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The effect of levamisole (L. tetramisole) treatment on the susceptibility to *Fasciola gigantica* infection in goats

GORAISH (I. A.), ABDELSALAM (E. B.), TARTOUR (G.), ABBAS (B.), ARADAIB (I. E.). Effet du traitement au lévamisole (L. tétramisole) sur la réceptivité des chèvres à la fasciolose à *Fasciola gigantica*. *Revue Elev. Méd. vét. Pays trop.*, 1988, 41 (3) : 283-287.

L'administration répétée d'un total de six doses hebdomadaires de lévamisole (L. tétramisole, 7,5 mg/kg S/C) s'est avérée augmenter la résistance à la fasciolose à *Fasciola gigantica* chez les chèvres. Cette résistance accrue a été révélée par une baisse du nombre de vers découverts à l'autopsie et par le développement de lésions hépatiques moins graves. L'élévation de l'activité enzymatique du plasma, de même que les dégradations du foie provoquées par *Fasciola*, étaient moins marquées chez les chèvres traitées au lévamisole. Le traitement au lévamisole était aussi associé à une plus grande réponse des anticorps contre l'infection à *Fasciola gigantica*. *Mots clés* : Chèvre - Lévamisole - Anthelminthique - Fasciolose - *Fasciola gigantica* - Trématode - Soudan.

INTRODUCTION

The stimulatory effect of the anthelmintic levamisole (L. tetramisole) upon the mammalian immune system has been extensively investigated (5, 15, 19, 21). However, the veterinary use of levamisole as immunomodulator was only tried with certain bacterial and viral diseases including bovine mastitis (7, 16), endometritis (7) and bovine rhinotracheitis (4). An increased resistance to *Fasciola hepatica* in sheep was also induced by the combined treatment with levamisole and prior infection with other helminths (14). However, the results were mainly attributed to the immunomodulatory action of the anthelmintic levamisole which is well known to be ineffective against trematodes.

The present work was designed to investigate the possible immunomodulatory effect of levamisole in goats experimentally infected with the liver fluke *Fasciola gigantica*.

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MATERIALS AND METHODS

Experimental animals and infective material

Eight 9 month-old male Nubian goats were purchased from Kuku livestock market, an area known to be free from fascioliasis. That was checked by faecal examination according to the sedimentation method described by SOULSBY (20). Metacercariae of *Fasciola gigantica* were obtained from infected laboratory colony of *Lymnaea natalensis*. The cysts were collected in cellophane sheets and transferred into gelatinous capsules for oral administration.

Experimental design

The animals were allotted into three groups as indicated in Table I. Groups I and II were both infected with 250 viable *F. gigantica* metacercariae and killed 8 weeks post infection. However, group I goats received a total of 6 subcutaneous doses of 7.5 mg/kg levamisole (Pamizole TM, Fatro spa.) given at weekly intervals (four doses were given before infection and two doses after). Group III goats were kept as uninfected controls but they were also treated with a total of 6 weekly doses of levamisole (7.5 mg/kg S/C). The animals were observed for clinical signs and heparinized blood samples were collected at weekly intervals before and after the experiment commenced.

Liver pathology and worm recovery

The livers were immediately removed at necropsy and examined for gross pathological lesions. Small pieces were fixed in 10 p. 100 formol-saline for routine histological processing and staining with haematoxylin and eosin (H & E). The flukes were recovered from the liver by slicing and squeezing in warm saline (37 °C) and they were counted individually.

Plasma analysis

The plasma activity of aspartate aminotransferase (AST), glutamate dehydrogenase (GD) and sorbitol

dehydrogenase (SD) was determined according to the methods described by REITMAN and FRANKEL (18), FORD (8) and FORD and BOYD (9) respectively. The enzyme-linked immunosorbent assay (ELISA) was used for the determination of antibody response as described by BURDEN and HAMMET (6) using crude *Fasciola gigantica* antigens and rabbit anticaprine IgG (H+L) conjugated to horse radish peroxidase (Miles Laboratories). The plasma was diluted 1:40 and the results were obtained in terms of optical densities.

