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## Susceptibility to homologous reinfection with *Fasciola gigantica* in goats

GORAISH (I.A.), ABDELSALAM, (E.B.), TARTOUR (G.). Sensibilité des chèvres à une réinfestation homologue par *Fasciola gigantica*. *Revue Elev. Méd. vét. Pays trop.*, 1991, 44 (1) : 69-73

Des chèvres infestées auparavant avec 100 métacercaires vivants de *Fasciola gigantica* et traitées avec du rafoxanide (Ranide, 7,5 mg/kg) à la quatrième semaine n'ont pas été protégées contre une invasion homologue de 250 métacercaires administrés deux semaines plus tard. La réinfestation s'est traduite par des lésions hépatiques plus sévères et un fort pourcentage de trématodes a été retrouvé par rapport à la première infestation. Cependant, la taille des trématodes de la seconde infestation (épreuve) était considérablement réduite. L'activité de l'enzyme plasmatique de l'aspartate amino-transférase (AST), du glutamate-déhydrogénase (GD) et du sorbitol déhydrogénase (SD) s'est élevée à un niveau comparable au cours des deux infestations. Cependant, la réponse des anticorps plasmatiques de *F. gigantica* était moins prononcée chez les chèvres réinfestées. *Mots clés* : Chèvre - Infestation expérimentale - Distomatose - *Fasciola gigantica* - Trématode - Anthelminthique.

### INTRODUCTION

Fascioliasis is an important helminthic disease of farm animals with a wide prevalence throughout the world. However, in goats, the disease has not been adequately studied as in cattle and sheep in which it is responsible for considerable economic losses. Evidence for the caprine susceptibility to the disease was based on data collected from the abattoir (1, 15) and was supported by a further experimental approach (8). However, a question remains whether goats are capable of acquiring resistance to repeated infection with the disease. One answer was provided by ELSANHOURI (5) who demonstrated significant resistance to a homologous reinfection with *F. gigantica*. However, more recent findings with *F. hepatica* have stated otherwise (12). The present work was therefore designed to determine the susceptibility of goats to homologous reinfection with *F. gigantica*.

### MATERIALS AND METHODS

#### Experimental animals

Ten 9-12 month old male Nubian goats were purchased from a fluke-free area around Khartoum province. They received prophylactic doses of anthelmintics (Pamizole,

Fatro/Ranide ; MSD) and blood and faecal samples were examined so as to confirm their clinical soundness and absence of parasitism.

#### Infective material

Metacercariae of *F. gigantica* were obtained from laboratory-infected colonies of *Lymnaea natalensis*. The cysts were collected in cellophane sheets and transferred into gelatinous capsules for oral administration.

#### Experimental design

The animals were divided into two equal groups as indicated in table I. Group I goats were initially infected with 100 *F. gigantica* metacercariae per animal and subsequently treated with a single dose of 7.5 mg/kg rafoxanide (Ranide, MSD) at week 4. The animals were further challenged with 250 metacercariae two weeks after the drug treatment and they were then killed 8 weeks post-challenge.

Group II goats were used as primarily infected controls (*i.e.* infected with 250 *F. gigantica* metacercariae and killed 8 weeks post-infection). The animals were observed for clinical changes and blood samples were collected at week intervals for biochemical and serological investigations.

TABLE I Detailed pathological lesions in the liver of *F. gigantica*-infected goats (primary and challenge).

Lesion	Group I (reinfected)	Group II (primarily infected)
Congestion	+	+
Enlargement	(-)	+
Fibrinous strands	+++	++
Fibrous adhesions with other organs	+++	(-)
Thickening of the liver capsule	++	+
Distortion of the liver surface	+++	++
Haemorrhagic tracts	++	+++
Necrotic foci	+++	++
Fibrosis (cirrhosis)	+++	+
Hyperplasia of the bile duct epithelium	+++	++

+, ++, +++ : severity of lesion. (-) : ... absence of lesion.

#### Liver pathology and worm recovery

The livers were immediately removed at necropsy and examined for the presence of pathological lesions and flukes. Small pieces of the liver were fixed in 10 % formol-

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Reçu le 16.10.1990, accepté le 12.12.1990.

saline for routine histological processing and staining with haematoxylin and eosin (H & E). The flukes were recovered by slicing and squeezing in warm saline (37 °C) and they were counted individually.

