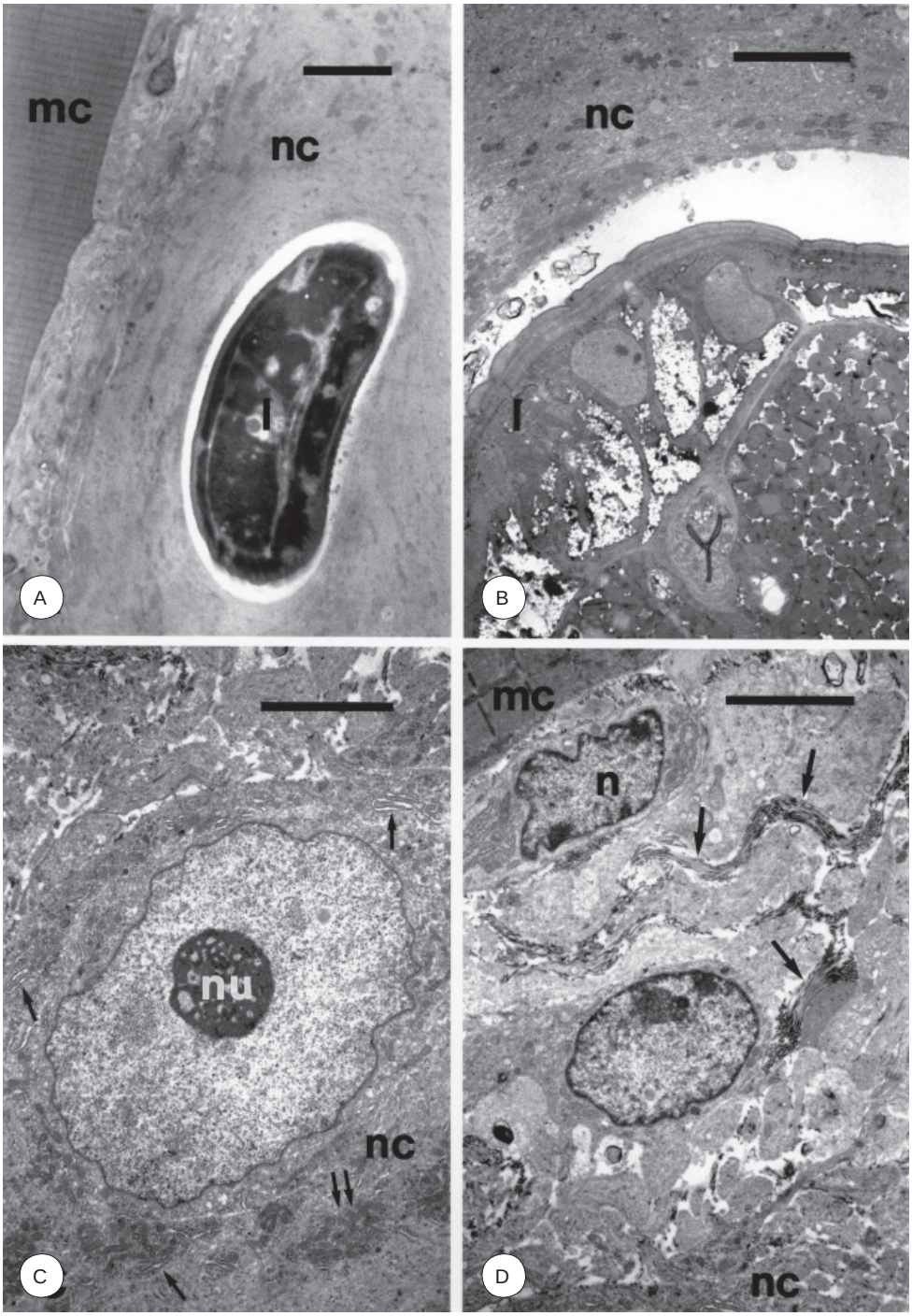

FIG. 2 Larvae of *Trichinella zimbabwensis* in muscles of a baboon 51 days post infection

A Longitudinal section of an intracellular larva (l), surrounded by a nurse-cell-like structure (nc). Note the absence of the typical collagen capsule. No inflammatory cells were observed around the infected muscle fibres. mc = normal muscle cell. Bar = 200 µm

