Transmission studies on *Trichinella* species isolated from *Crocodylus niloticus* and efficacy of fenbendazole and levamisole against muscle L1 stages in Balb C mice

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ABSTRACT

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Forty-four Balb C mice, aged 18 weeks were infected with crocodile (*Crocodylus niloticus*)-derived *Trichinella* species. Of the infected mice, 32 were randomly divided into two groups each containing equal numbers of males and females; levamisole treated group and fenbendazole treated group. Each group was randomly subdivided into two subgroups as follows: levamisole group (subgroup 1: treated with levamisole on day 35 post infection, and subgroup 2: treated with levamisole on days 35 and 42 post infection) and fenbendazole group (subgroup 1: treated with fenbendazole on day 35 post infection and subgroup 2: treated with fenbendazole on days 35 and 42 post infection). The first subgroups treated on day 35 post infection were slaughtered on day 42 post infection and the second subgroups were treated on day 35 and day 42 post infection and slaughtered on day 49 post infection. Two female mice were infected a day after mating and were slaughtered together with the offspring on day 64 post-infection. Ten infected control mice were given 1 mℓ distilled water orally as placebo, and five of these were slaughtered on day 42 post infection. The results showed that the mean reproductive capacity index of this strain (RCI) in Balb C mice was 110. There was a significant reduction ($P < 0.01$) in larval counts in the single treatment groups (day 35) and in the double treatment groups (days 35 and 42) for both anthelmintics when compared the number of parasites in the control groups. After a single treatment, levamisole reduced the infection by 79.9 % and fenbendazole by 76.7 %. Following double treatments, levamisole reduced the infection by 95.5 % and fenbendazole by 99.1 %.There was evidence that the infected pregnant mice transmitted the parasite to their offspring. It is not certain whether the parasite was transmitted congenitally or transmammary. Alternative ways of controlling the parasite in crocodile farms in Zimbabwe are discussed.

Keywords: Balb C mice, crocodile, fenbedazole, levamisole, transmission, *Trichinella* sp.

INTRODUCTION

Recently, the crocodile (*Crocodylus niloticus*) has been found to be a host of a *Trichinella* species (Foggin, Vassilev & Widdowson 1997) which has yet to be identified. It is postulated that the feeding of offal and the recycling of crocodile meat enhances the transmission of this parasite (Foggin *et al.* 1997). Studies on the transmission and infectivity of this parasite to the indigenous Zimbabwean pig (*Sus scrofa*) and rat (*Rattus norvegicus*) have been reported by Mukaratirwa & Foggin (1999).

The *Trichinella*/mouse model has been widely used to assay the efficacy of benzimidazole-carbamate and imidazothiazole anthelmintic activity (Campbell & Denham 1983; McCracken 1978). This model makes it possible within a short time to test the efficacy of drugs against different parasite stages in various locations within the host.

Levamisole is an imidazothiazole that acts by interfering with the parasites nerve transmission causing

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muscular paralysis and its rapid expulsion (Van Nuten 1972). The therapeutic index of levamisole is low in animals.

Fenbendazole is a benzimidazole compound which disrupts parasite energy metabolism by binding to tubulin, a protein required for the uptake of nutrients and other functions (Abo-Shehada & Herbert 1984). Its activity is related to the duration of therapeutic blood concentration.

The efficacy of levamisole and fenbendazole to the crocodile-derived *Trichinella* strain has not been tested, although Boczon, Olba & Olaszek (1984) have reported on the efficacy of the two anthelmintics against the adult and larval stages of *T. spiralis* and *T. pseudospiralis*. Under natural conditions it is difficult to detect infection in its early stages, hence the need to search for a better drug which is effective on the muscle stage (first stage larvae-L1) of the parasite.

The objective of this study was to evaluate the efficacy of fenbendazole and levamisole against the larval stages of the crocodile-derived *Trichinella* sp. in muscle and to determine if vertical transmission of the *Trichinella* sp. strain occurs in Balb C mice.

MATERIALS AND METHODS

Experimental animals

Forty-four Balb C mice were bred at the Faculty of Veterinary Science, University of Zimbabwe animal unit. Equal numbers of females and males aged 18 weeks were randomly selected into groups and subgroups according to the experimental design (cf. Table 1). The mice in two of these groups served as untreated controls.

Parasite

The crocodile-derived strain of *Trichinella* sp. used, has been maintained under laboratory conditions by periodical passages through crocodiles. The methods followed for infecting mice and for counting larvae were those described by Kapel, Webster, Bjørn, Murrell & Nansen (1998). Each mouse was infected with ten *Trichinella* first stage larvae.