RESULTS

Clinical observations

Control goats (group III) appeared normal throughout the experimental period and did not show any adverse clinical reaction to the repeated dosing with levamisole. Clinical changes in the other groups (I, II) were hardly noticed before 4 weeks of infection. However, the animals started to lose appetite by the 6th week and appeared slightly dull at the time of slaughter (8 weeks).

Pathological findings

The hepatic lesions were basically similar in both groups of *F. gigantica*-infected goats although they were less severe in levamisole-treated ones.

The livers of group II goats (infected/untreated) were congested, diffusely fibrotic and had slightly thickened capsule covered with fine fibrinous strands. A large number of necrotic lesions and raised plaques of haemorrhagic tracts were scattered all over the surface. The cut surface was hard and occasionally distorted. The gall bladders were distended with thick bile containing a small number of flukes. The main

bile ducts were also dilated and stuffed with flukes. On the other hand, levamisole-treated goats (group I) showed relatively mild hepatic lesions including a few necrotic foci and scattered haemorrhagic tracts. The cut surface was normal in consistency. The gall bladder and the main bile ducts were slightly dilated but no flukes were recovered from them.

Microscopically, the migratory tracts consisted of central zones of cell debris and erythrocytes surrounded by degenerating cells and infiltrated with macrophages and eosinophils. The portal area was considerably enlarged in group II goats (infected/untreated) due to extensive periductal fibrosis, hyperplasia of the bile duct epithelium and diffuse infiltration with mononuclear cells.

Worm recovery

The results of worm recovery in goats infected with *F. gigantica* with or without levamisole treatment are also shown in table I.

The individual and mean recoveries of liver flukes in levamisole-treated goats (group I) were lower than in untreated animals (group II). The mean percentage recovery was reduced from 27.3 p. 100 in infected goats to 8.8 p. 100 as a result of the repeated administration of levamisole.

Plasma enzymes

The mean plasma activities of AST, SD and GD in *F. gigantica* infected and control goats are shown in figure 1.

There was no change in the plasma activity of AST, SD and GD as a result of repeated dosing with levamisole (group III).

TABLE I Experimental design and worm recovery in goats infected with 250 *F. gigantica* metacercariae with or without levamisole treatment.

Group	Infective dose (metacercariae)	No. of worms recovered at necropsy per individual animal	Mean recovery	Recovery (p. 100)	Remarks
I - Levamisole + infection (Nos, 1, 2, 3)	250	19, 36, 12	22,3	8,9	Killed after 8 weeks
II - Infection (Nos, 4, 5, 6)	250	95, 40, 70	68,3	27,3	Killed after 8 weeks
III - Levamisole alone (Nos, 7, 8)	—	—	—	—	Survived

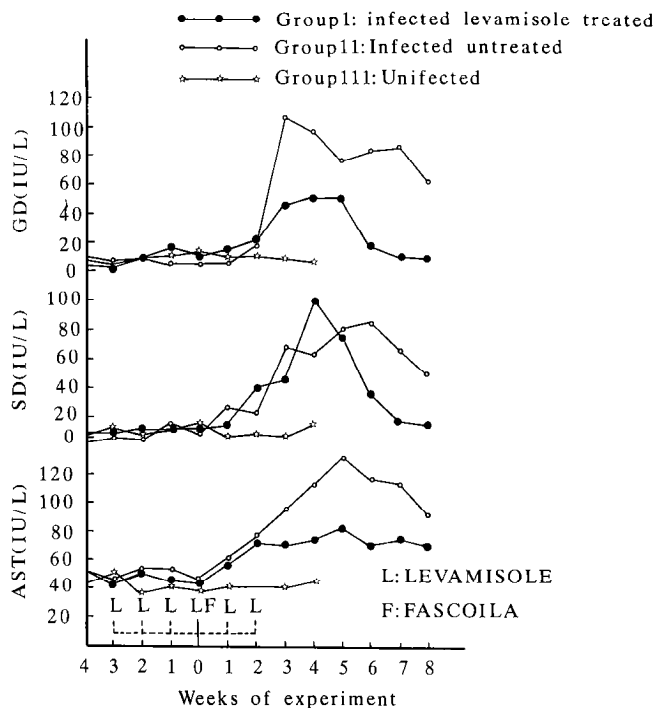


Fig. 1: Serum enzyme activity of AST, SD and GD in *F. gigantica*-infected and in control goats.