### Plasma analysis

The plasma enzyme activities of aspartate amino-transferase (AST), glutamate dehydrogenase (GD) and sorbitol dehydrogenase (SD) were determined according to the methods described by REITAMAN and FRANKEL (13), FORD and BOYD (7) and FORD (6) respectively. The enzyme-linked-immunosorbent-assay (ELISA) was used for the determination of the plasma antibody response as described by BURDEN and HAMMET (3) using crude *F. gigantica* antigens and rabbit anticaprine IgG (H + L) conjugated to horse radish peroxidase (Miles labs). Orthophenylene diamine (OPD) was used as a substrate. The reaction was carried out in polystyrene microplates (Linbro chemicals) and the results were read in Titerek uniskan spectrophotometer and expressed as optical densities (OD).

## RESULTS

### Clinical observations

Group I goats did not show significant clinical changes during the course of the initial infection and treatment. However, they started to lose appetite 4 weeks after reinfection followed by a gradual loss of condition until the time of slaughter. One animal was severely affected and died 3 weeks after challenge. On the other hand, group II goats appeared normal during the first 4 weeks of infection. However, their appetite was reduced by the 6th week and they appeared slightly dull at the time of slaughter.

### Liver pathology

The detailed pathological findings in the liver of goats primarily infected or reinfected with *F. gigantica* are shown in table I. The liver of group II goats (primarily infected

controls) was congested and covered with fine fibrinous strands. The liver capsule was slightly thickened and the surface contained a number of haemorrhagic tracts and necrotic foci. The main bile ducts were dilated and slightly thickened. The gall bladder was distended by thick dark greenish bile containing some mature flukes. Histologically, the migratory tracts consisted of central cores of necrotic tissues infiltrated with erythrocytes and inflammatory cells. The portal area was thickened and infiltrated with fibrous connective tissue elements and mononuclear cells. In addition, there was a moderate hyperplasia of the bile duct epithelium and periductal fibrosis.

Group I goats (reinfected) showed more severe pathological lesions. The liver was congested, mottled and shrunken. The capsule was very much thickened, irregular and occasionally perforated. The cut surface was distorted and hard in consistency. Fibrinous adhesions with other organs were frequently observed.

The necrotic lesions were more extensive than the haemorrhagic ones forming raised plaques. A number of depressed areas representing contracted fibrous tissue were also present. Diffuse haemorrhages were also seen and blood clots were occasionally present. The gall bladder and intrahepatic bile ducts were extremely thickened and distended with flukes and exudate. Black pigment with characteristic foul odour was occasionally seen in the vicinity of the fluke and in the surrounding areas. Microscopically, the liver tissue showed varying degrees of degeneration, necrosis, cellular infiltration and regenerative changes of the hepatocytes. The migratory tracts were similar to those seen in group II goats (primarily infected) but the old lesions were mostly dominated by fibrotic changes and chronic inflammatory reactions. In addition, the portal area was markedly thickened due to extensive fibrosis and mononuclear cellular infiltration and there was considerable hyperplasia of the bile duct epithelium, extensive periductal fibrosis and heavy leucocyte infiltration.

### Worm recovery

The results of worm recovery in goats primarily infected or reinfected with *F. gigantica* are also shown in table II. The worms recovered from primarily infected goats

TABLE II Experimental design and worm recovery in *F. gigantica*-infected or reinfected goats.

Group	Infective dose ( <i>Metacercariae</i> )		Mean fluke recovery (C + SD)		Overall percentage recovery	Remarks
	Primary	Challenge	Primary	Challenge		
Infected, treated and challenged (n = 5)	100	250	20 ± 3.8 (large)*	93.2 ± 13.5 (small)*	32.4	One animal died 3 weeks after challenge. Others were killed on week 8
Primarily infected controls (n = 5)	0	250	—	73.0 ± 15	29.2	All survived and were killed 8 weeks post infection

Large flukes (30-35 mm) originating from the previous infection.  
\* Small flukes (10-15 mm) belonging to challenge infection.

(group II) were all uniform in size ranging from 15 to 20 mm in length. However, in reinfected goats (group I) the majority of worms were reduced in size (10-15 mm) although a number of large flukes (30-35 mm) belonging to the initial infection were also encountered. The overall percentage fluke recovery was slightly higher in reinfected than in primarily infected controls.

### Plasma enzyme activity

The plasma enzyme activity of AST, SD and GD in *F. gigantica* infected goats is shown in figure 1.

