

VIABILITY OF *ASCARIS LUMBRICOIDES* EGGS ELIMINATED AFTER ANTI-HELMINTHIC THERAPY

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The viability of Ascaris lumbricoides eggs passed in the feces was evaluated after treatment of patients with one of the anti-helminthic drugs (thiabendazole, levamisole, cambendazole, pyrantel pamoate, mebendazole or praziquantel). For each drug, a group of 5 children was selected and their feces collected 24 h before treatment and 24, 48 and 72 h after drug administration, except for mebendazole, with the feces being collected throughout the period of treatment.

After sedimentation, the total amount of eggs from each collection was transferred to tissue culture flasks containing 10 ml H₂SO₄ 0,1N, with the addition of 3 drops of a miconazol solution, and incubated at 28 °C, individually, for 80 days. The flasks were maintained open and the culture were oxygenated daily by manual agitation.

On the 80th day of culture, 20-days-old albino mice were inoculated with 3,200 embryonated eggs, per os. Larvae were recovered from their lungs and hearts, on the 8th day after infection, according to Baerman's method (Morais, 1948).

Thiabendazole showed 100.0% ovicidal capacity as early as 48 h after treatment.

Inhibition of embryonic development was observed when thiabendazole was used. This drug also had an effect on the eggs infectivity when inoculated into normal mice. No significant effect on embryonic development was observed for the other drugs tested.

Key words: *Ascaris lumbricoides* – therapy – egg embryonation

Ascaris lumbricoides infection is known to be a serious public health problem, mainly due to the facility of infection and high prevalence in the population.

The damages caused by *A. lumbricoides* infection range from alterations in the intestinal tract to weakness of the whole organism, retardation of the somatic and intellectual

development, hepatic abscess, and obstruction of the common bile duct, very often leading to surgical intervention (WHO, 1967).

Attempts have been made by different investigators to control the infection with ascaricidal drugs (Katz et al., 1972b; Metene et al., 1972; Souza et al., 1972; Katz et al., 1973; Cimerman, 1984). Although several laboratories are working on the development of an experimental model for *A. lumbricoides* (Alicata, 1934; Vanhaelen-Lindhout & Smit, 1971; Moreira & Souza, 1973; Wagner Pena Chavarria, 1974a, b; Souza et al., 1977, 1985), we do not have yet a susceptible host, except man, in which the parasite life cycle can be maintained. However, our laboratory (Massara et al., 1990) working with viable eggs recovered from the feces of untreated children showed

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that after appropriate incubation and inoculation in mice, the eggs were able to generate viable larvae that could be recovered from the lungs and hearts 8 days after infection.

This paper describes the effect of different anti-helminthic drugs on the viability of *A. lumbricoides* eggs collected in the feces.

MATERIALS AND METHODS

Drugs used – Children (age group between 7 and 14 years) were distributed into 6 groups of 5 children each. They were submitted to antiparasitic therapy (one group for each drug), under medical assistance. Thiabendazole, levamisole, cambendazole, pyrantel pamoate, and mebendazole were administered at the usual doses; praziquantel, at 60 mg base salt/kg, single dose, administered to children with ascariasis associated with schistosomiasis.

Collection of feces – Feces were collected from 30 children infected with *A. lumbricoides*, diagnosed by Kato's method, modified by Katz et al. (1972), 24 h before (control), and 24, 48 and 72 h after treatment.

Collection of eggs – Eggs per gram of feces (EPG) were determined in every collection, by the method described by Beaver (1950). The remaining material was diluted in water, homogenized, and collected in sedimentation flasks (1,000 ml), after passing through a "tamis" placed on a PVC (polyvinyl chloride) tube, with different size mesh (900, 150 and 112 μ) nylon nets. This material was then washed several times until a clear supernatant (Lutz, 1919) was obtained.

Culture for collection of embryonic eggs – Sediments with eggs were transferred to tissue culture flasks, with about 10 ml of H₂SO₄ 0,1N (Fairbairn, 1961), after an addition of 3 drops of miconazol. Feces were individually cultured at 28 °C, for 80 days. The flasks were kept opened and oxygenated daily by manual agitation.

Determination of egg embryonation ratio – Every 10 days, starting from 20th up to the 80th day, egg embryonation ratio was determined in the eggs kept in 0.025 ml culture

medium. The result obtained corresponded to the average of 3 different evaluations.

