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# RESISTANCE TO *SCHISTOSOMA BOVIS* IN SHEEP INDUCED BY AN EXPERIMENTAL *FASCIOLA HEPATICA* INFECTION

**Matilde Rodríguez-Osorio, Victoria Gómez-García, José Rojas-González, Vicente Ramajo-Martín*, M. Yolanda Manga-González,† and Camino González-Lanza†**

Department of Immunology, Instituto López Neyra de Parasitología, c/ Ventanilla 11, 18001 Granada, Spain

ABSTRACT: Sheep infected with *Fasciola hepatica* for 10 wk acquired a substantial level of resistance to challenge with *Schistosoma bovis*. The worm burden was reduced by 87.2% ($P < 0.01$) compared with that of a control group. But when sheep primarily were infected with *S. bovis* and 6 wk later with *F. hepatica*, no significant reduction in the *S. bovis* burden was observed.

Cross-resistance between *Schistosoma mansoni* and *Fasciola hepatica* has been demonstrated in mice by Hillyer (1979, 1981) and Christensen et al. (1978, 1980). Calves harboring mature primary infection with *Schistosoma bovis* also showed significant resistance to challenge with *F. hepatica* (Sirag et al., 1981). Reciprocal resistance between *S. bovis* and *Fasciola gigantica* was detected in Sudanese zebu calves (Yagi et al., 1986). Monrad et al. (1981) found a high level of resistance to *F. hepatica* in sheep with 2–3-wk-old and 7–8-wk-old *S. bovis* infections; however, older infections were not protective. Similarly, primary infection of sheep with *S. mansoni* followed by oral infection with *F. hepatica* resulted in a reduction of the *F. hepatica* burden (Haroun and Hillyer, 1988). Immunity to schistosomes using heterologous trematode antigens was reviewed by Hillyer (1984).

This is the first demonstration of cross-resistance induced by a primary infection of *F. hepatica* in sheep to challenge with *S. bovis*.

## MATERIALS AND METHODS

*Fasciola hepatica* metacercariae were produced from laboratory-bred *Lymnaea truncatula* infected with 5–10 miracidia per snail derived from eggs of laboratory-maintained sheep.

The Salamanca strain of *S. bovis* was maintained in Castellana sheep, and *Planorbarius metidiensis* was used. Recently emitted cercariae were used to infect animals.

Three groups of 6 3-mo-old Castellana sheep were used. Figure 1 illustrates the experimental plan. Control animals were infected percutaneously with 400 *S. bovis* cercariae each for 30 min by submerging the forelimb in a plastic receptacle containing the cercarial suspension of *S. bovis*. The leg was sheared and washed with water immediately before infection, according to the technique of Van Wyk et al. (1975). Animals in experiment I primarily were infected with 80 *F. hepatica* metacercariae administered by esophageal probe and challenged after 10 wk with a single exposure to 400 *S. bovis* cercariae. In experiment II, animals were exposed to 400 *S. bovis* cercariae and infected 6 wk later with 220 *F. hepatica* metacercariae.

Necropsies were performed 24 wk after last infection. *Schistosoma bovis* adults were recovered from the mesenteric and gastric radicles, the intrahepatic branches of the portal vein and the internal iliac veins, using a modification of the perfusion technique developed for use in sheep by McCully and Kruger (1969). The worms remaining in the veins beneath the serosa of the intestine were counted in situ. *Fasciola hepatica* worms were recovered by dissection of the bile ducts. The degree of resistance was evaluated by comparing the number of worms recovered in infected and control groups.

Statistical analysis of the results was carried out using a 1-way ANOVA.

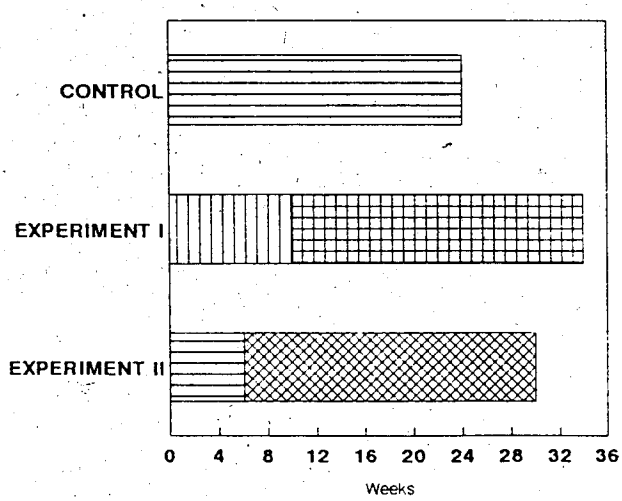


FIGURE 1. Experimental plan: fill patterns indicate infection with 400 *Schistosoma bovis* cercariae (▤), infection with 80 *Fasciola hepatica* metacercariae (▥), challenge infection with 400 *S. bovis* cercariae (▦) or challenge infection with 220 *F. hepatica* metacercariae (▨).