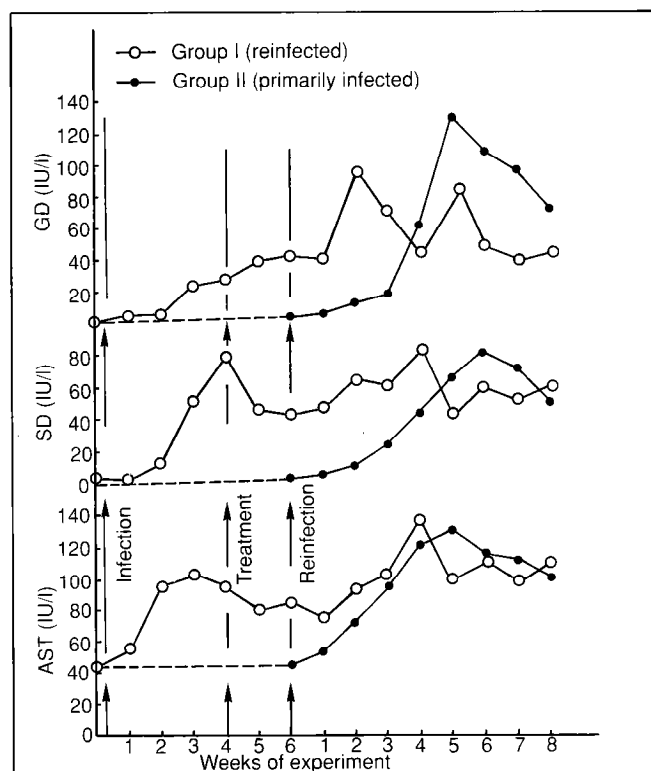


Fig. 1 : Plasma enzyme activity of AST, GD and SD in *F. gigantica*-infected or reinfected goats.

The plasma enzyme activity of AST and SD was slightly increased during the second week of the initial infection in group I goats, and remained high until the animals were drug-treated. They again increased four weeks after challenge and then remained high until the time of slaughter. GD activity also started to increase two weeks after the initial infection and remained slightly elevated even after treatment. A sharp increase in activity was then observed two weeks after challenge and at the fifth week as well.

In primarily infected controls (group II) the plasma enzyme activity of AST, SD and GD increased during the second week of infection, peaked in the 5th or 6th week

and then started to decline. However, the terminal activity was still higher than the pre-infection level.

### Immune response

The antibody response to *F. gigantica* in primarily infected and in reinfected goats is shown in figure 2.

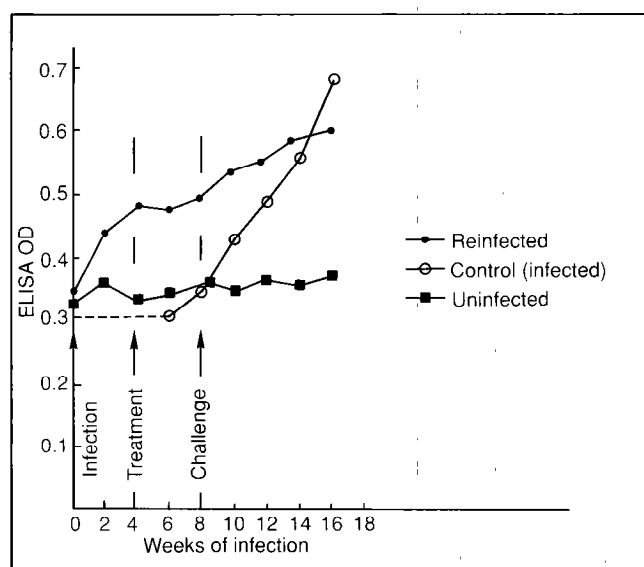


Fig. 2 : Plasma antibody response to *F. gigantica*-infected and reinfected goats.

Results obtained from a separate group of uninfected goats ( $n = 5$ ) were considered as a base-line for negative values. In primarily infected goats (group II) a positive antibody response (above base-line) was detected as early as the second week of infection. The level then continued to rise progressively until the time of slaughter (8 weeks). Group I goats (reinfected) also showed a steady rise in antibody level after the initial infection which continued until week 4. However, the antibody level was slightly suppressed by the treatment and then continued to rise to a lesser extent following reinfection.

### DISCUSSION

Resistance of fascioliasis is usually evaluated by the relative reduction in the parasitic burden of infected animals at necropsy. However, other manifestations such as retardation of the fluke development (*i.e.*) reduction in fluke size), reduction of faecal egg count, delay of onset of the clinical and haematological changes and elevated antibody titres were also considered (9). In the present work homologous reinfection of goats with *F. gigantica* metacercariae was associated with a higher percentage reco-

very of liver flukes and more severe pathological lesions. However, the size of challenging flukes was considerably reduced, but that was not sufficient to improve the severity of hepatic lesions and other clinico-pathological changes. In addition, the plasma antibody response was less pronounced in reinfected goats. The results therefore contradict the previous findings of ELSANHOURI (5) who reported a significant resistance to homologous reinfection with *F. gigantica* in goats. His experiments were similar to those described in the present work, but he used a higher sensitizing initial dose of 200 metacercariae. The antigenic stimulation produced by such a higher dose was probably more capable of inducing a sufficient degree of acquired resistance to challenge. On the other hand, our findings were similar to those obtained by REDDINGTON *al.* (12) who used *F. hepatica*. These authors demonstrated an increased susceptibility to challenge infection and they further concluded that goats are probably similar to sheep in their reduced ability to acquire sufficient immune protection against the disease. Further studies are therefore required to determine the extent of the immunological response in connection with caprine fascioliasis.