Infection of mice – On the 80th day of culture, 0.025 ml of each sample was separated for *A. lumbricoides* eggs countings, the volume corresponding to 3,200 embryonic eggs determined after triplicate countings.

Twenty days old albino mice, were infected orally, 5 mice per culture. Five uninfected animals were kept as control.

Recovery of larvae – Mice were sacrificed by cervical fracture, 8 days after inoculation. Their lungs and heart were isolated after thoracic incision, minced and the larvae recovered by the modified Baerman's method (Morais, 1948) and 40 min later, samples of the liquid were collected in watch-glasses. Similar procedure was performed with the control group.

Statistical analysis – The results were submitted to the analysis of variance, taking into account the data obtained before (control) and after every treatment.

RESULTS

Each group is represented by the mean of egg count of 5 children. The number of *A. lumbricoides* eggs obtained before, during and after the anti-helminthic drug treatment is shown on Table I. Every drug, except praziquantel, reduced the number of eggs in the feces. The mean embryonation of the *A. lumbricoides* eggs on the 80th day of culture may be seen on Table II. Pyrantel pamoate and mebendazole reduced over 60.0% the embryonation rate of the eggs. Thiabendazole inhibited 100.0% the embryonation of eggs 48 h after drug administration. The other drugs did not show any ovidical activity. The recuperation of larvae from lungs and hearts of mice after 8 days of infection may be observed on Table III. Treatment with cambendazole, pyrantel pamoate and mebendazole reduced up to 90.0% the recuperation of larvae. A reduction of 100.0% on the larvae recuperation was observed with treatment by thiabendazole. Levamisole and praziquantel did not interfere on the larvae development.

TABLE I

Mean *Ascaris lumbricoides* eggs per gram of feces (EPG) before and at different times after treatment

Drugs	24 h before treatment		During treatment (days)				After treatment (hours)					
	(control)		2nd		3rd		24		48		72	
	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)
Thiabendazole	37,600	(100.0)	—	—	—	—	29,875 (-20.5)	19,667 (-47.7)	34,200 (-9.0)			
Levamisole	49,450	(100.0)	—	—	—	—	19,050 (-61.5)	11,312 (-77.1)	8,667 (-82.5)			
Cambendazole	14,550	(100.0)	—	—	—	—	8,900 (-38.8)	13,800 (-5.1)	9,625 (-33.8)			
Pyrantel pamoate	25,450	(100.0)	—	—	—	—	22,000 (-13.5)	9,200 (-63.8)	650 (-97.4)			
Mebendazole	116,700	(100.0)	85,950 (-26.3)	34,625 (-70.3)			80,250 (-31.2)	33,500 (-71.3)	18,937 (-83.7)			
Praziquantel	23,200	(100.0)	—	—	—	—	54,600 (+135.3)	45,700 (+97.0)	50,250 (+116.6)			

() Reduction or increase rates in relation to the control group.

TABLE II

Mean embryonation rates for *Ascaris lumbricoides* eggs passed in the feces of treated children, on the 80th day of culture

Drugs	24 h before treatment		During treatment (days)				After treatment (hours)					
	(control)		2nd		3rd		24		48		72	
	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)
Thiabendazole	36.6	(100.0)	—	—	—	—	30.7 (-16.1)	0.0 (-100.0)	0.0 (-100.0)			
Levamisole	25.4	(100.0)	—	—	—	—	44.8 (+76.4)	69.0 (+171.6)	48.5 (+90.9)			
Cambendazole	7.8	(100.0)	—	—	—	—	6.0 (-23.1)	2.2 (-71.8)	8.5 (+9.0)			
Pyrantel pamoate	11.2	(100.0)	—	—	—	—	12.7 (+13.4)	16.5 (+47.3)	4.0 (-64.3)			
Mebendazole	14.6	(100.0)	5.0 (-65.7)	5.2 (-64.4)			17.5 (+19.9)	8.0 (-45.2)	4.5 (-69.2)			
Praziquantel	16.8	(100.0)	—	—	—	—	19.8 (+17.8)	41.2 (+145.2)	57.0 (+239.3)			

() Reduction or increase rates in relation to the control group.