In *F. gigantica*-infected goats (groups I, II), the plasma activity of AST was increased as early as the second week of infection, peaked on the 5th week and remained high at the time of slaughter (8 weeks). However, the increase in AST activity was higher in group II (infected/untreated) than in group I (levamisole-treated) goats.

SD and GD activities showed a similar pattern of change. They both started to increase by the 3rd week of infection, peaked at the 5th or 6th week and then declined. However, their activity was also higher in group II goats.

Antibody response

The antibody response in *F. gigantica*-infected goats with or without levamisole treatment is shown in figure 2. Results obtained from control uninfected goats (group III) were considered as a base-line indicating negative values (6, 17, 23).

In group I goats (infected/levamisole-treated) antibodies were detected as early as the second week of infection (above the base line). Their levels increased rapidly and progressively until the animals were slaughtered by the 8th week.

Group II goats (infected/untreated) showed relatively little increase in antibody level during the first 4 weeks

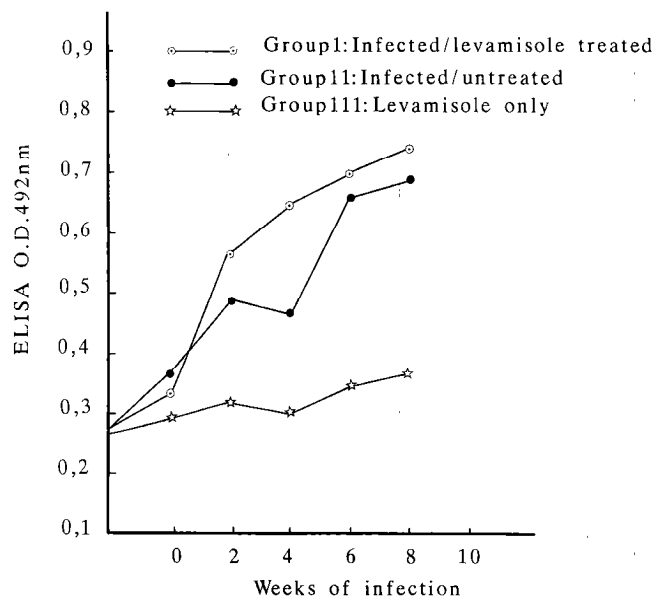


Fig. 2: Serum antibody response (ELISA O.D.) in *F. gigantica*-infected goats with or without levamisole treatment.

of infection. However, a sharp rise occurred on the 6th week and the highest antibody level was reached by the 8th week. The terminal increase in antibody level was higher in group I than in group II goats.

DISCUSSION

The present work has apparently indicated that the repeated treatment with levamisole (7.5 mg/kg weekly for 6 weeks) increased the resistance to *F. gigantica* infection goats. The increased resistance was reflected by reduced worm recovery at necropsy and by the development of less severe liver lesions. A higher antibody response was also observed in infected levamisole-treated goats.

Levamisole (L-tetramisole) has been extensively used in veterinary medicine as a broad spectrum anthelmintic as well as immunopotentiator (1, 15). However, the drug was found to be inactive against trematode and cystode infections in various species of domestic animals (12, 22). For this reason, the increased resistance of goats to *F. gigantica* infection in the present study was probably due to the immunostimulatory action of the drug. Similar findings of levamisole-induced resistance to *F. hepatica* have been previously reported in sheep (14) in the presence of prior infection with other helminth species. Immunomodulation by levamisole in hosts with other parasitic diseases has also been observed (13) in rats infected with the nematode *Nippostrongylus brasiliensis* and challenged with whole worm antigen.

