

## LEVAMISOLE-INDUCED RESISTANCE TO RAILLIETINA TETRAGONA INFECTION IN YOUNG CHICKS

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### A b s t r a c t

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The repeated pre-treatment with levamisole (L-tetramisole) was found to induce significant resistance to Raillietina tetragona infection in 7 day old chicks. A total of 6 twice weekly oral doses of 2.5 mg/kg reduced the parasitic burden from 40% in primarily infected controls (n = 10) to 13% in levamisole-treated chicks (n = 10). Resistance to R. tetragona was also observed in reinfected chicks (n = 9) when the intial infection was subsequently treated with niclosamide (Yomesan, 100 mg/kg). In this respect, the parasitic burden was reduced to 20%. In addition, a further reduction to 8.8% of the parasitic burden was obtained in reinfected chicks (n = 9) when they were also treated with levamisole at 6 twice weekly doses of 2.5 mg before challenge. The increased resistance of the young chicks to R. tetragona infection and/or reinfection was attributed to the immunomodulatory action of levamisole.

Raillietina tetragona (cestode), chicks, Levamisole, resistance.

The imidazole compound levamisole (L-tetramisole) has enjoyed considerable interest in veterinary medicine due to its broad spectrum anthelmintic activity and non-specific immunomodulatory effect (A b d e l s a l a m 1986). The latter property was initially discovered by R e n o u x and R e n o u x (1971) and further substantiated by numerous investigations and reports (J a n s s e n 1976; S y m o e n s and R o s e n t h a l 1977; R e n o u x 1978; B r u n n e r and M u s c o p l a t 1980; G u o r r e r o 1980; M u l g a h y and Q u i n n 1986). However, the use of levamisole in poultry was still limited to its anthelmintic efficacy against certain parasitic nematodes including Ascaridia, Capillaria and Heterakis spp. (C l a r k s o n and B e g 1970; A l t a i f 1972; P a n k a v i s h et al. 1973). The modulatory effect of levamisole upon the avian immune system has not been apparently investigated and the present report, therefore, describes the effect of levamisole pre-treatment on the susceptibility to Raillietina tetragona infection in young chicks. The parasite belongs to the cestode group which does not normally respond to the chemotherapeutic action of the drug.

## Materials and Methods

**B i r d s :** Newly-hatched White Leghorn chicks were purchased from commercial farms (Almasara Co. Ltd, Khartoum North) and raised on standard grower diet with free access to water. The chicks were kept for one week before experiments commenced.

**D r u g s :** Levamisole (Nilverm, ICI solution containing 7.5% W/V L-tetramisole) Niclosamide (Yomesan 500 mg tablets, Bayer). Levamisole was further diluted to 1 : 10 in water. Niclosamide was also dissolved in water and both drugs were administered orally.

**I n f e c t i v e m a t e r i a l :** Cysticercooids of *R. tetragona* were recovered from naturally-infected ants (*Pachycondyla sennaarensis*) collected from soil in the neighbouring farms. The required number of cysticercooids was suspended in saline and transferred into gelatinous capsule for oral administration.

**E x p e r i m e n t a l d e s i g n :** Two complementary experiments were performed on the effect of levamisole against primary infection and reinfection with the homologous cestode.

**E x p e r i m e n t 1 : (Primary infection)**

Twenty chicks were divided into two equal groups (A & B). The first group (A) was pre-treated with 2.5 mg/kg levamisole twice weekly for three weeks. Group B was left without treatment for the same period of time. Both groups were then infected with *R. tetragona* (15 cysticercooids/chick) and killed two weeks post infection (see Table 1).

Table 1

Experimental design and results of experiment I (Primary infection)

Group	Infective dose (cysticercooids)	No. of worms recovered at necropsy per individual chick	Total No.	Group mean ( $\pm$ SD)	% Re- covery
A - Levami- sole treated (n = 10)	15	2,1,2,1,2,3,2,3,1,3	20	2.0 $\pm$ 0.82	13.3%
B - Untreated controls (n = 10)	15	4,8,9,8,5,6,4,3,7,6	60	6.0 $\pm$ 2.0	40%

**E x p e r i m e n t 2 : (Reinfection)**

Three equal groups of chicks ( $A_1$ ,  $B_1$  &  $B_2$ ) were used. Each group was composed of 9 chicks of the same age (7-day old). Groups  $B_1$  and  $B_2$  were initially infected with 20 cysticercooids per chick and subsequently treated with niclosamide (100 mg/kg) a week after. Both groups were then reinfected with the same number of cysticercooids and killed after another two weeks (Table 2). However, group  $B_2$  chicks were first pre-treated with levamisole (2.5 mg/kg twice weekly) for 3 weeks before reinfection. On the other hand, group  $A_1$  chicks were used as primarily infected controls (i.e infected with 20 cysticercooids each and killed two weeks post - infection for comparison).

Table 2

Experimental design and results of experiment 2 (Reinfection)

Group	Infective dose (cysticercoids) Primary Challenge	No. of worms recovered at necropsy per individual chick	Total No.	Group mean ( $\pm$ SD)	% Re- covery	
A <sub>1</sub> - Prima- rily infec- ted (n=9)	20	0	12,9,11,9,7,8,12,10,8	86	9.6 $\pm$ 1.81	47.8%
B <sub>1</sub> - Reinfec- ted (n=9) (treated with niclos- amide)	20	20	4,6,2,4,3,5,4,5,3	36	4.0 $\pm$ 1.22	20%
B <sub>2</sub> -Rein- fected (treated /leva- misole treated (n=9) niclos- amide)	20	20	2,1,1,2,3,1,1,3,2	16	1.8 $\pm$ 0.83	8.8%

### W o r m   r e c o v e r y

The whole intestines were immediately removed at necropsy and opened into plastic dishes containing normal saline. The content was evacuated and the recovered worms were collected and preserved in Roudabush solution. They were then identified under light microscope and counted individually. Student's t-test was employed for the statistical evaluation of the results.