TABLE III

Mean larva recovery from lungs and heart of mice infected^a with *Ascaris lumbricoides* eggs obtained from treated children on the 8th day after infection

Drugs	24 h before treatment		During treatment (days)				After treatment (hours)					
	(control)		2nd		3rd		24		48		72	
	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)	\bar{x}	(%)
Thiabendazole	206.0	(100.0)	—	—	—	—	332.7 (+61.5)	0.0 (-100.0)	0.0 (-100.0)			
Levamisole	424.2	(100.0)	—	—	—	—	520.0 (+22.6)	411.0 (-3.1)	442.5 (+4.3)			
Cambendazole	1,274.2	(100.0)	—	—	—	—	636.8 (-50.0)	78.6 (-93.8)	43.7 (-96.6)			
Pyrantel pamoate	59.6	(100.0)	—	—	—	—	156.0 (+161.7)	29.2 (-51.0)	4.7 (-92.1)			
Mebendazole	174.8	(100.0)	158.0 (-9.6)	34.2 (-80.4)			263.5 (+50.7)	152.3 (-12.9)	12.7 (-92.7)			
Praziquantel	282.4	(100.0)	—	—	—	—	269.0 (-4.7)	529.8 (+87.6)	1,394.0 (+393.6)			

() Reduction or increase rates in relation to the control group.

a: groups of 25 mice with inoculum of 3,200 egg/animal.

DISCUSSION

Studies on the activity of thiabendazole on *A. lumbricoides* eggs isolated from soil were performed by Egerton (1961), Kutsumi & Komyia (1965), and Kutsumi et al. (1967). Results obtained by these authors suggest high ovicidal activity of that drug.

Our results further support these observations and also show that high ovicidal activity is also detected *in vivo* studies. These results show that eggs obtained from feces of thiabendazole treated children did not embryonate when collected as early as 48 h post-treatment.

Levamisole did not inhibit egg evolution. On the contrary an increase in larva development in the 3 collections performed after treatment was observed. These results are in agreement with those described by Moreira & Souza (1973) and Souza et al. (1977, 1985). Similar results were obtained by Wagner & Rexinger (1978), when they failed to detect embryonation inhibition of *A. lumbricoides* eggs recovered from patients treated with levamisole. Only a low percentage (11.6%) inhibition of *Trichuris trichiura* eggs was detected.

As far as pyrantel pamoate is concerned, the drop on recovery rate of the larvae seems to be related to the elimination of the parasites, consequently diminishing the size of imoculum. Moreira & Souza (1973), and Souza et al. (1977, 1985) tested the viability of *A. lumbricoides* eggs isolated from females that were eliminated after treatment with pyrantel pamoate however these authors did not quantify the larva recovery.

When mebendazole was used, the eggs obtained 72 h after treatment showed an embryonation reduction of 69.2% in agreement with the results obtained by Wagner & Rexinger (1978), who observed an ovicidal activity of 64.8% for *A. lumbricoides* and 11.6% for *T. trichiura* eggs, on the 5th day after treatment.

The results obtained in this study together with those previously described suggest that these drugs lead to an inhibition of egg evolution after treatment since in experimentally infected mice larval recovery was reduced at 92.7% when compared to infected control with eggs from untreated individuals.

Praziquantel did not show any activity on *A. lumbricoides* eggs. The 3 collections performed after treatment presented an average development of 135.3, 97.0 and 116.6% for EPG. Intolerance demonstrated by the occurrence of dizziness, epigastric pain, diarrhea, headache, fever and fainting were also observed, however change on embryonation rate was not observed. This drug seems to exert some kind of stimulus on the larvae development, in such a way that the recovery rates showed development indexes of 87.6% and 393.6% for the material collected 48 and 72 h after treatment, respectively. The results obtained in this study for *A. lumbricoides* differ from those reported by Matsuda et al. (1983) for *Schistosoma japonicum* eggs.

Cambendazole – not yet studied in relation to its ovicidal activity – presented an embryonation reduction of 71.8% for *A. lumbricoides* eggs in the collection performed 48 h after treatment.

CONCLUSIONS

Although thiabendazole is not the drug of choice for the treatment of *A. lumbricoides* infection, it showed 100.0% ovicidal activity, from 48 h after its administration onwards.

Levamisole does not seem to exert an inhibitory effect either on the development of *A. lumbricoides* eggs or on the infectivity for mice.

Cambendazole, pyrantel pamoate and mebendazole need further studies concerning inhibition of the embryony development of eggs, besides the infectivity for mice.

Praziquantel did not act either as an anti-helminthic drug for *A. lumbricoides*, or as an inhibitor of the embryony development and infectivity of eggs.

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