Resistance to reinfection with *Fasciola* spp. has long been suggested to result from some kind of a physical barrier imposed by the presence of pre-existing damage and fibrosis of the previously infected liver (2).

However, the extensive fibrosis of the liver of reinfected goats observed in the present work was only associated with a reduction in the size of challenging flukes without any reduction in their number, and that would probably indicate a minimal role of hepatic fibrosis in this respect. The resistance therefore appears to be a real immunological process rather than physically imposed by hepatic

fibrosis. Sufficient evidence for the immunological involvement in the pathogenesis of fascioliasis was further obtained (10, 11, 14) in sheep, cattle and rats.

The plasma antibody response to *F. gigantica* showed a characteristic pattern in primarily infected and in reinfected goats. In both groups, a positive antibody response was detected as early as the second week of infection and continued to increase with time. However, reinfected goats produced less pronounced antibody response to their challenge infection. A similar phenomenon was also observed in reinfected cattle (10) and sheep (14) attributed to a lower antigenic stimulation by the second infection. Furthermore the presence of adult flukes in the main bile ducts was found to suppress the antibody response to subsequent infections (14).

Although the results of the present work generally indicate a reduced ability of goats to resist reinfection with *F. gigantica*, however, a number of factors were also found to influence the degree of resistance to reinfection in various species of animals. The most important of them include the size of the initially infective dose and the duration of the sensitizing period (4, 9) and these factors should be subject to further investigations.

## ACKNOWLEDGEMENTS

This work was supported by research grants from the Graduate College, University of Khartoum and from the National Council for Research, Sudan Government. We are grateful to Dr. B. ABBAS for his help with ELISA and to Miss N. ABDEL FATTAH for technical assistance.

**GORAISH (I.A.), ABDELSALAM, (E.B.), TARTOUR (G.).** Susceptibility to homologous reinfection with *Fasciola gigantica* in goats. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (1) : 69-73

Goats previously infected with 100 viable *Fasciola gigantica* metacercariae and treated with rafoxanide (Ranide, 7.5 mg/kg) at week 4 were not protected against subsequent homologous challenge with 250 metacercariae administered two weeks later. Reinfection resulted in more severe hepatic lesions and a higher percentage of flukes recovered as compared with primarily infected controls. However, the size of flukes originating from the second (challenge) infection was considerably reduced. The plasma enzyme activity of aspartate aminotransferase (AST), glutamate dehydrogenase (GD) and sorbitol dehydrogenase (SD) increased to a similar extent with primary and challenge infections. However, the plasma antibody response to *F. gigantica* was less pronounced in reinfected goats. *Key words* : Goat - Reinfection - Distomatosis - *Fasciola gigantica* - Trematode - Anthelmintics.

**GORAISH (I.A.), ABDELSALAM, (E.B.), TARTOUR (G.).** Sensibilidad a una reinfestación homóloga por *Fasciola gigantica* en cabras. *Revue Élev. Méd. vét. Pays trop.*, 1991, **44** (1) : 69-73

La administración de rafoxanide (Ranide, 7,5 mg/kg) en cabras durante la cuarta semana de una infestación experimental con 100 metacercarias vivas de *Fasciola gigantica* no las protegió contra una invasión homóloga de 250 metacercarias administradas dos semanas más tarde. La reinfestación causó lesiones hepáticas más graves y se encontró un porcentaje más elevado de trematodos que durante la primera infestación. Sin embargo, la dimensión de los trematodos de la segunda infestación experimental estaba considerablemente reducida. La actividad de la enzima plasmática del aspartato amino-transferasa, del glutamato-dehidrogenasa y del sorbitol dehidrogenasa llegó a un nivel comparable durante las dos infestaciones. La repuesta de los anticuerpos plasmáticos de *F. gigantica* era menos importante en las cabras infestadas. *Palabras claves* : Cabra - Infestación experimental - Distomatosis - *Fasciola gigantica* - Trematoda - Antihelmíntico.

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