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The precise mechanism by which levamisole is capable of stimulating the mammalian immune system is not fully understood. However, the drug was found to enhance the lymphocyte proliferating response, increase lymphoid production and promote macrophage function *i.e.* phagocytosis (2). In addition, the immunostimulatory effect of levamisole was found to be more pronounced in hosts with depressed immune system (11, 18). An immunosuppressive component was found to be involved in the pathogenesis of fascioliasis in animals (10). Therefore, levamisole might have also acted by correcting or restoring the immunosuppression induced by *F. gigantica* infection in goats in the present work. This assumption was further substantiated by the detection of a higher antibody response in levamisole-treated goats.

The role of the plasma enzyme activity in the diagnosis of animal fascioliasis is now well established (3). The disease is generally characterized by considerable elevation of the plasma activities of SD, GD, and AST indicating parenchymal cellular liver damage caused by the migrating flukes. However, in the present work, the increase in the plasma enzyme activity was less marked in *F. gigantica*-infected/levamisole-treated goats indicating the development of less severe liver lesions. Moreover, the lack of change in the plasma enzyme activity after the repeated administration of 6 weekly doses of 7.5 mg/kg levamisole in uninfected goats (group III) indicates that the drug is well tolera-

ted as this dose level even when given at multiple repeated doses.

CONCLUSION

Although the present results have generally suggested a possible protective effect of levamisole (as immunomodulator) against *F. gigantica* infection in goats, however, such treatment with the drug at 6 weekly intervals is probably unlikely to be encountered for the routine nematode control under field conditions. Further research is therefore required to determine the immunomodulatory action of levamisole in connection with parasitic infections other than those produced by nematode helminths in domestic animals.

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GORAISH (I. A.), ABDELSALAM (E. B.), TARTOUR (G.), ABBAS (B.), ARADAIB (I. E.). The effect of levamisole (L. tetramisole) treatment on the susceptibility to *Fasciola gigantica* infection in goats. *Revue Elev. Méd. vét. Pays trop.*, 1988, 41 (3) : 283-287.

The repeated administration of a total of six weekly doses of levamisole (L. tetramisole, 7.5 mg/kg S/C) was found to increase the resistance to *Fasciola gigantica* infection in goats. The increased resistance was reflected by reduced worm recovery at necropsy and by the development of less severe hepatic lesions. Elevation of the plasma enzyme activity associated with *Fasciola*-induced liver damage was less marked in levamisole-treated goats. Levamisole treatment was also associated with a higher antibody response against *Fasciola gigantica* infection. **Key words :** Goat - Levamisole - Anthelmintic - Distomatosis - *Fasciola gigantica* - Trematode - Sudan.

GORAISH (I. A.), ABDELSALAM (E. B.), TARTOUR (G.), ABBAS (B.), ARADAIB (I. E.). Efecto del tratamiento con el levamisole (L. tetramisole) sobre la sensibilidad de las cabras a la fasciolosis a *Fasciola gigantica*. *Revue Elev. Méd. vét. Pays trop.*, 1988, 41 (3) : 283-287.

De la administración repetida de un total de seis dosis semanales de levamisole (L. tetramisole, 7,5 mg/kg por vía subcutánea) resultó la aumentación de la resistencia a la fasciolosis a *Fasciola gigantica* en las cabras. Se demostró esta resistencia acrecentada por una disminución del número de los helmintos observados durante la autopsia y por el desarrollo de las lesiones hepáticas menos graves. La aumentación de la actividad enzimática del plasma y la deterioración del hígado causadas por *Fasciola* eran menos importantes en las cabras tratadas. El tratamiento con el levamisole era también asociado a una respuesta más grande de los anticuerpos contra la fasciolosis a *Fasciola gigantica*. **Palabras claves :** Cabra - Levamisole - Antihelmintico - Fasciolosis - *Fasciola gigantica* - Trematodo - Sudán.

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