Anthelmintic experiments

To evaluate the anthelmintic activity of levamisole and fenbendazole, the mice were orally infected with ten first stage (L1) muscle larvae. Information on the anthelmintics and administration are shown in Table 2. Different groups of mice were anaesthetized by ether and then slaughtered on day 42 and day 49 and the other comprising two females and their offspring on day 49 (vide infra). The slaughtered mice were eviscerated and their carcasses processed to free the muscle larvae according to method described by Kaufmann (1996). The procedure to estimate the effectiveness of the anthelmintic drugs was done according to that described by Martinez-Fernandez (1978).

Transmission experiments

Two female mice were mated on the 19/01/00 and were infected on the following day (Table 5). The offspring born on 08/02/2000 together with the adult females were slaughtered on 24/03/00, and carcasses were processed and the larvae counted as described above.

Statistical analysis

The number of L1 recorded in each treated group was expressed as a percentage of the mean value

TABLE 1 Summary of anthelmintics used, number of animals per group, and day of treatment and slaughter post-infection

Group	No. of mice	Subgroup	Treatment post-infection	Slaughter
Trial 1				
Levamisole	16	1 (n = 8)	Day 35	Day 42
		2 (n = 8)	Days 35, 42	Day 49
Fenbendazole	16	1 (n = 8)	Day 35	Day 42
		2 (n = 8)	Days 35,42	Day 49
Placebo	10	–	–	Day 42
Trial 2				
(Pregnant mice)	2	–	–	Day 64

for the control group, and the anthelmintic effect of the various treatment groups were compared by the 1-way ANOVA.

RESULTS

Both single (day 35, Table 3) and double (days 35 and 42, Table 4) treatments with each compound significantly reduced the numbers of L1 in the muscles of the mice ($P<0.01$). While the difference in efficacy of the two anthelmintics was not significant after a single treatment, fenbendazole was significantly more effective than levamisole after double treatments ($P < 0.01$).

Double treatments eliminated 95.5 % of the larvae in levamisole and 99.1 % in fenbendazole-treated animals (Table 4). There was a significant reduction ($P < 0.01$) in the worm counts of double treated groups (days 35 and 42) (cf. Table 4). Fenbendazole was more effective than levamisole at double treatments ($P < 0.01$).

TABLE 2 Information on the anthelmintics used for treatment

Anthelmintic	Fenbendazole	Levamisole
Trade name	Zerofen	Tramisol
Manufacturer	Caps Zimbabwe	Milborrow
Date of manufacture	11/1998	10/1999
Date of expiry	11/2001	10/2002
Batch number	900010	9981
Concentration	10 %	2.5 %
Dosage	7.5 mg/kg, orally	7.5 mg/kg, orally

All the young mice looked healthy and there were no indications of changes in their tissues and organs examined at necropsy. Mother 1 gave birth to four offspring of which three were infected with the parasite and mother 2 gave birth to eight offspring of which five were infected (Table 5). Thus, the mice had apparently transmitted the parasite to their offspring but it is not certain whether this occurred congenitally or transmammary route.

DISCUSSION

Our observations showed that the crocodile isolate of *Trichinella* sp. is highly infective to Balb C mice, as also shown previously for pigs (Mukota breed) *(Sus scrofa)* and rats *(Rattus norvegicus)* (Mukaratirwa & Foggin 1999). The parasite was also found to be non-infective to birds (S. Mukaratirwa, unpublished data 2000). Pozio, La Rosa, Murrell & Lichtenfels (1992a) reported that host species seem unimportant for distinguishing *Trichinella* species, excepting that *Trichinella spiralis* is the only species that has a high reproductive capacity index (RCI) for pigs, rats and mice and that *Trichinella pseudospiralis* is the only species infective to birds. The inability of the present isolate to infect birds makes it highly unlikely to be *T. pseudospiralis*, but our results are in agreement with work by Foggin *et al.* (1997), who tentatively identified the parasite as a subspecies of *T. spiralis*.