### Results

The results of tapeworm recovery in primary infection and reinfection with R. tetragona in chicks are also shown in tables 1 and 2.

#### P r i m a r y   i n f e c t i o n (Table 1):

The mean parasitic burden in levamisole-treated chicks (group A) was significantly lower ( $P < 0.001$ ) than in untreated controls (group B). The mean percentage tapeworm recovery was reduced from 40% in the control group to 13% in levamisole treated chicks.

#### R e i n f e c t i o n (Table 2):

The total worm burden in the reinfected groups (B<sub>1</sub> and B<sub>2</sub>) was significantly lower ( $P < 0.001$ ) than in primarily infected chicks (group A<sub>1</sub>). The overall percentage tapeworm

recovery was reduced to 20 % in the reinfected chicks (group B<sub>1</sub>) without levamisole treatment. However, a further reduction<sup>1</sup> to 8.8 % of the overall percentage tapeworm recovery was obtained by levamisole treatment in reinfected chicks (group B<sub>2</sub>).

The results of the present work have generally indicated that the pretreatment of young chicks with repeated doses of levamisole (2.5 mg/kg twice weekly for three weeks) increased their resistance to R. tetragona infection as judged by the significant reduction of worm recovery (Table 1). The result was probably due the immunomodulatory action of levamisole, since the drug was not found to have any anthelmintic efficacy against tapeworms (Thienpont et al. 1966; Janssen 1976). Although the modulatory effect of levamisole upon the mammalian immune system is now well recognized (Muleahy and Quinn 1986), however, the exact mechanisms by which the drug can exert its immunological effects are not fully understood. Nevertheless, the drug was suggested to enhance the lymphocyte proliferative responses, increase lymphokin production and promote macrophage function (phagocytosis) (Al-Ibrahimi et al. 1977). In addition, the drug was found to be more effective in young hosts (Janssen 1976) and in those with hypofunctional T-lymphocytes (Guerrero 1980). In the present report, the chicks were used as young as 7-day old and that would probably account for the relative success of the drug in inducing significant immune protection against the parasite (R. tetragona).

Acquired resistance to homologous reinfection with cestode parasites has been previously reported (Heath et al. 1979). In the present work, a significant resistance to reinfection with R. tetragona was also observed in young chicks (Table 2). The results, therefore, indicate that chicks are capable of developing acquired resistance to homologous reinfection with the cestode parasites. Such resistance was probably due to the antigenic stimulation caused by the destruction and disintegration of the dead parasite of the previous infection as a result of niclosamide treatment. Resistance to reinfection was also enhanced by levamisole pre-treatment (Table 2).

It is finally concluded that the routine use of levamisole in poultry management is highly beneficial in terms of its broad-spectrum anthelmintic activity and non-specific immunomodulation.

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Levamisolem indukovaná rezistence  
k infekci Raillietina tetragona u kuřat

Opakovaná premedikace levamisolem (L-tetramisole) vedle k signifikantní resistenci vůči infekci Raillietina tetragona u 7denních kuřat. Celkem 6 orálních dávek po  $2,5 \text{ mg.kg}^{-1}$  zredukovalo 40%ní parazitární invazi u primárně infikovaných kontrol ( $n = 10$ ) na 13 % u kuřat ošetřených levamisolem. Resistence vůči R. tetragona byla také pozorována u reinfikovaných kuřat ( $n = 9$ ), když byla počáteční infekce léčena niclosamidem (Yomesan,  $100 \text{ mg.kg}^{-1}$ ). V tomto případě byla parazitární invaze zredukována na 20 %. Další redukce na 8,8 % nastala u reinfikovaných kuřat ( $n = 9$ ), která byla také ošetřena levamisolem za použití 6 dávek po  $2,5 \text{ mg}$  2 x týdně před čelenží. Zvýšená rezistence kuřat k infekci R. tetragona a reinfekci je přisuzována imuno-modulačnímu účinku levamisolu.

Индукцированная левамизолом резистентность  
к инфекции Raillietina tetragona у цыплят

Повторная премедикация левамизолом (L-tetramisoleä) вылилась в значимую резистентность к инфекции Raillietina tetragona у цыплят в возрасте 7 суток. В итоге 6 оральных доз по  $2,5 \text{ мг.кг}^{-1}$  редуцировало 40% паразитарную инвазию у первично инфицированных контрольных групп ( $n=10$ ) в случае 13% цыплят, принимающих левамизол. Резистентность к R. tetragona наблюдали также у реинфицированных цыплят ( $n=9$ ), когда начальную  $-1$  инфекцию лечили никлосамидом (емесан,  $100 \text{ мг.кг}^{-1}$ ). В данном случае паразитарную инвазию редуцировали до 20%. Последующая редукция до 8,8% имела место у повторно инфицированных цыплят ( $n=9$ ), принимающих левамизол

в 6 дозах по 2,5 мг два раза в неделю перед член-жированием. Повышенная резистентность цыплят к инфекции R. tetragona и реинфекции связывается с имму-номодуляционным воздействием левамизола.

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