B TEM micrograph showing a *Trichinella* larva (l) surrounded by a nurse-cell-like structure (nc). Note the loss of contractile elements. Bar = 5 µm

C Details of the nurse-cell-like structure (nc) showing a hypertrophic nucleus with the nucleolus (nu) in the transcriptional phase. Bar = 5 µm

D Details of the outer zone of the nurse-cell-like structure (nc) showing bands of collagen fibres (arrows). Mc = normal muscle cell, n = fibroblast nucleus, nc = nurse-cell-like structure. Bar = 5 µm

TABLE 3 Larvae per gram (lpg) of masseter muscle following treatment with oxfendazole-levamisole combination and subsequent use of ivermectin in three monkeys and one baboon infected with *Trichinella zimbabwensis*

Animal code	lpg after oxfendazole-levamisole treatment	lpg after one ivermectin treatment	lpg after second ivermectin treatment
BMM	11.1	0	–
SMM	9.5	0	–
*SFM	6.4	–	–
BFB	44.1	7.5	0

* Died 36 days post infection

were replaced by a sarcoplasmic reticulum (Fig. 2B). At higher magnification, in the cytoplasm of this structure, characterized by the presence of a smooth and rough endoplasmic reticulum, it was possible to observe hypertrophic nuclei each of which contained a prominent nucleolus (Fig. 2C). This detail was indicative of the presence of an intense transcriptional activity. At the peripheral zone of the nurse-cell larva complex, bundles formed by irregularly arranged collagen fibres were observed (Fig. 2D).

DISCUSSION

In this study, *T. zimbabwensis* was able to establish in both baboons and monkeys and the clinical signs observed did not differ from those already reported in humans infected with the non-encapsulated species *T. pseudospiralis* (Ranque, Faugére, Pozio, La Rosa, Tamburrin, Pellissier & Brouqui 2000).

The normal rectal temperatures of baboons and monkeys are 36–39 °C and 36–40 °C, respectively (Poole 1987). In the course of trichinellosis in humans, fever is one of the most common sign expected (Clausen *et al*. 1997) and in this study it was recorded in both infected baboons and monkeys.

The observation of diarrhoea in infected animals in this study around Day 16 pi could be related to the early stages of intestinal invasion which manifests clinically as diarrhoea of several days' duration and abdominal pains (Kociecka 2000). The pathophysiology of trichinellosis in the small intestines includes restricted absorption, disturbed motility of the intestines, diarrhoea or constipation and the presence of cellular infiltrates in the lamina propia (Ruitenberg, Elgersma, Kruizing & Leenstra 1977; Castro & Bullick 1983; Gustowska, Ruitenberg, Elgersma & Kociecka 1983; Ruitenberg & Buys 1986). In humans infected with *T. spiralis*, pathomorphology of the intestinal mucosa includes lesions to the epithelium involving the brush border, lamina propia and smooth muscles of the jejunum, deformation of villi, stimulated enterocyte proliferation at villi margins, hyperplasia in the crypts of Lieberkuhn and the presence of massive cellular infiltrates in the mucosal sublayer (Castro & Bullick 1983). Lesions may persist until Day 65 pi (Kociecka 1981a; Gustowska *et al.* 1983). In this study, similar intestinal lesions were noted from a baboon that died on Day 49 pi. Periorbital and facial oedema is a common clinical sign of *T. spiralis* and *T. pseudospiralis* trichinellosis (Kociecka 1981a) and this agrees with the findings from this study where all affected animals manifested periorbital oedema.

The observation that the intensity of clinical disease and the muscle larval yields were not proportional to the initial infective doses given to the animals might suggest host factors that determine disease resistance since the parasite and environment factors were similar for all the animals.

The experimental infection of Swiss CD1 mice reveals the existence of structural changes of the infected muscle cells 4 months pi. These structures were very similar to those observed in crocodiles 18 months pi (Pozio *et al*. 2002). In the baboon infected with the same species of *Trichinella*, the myofilaments of the infected muscle cell had lost their integrity and appear transformed in a nurse-cell-like structure surrounded by a bundle of collagen fibres irregularly arranged (a true collagen capsule being absent) 51 days pi. These results suggest that *T. zimbabwensis* is able to infect different mammalian species in which it reproduces the same morphological changes as have been described in reptiles (Pozio *et al*. 2002, 2004b).