The RCI from the untreated group was 110, which is classified as a high index, in agreement with a corresponding value of 145 for *T. spiralis* in NIH mice (Wakelin & Goyal 1996). The RCI for *Trichinella nativa* in these mice was found to be low (14). Bolas-Fer-

TABLE 3 Summary of mean larval count(LC), mean RCI and mean % larval reduction following on a single treatment at day 35 after infection

Group	No.	Dosage	Mean LC	Reduction (%)	Mean RCI
Levamisole	8	7.5 mg/kg	$220.9^a \pm 72.8$	79.9	–
Fenbendazole	8	7.5 mg/kg	$255.8^a \pm 123.0$	76.7	–
Control	5	–	$1107.4^b \pm 538.3$	–	110 ± 53.8

Values with different superscript within a column are significantly different $P < 0.01$
RCI = number of larvae recovered/number of larvae given
% larval reduction = 100 – (mean larval count for treated group/mean larval count for control group)/100

TABLE 4 Summary of mean larval count (LC), mean RCI and mean % larval reduction control group and treatment groups at days 35 and 42

Group	No.	Dosage	Mean LC	Reduction (%)	Mean RCI
Levamisole	8	7.5 mg/kg	$49.5^a \pm 27.3$	95.5	–
Fenbendazole	8	7.5 mg/kg	$9.9^b \pm 4.7$	99.1	–
Control	5	–	$1107.4^c \pm 538.3$	–	110 ± 53.8

Values with a different superscript within a column are significantly different $P < 0.01$
RCI = number of larvae recovered/number of larvae given
% larval reduction = 100 – (mean larval count for treated group/mean larval count for control group)/100

TABLE 5 Data on the two mice that were infected a day after having been mated, and on their offspring

Identification	Mass (g)	No. of L1
Mother 1	19.6	449.0
Offspring 1	7.1	0.0
Offspring 2	6.7	7.0
Offspring 3	10.2	12.0
Offspring 4	9.4	4.0
Mean larvae/offspring	–	5.8
Mother 2	20.1	414.0
Offspring 1	9.2	6.0
Offspring 2	10.2	273.0
Offspring 3	9.6	0.0
Offspring 4	8.9	5.0
Offspring 5	8.3	0.0
Offspring 6	9.5	3.0
Offspring 7	10.1	0.0
Offspring 8	9.7	9.0
Mean larvae/offspring	–	37.0

nandez & Wakelin (1989) also reported RCI values to be high for *T. spiralis*, low for *T. nativa* and intermediate for *T. pseudospiralis*, while the values for *Trichinella nelsoni* and *Trichinella britovi* were low in pigs and rats (Murrell, Lichtenfels & Rausch 1991).

From tissue sections of the muscles of the mice, the parasite was found to be cyst forming. This eliminates *T. pseudospiralis*, which lacks the ability to form a nurse cell and remains unencysted in the muscle (Pozio, La Rosa, Rossi, Murrell & Lichtenfels 1992).

In female mice that were first mated and then infected with the *Trichinella* parasite strain, the infection passed to their offspring, but it is not known whether this occurred vertically (transplacental) or transmammary.

While both anthelmintics used considerably reduced the muscle larval stages in all four treatment groups, none of the treatments were fully effective after a single and double treatments. For a drug to be recommended for use against *Trichinella* parasites, it has to clear the infection completely. We suggest that a serological test for diagnosis of this parasite in crocodiles be developed for the detection of reservoirs or carrier animals. Once detected, these can be culled and the meat disposed of by incineration and so that the meat is not recycled.

The present results with levamisole and fenbendazole were similar to those reported for related anthelmintics, which failed to completely eliminate muscle L1 stages. Flubendazole in Swiss albino mice reduced muscle L1 stages by 61–64 %, and the intestinal phase 3 days post-infection by 89.9 % (El-Temsahi & El-Mansoury 1995). Thiabendazole and mebendazole treatment resulted in complete elimination of *Trichinella spiralis* adult worms in the small intestine and marked reduction of larval infection of muscle larval stages in albino rats (El-Ridi, Abou-Ragab, Ishmail, Shehata, Ramadan & Etewa 1990). Albendazole is ranked as one of the most effective anthelmintics against muscle L1 stages of *Trichinella*, but displays a low solubility in aqueous solutions (Hrčkova, Velebny & Horák 1993). An increased number of dosages in a liposomised form to improve solubility, resulted in an increased reduction in the number of larvae (Hrčkova *et al.* 1993). Although some anthelmintics have been found to clear the intestinal stage, this is of little practical use because under natural conditions it is difficult or impossible to detect this stage.

In view that this parasite is infective to pigs, public health education and economic implications of this parasite has to be reemphasised. Introduction of legislative measures may be required to control this parasite. Although a number of biological characteristics of this parasite have been documented, more studies need to be done to identify this strain of *Trichinella* to species level.

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