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* Department of Animal Pathology, Instituto de Recursos Naturales y Agrobiología, c/ Cordel de Merinas 40-52, 37001 Salamanca, Spain.

† Department of Animal Parasitology, Estación Agrícola Experimental, Apdo. 788, 24080 León, Spain.

TABLE I. Recovery of *Fasciola hepatica* and *Schistosoma bovis* adult worms in experiments I and II and control groups.*

| | Animals | Mean worm recovery ± SD | | R† (%) | |
|---|---|---|---|---|---|
| | | *F. hepatica* | *S. bovis* | | |
| Control | 6 | – | 107.8 ± 9.4 | – | |
| Experiment I | 6 | 55.5 ± 23.6 | 13.8 ± 5.2 | 87.2 | ($P < 0.01$)‡ |
| Experiment II | 6 | 192.3 ± 14.9 | 104.2 ± 29.4 | 3.3 | ($P > 0.7$)‡ |

* Experiment I, sheep infected with 80 *F. hepatica* metacercariae and challenged with 400 *S. bovis* cercariae; experiment II, sheep infected with 400 *S. bovis* cercariae and challenged with 220 *F. hepatica* metacercariae; control, sheep infected with 400 *S. bovis* cercariae.

† R, percentage reduction of *S. bovis* burden. R = ([number of worms in control − number of worms in experiment] ÷ number of worms in control) × 100.

‡ One-way analysis of variance.

## RESULTS

Worm recoveries are indicated in Table I. A substantial level of protection to *S. bovis*, in terms of worm reduction, developed in sheep infected with a single dose of 80 metacercariae. The mean burden of *S. bovis* in the group previously infected with *F. hepatica* was much lower than that in the control group, corresponding to an 87.2% reduction. ANOVA showed that the difference between the means was significant ($P < 0.01$). The results of *S. bovis* recovery in experiment II showed that a *F. hepatica* infection did not have influence on a previously established *S. bovis* infection, as the difference between the mean burdens of *S. bovis* in experiment II and the control group was not significant ($P > 0.7$).

## DISCUSSION

There is no evidence that primary infection of sheep with *F. hepatica* induces resistance to homologous challenge in terms of reduction in the number of worms recovered from this latter infection. This fact has been reported by numerous authors (Boray, 1967; Sinclair, 1971a, 1973; Knight, 1980; Sandeman and Howell, 1981). Attempts to stimulate resistance to *F. hepatica* by immunization with somatic or metabolic products (Ross, 1967; Sandeman et al., 1980) and homogenates of lymph nodes or spleen (Sinclair, 1971b) consistently were unsuccessful in inducing protection in sheep. However, the findings from experiment I presented here provide evidence of heterologous resistance. These facts suggest that *F. hepatica* might evade the host's immune system by eliciting humoral and cellular responses to nonessential epitopes that may prove vital for *S. bovis*.

Data from experiment II seem to indicate that the protective mechanisms triggered by *F. hepatica* act on the primary stages of *S. bovis* but not on established adult worms.

We have found no reference to the resistance to *S. bovis* in sheep induced by *F. hepatica* infections. Yet, Yagi et al. (1986) found that *F. gigantica* experimental infections protected cattle against *S. bovis* challenge. Studies (Monrad et al., 1981; Sirag et al., 1981) in line with the present one showed that a primary infection with *S. bovis* protected sheep and calves to a challenge with *F. hepatica*. Sheep infected with *S. mansoni* and challenged with *F. hepatica* also presented (Haroun and Hillyer, 1988) a reduction in the *F. hepatica* burden. Similarly, infection of mice with *F. hepatica* conferred significant resistance to a heterologous *S. mansoni* challenge (Christensen et al., 1978). It is not known whether the observed heterologous resistance involves immunological factors, mechanical barriers, or both.

Heterologous trematode antigens have been used (Hillyer, 1984) to induce immunity against *S. mansoni*. It has been established (Hillyer, 1979) that cross-reactive antigens isolated from *F. hepatica* protect against *S. mansoni* and that some of these antigens share common epitopes with other trematodes such as *S. bovis*.

It has been found (J. Rojas, 1991, pers. comm.) that 125-, 69-, 36-, and 17-kDa *F. hepatica* antigens were shared by *S. bovis*. These common antigens may well be responsible for the heterologous resistance reported here. Further studies and the isolation of these antigens will be necessary to elucidate their possible protective role.

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