The choice of treatment for trichinellosis in humans varies according to the clinical severity of the infection and the strain or species of *Trichinella* involved (Andrews, Ainsworth & Abernethy 1994). Drugs administered to infected humans include anthelmintics, glucocorticosteroids, immuno-modulating drugs and preparations which compensate protein and electrolyte deficits (Kociecka 2000). In this study, since

adult worms had long been expelled the treatment was targeted at the larvae in muscles. Injectable ivermectin was able to clear the infection after 7 days following a single dose unlike the oxfendazole-levamisole combination which failed to clear the infection in both the monkeys and baboons when administered for 5 consecutive days. However, it was not determined whether the effects of ivermectin were potentiated by the earlier use of the combination of oxfendazole-levamisole or were due solely to the anthelmintic effects of the drug.

Disturbances in blood muscle enzyme activities are related primarily to activities of those enzymes that are muscle bound, and these include CK, LDH and occasionally aspartate aminotransferase (AST). This follows the damage of the muscle cell by the L-1 resulting in an increase in permeability of the muscle cell membranes (Boczon, Winiecka, Kociecka, Hadas & Andrezejewska 1981). In 75–90 % of human cases of trichinellosis, an increase in the activity of CK was noted between Weeks 2 and 5 pi and involved an increase of several fold and LDH levels fluctuated from Week 1 to Week 6 pi. In this study, LDH and CK peaked around Day 42 pi in both baboons and monkeys although there were phases where the levels of both enzymes dropped. This is unsurprising since the increase in CK and LDH activity in blood is not correlated to the clinical severity of trichinellosis (Boczon *et al.* 1981) and there could also be some host differences in reacting to different *Trichinella* spp. The peak around Day 42 pi of both enzymes could be related to the fact that almost all of the parasites are expected to be in the muscles by that period resulting in marked muscle membrane damage (Boczon *et al*. 1981).

The settling of *Trichinella* larvae in the muscles results in the basophilic transformation of the muscle cells, encapsulation of the larvae and the development of a capillary network surrounding the affected cell (Gabryel & Blotna 1969; Gabryel, Gustowska & Blotna-Filipiak 1995). These changes appear early and persist for as long as the encapsulated larva remains viable. In this study the basophilic changes were observed together with an eosinophilic infiltration around the affected cell. The muscles that were preferred most by the parasite were the diaphragm, psoas, laryngeal, tongue and masseter muscles in that order. The masseter muscle, however, had the greatest histopathological changes attributable to the parasite. *Trichinella pseudospiralis* and *T. spiralis* larvae have been shown to have the massetter muscle as their main predilection sites in monkeys (Kociecka 1981b), and this probably explains the high histopathological changes in the massetter muscles from our animals.

Leukocytosis appears early and rapidly increases between Weeks 2 and 5 of the disease and subsides in parallel to clinical signs while eosinophilia persists (Kociecka 2000). However, an extremely severe course of trichinellosis may be accompanied by eosinopenia and/or lymphopenia which is a manifestation of immunosuppression (Dupoy-Camet, Paugam, Picard & Ancelle 1994). In our study, eosinophilia was noted, albeit between Weeks 4 and 8 pi. However, eosinophilia has been reported to regress slowly and may persist from several weeks to 3 months pi, and no relationship has been noted between the clinical course of disease and eosinophilia (Dupoy-Camet *et al.* 1994).

The clinical, parasitological, pathological, biochemical and haematological picture of *T. zimbabwensis* infection in the baboons and monkeys in this study closely simulates those reported for *T. pseudospiralis* and *T. spiralis* infection in humans and other primates (Clausen *et al*. 1997; Taratuto & Venturiello 1997; Ranque *et al*. 2000). The fact that *T. zimbabwensis* could successfully infect non-human primates to give clinical features characteristic of other *Trichinella* species in humans could have relevant implications in human infection as chances of humans getting infected with *T. zimbabwensis* are present in Zimbabwe where crocodile meat is consumed.

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