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Research note

Schistosoma bovis: vaccine effects of a recombinant homologous glutathione *S*-transferase in sheep

Denis Boulanger[a,*], Dominique Schneider[a], Jean-Philippe Chippaux[a], Bertrand Sellin[a], André Capron[b]

[a] *Centre de Recherche sur les Méningites et Schistosomoses (CERMES/OCCGE/ORSTOM), WHO Collaborating Centre for the Control of Schistosomosis, BP 10887, Niamey, Niger*
[b] *Institut Pasteur, Lille, France*

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Abstract

The economic importance of the trematode *Schistosoma bovis* in African livestock has justified the development of a specific vaccine. Administered preventively to sheep, rSb28GST—the only molecule cloned from *S. bovis* which has demonstrated vaccine potentialities in goats and cattle—reduced the mean worm burden in vaccinated animals and improved their health status compared with that of non-vaccinated controls. As in goats, but not in bovines, the fecundity of the settled worm pairs was not modified. Therefore, rSb28GST can be proposed as a universal tool for the prevention of clinical disorders engendered by the main schistosome species affecting domestic ruminants in the African continent.

Keywords: *Schistosoma bovis*; Vaccination; Sheep; Recombinant protein; Glutathione *S*-transferase

Schistosoma bovis is responsible for a helminthiasis affecting livestock in Africa, with prevalences reaching up to 90% in calves [1]. Its severe pathological impact, also demonstrated in small ruminants [2], has led to attempts to control the disease through vaccination. Irradiated larvae have been efficacious [3], but their large-scale utilisation remains logistically difficult. Recently, the preventive administration of a recombinant *S. bovis*-derived glutathione *S*-transferase (rSb28GST) resulted in dissociated effects according to the definitive host species: in cattle, it markedly diminished the fecundity of paired adult worms [4] whereas, in goats, it significantly reduced worm burden [5]. Because the ovine commercial market is of crucial importance in many sub-Saharan countries, it was considered important to check whether or not the rSb28GST was protective in sheep and, if so, what was the expression of the vaccine-induced immunity.

The same schedule of immunisations/infection as in the caprine experiments [5] was used. Briefly, eight male Sahelian sheep (14–26 kg at the start of the experiment) received two s.c. injections, 6 weeks apart, of 100 μg of rSb28GST [6] in PBS, with Freund's complete, then incomplete, adjuvant v/v.

* Corresponding author. Tel: +227-75-20-45; fax: +227-75-31-80; e-mail: boulange@niamey.orstom.ne.

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Table 1
Clinical and parasitological data in sheep twice-immunised with the recombinant Sb28GST and in controls receiving adjuvant alone[a]

	rSb28GST	Controls	p[b]	*P*
Clinical status				
PCV (%)[c]	−8.2±3.7	−13.8±4.2	−41%	<0.05
Body weight (kg)[c]	−2.9±1.3	−3.8±1.2	−23%	<0.05
Faecal egg excretion				
Per 24 h	2511±1216	4786±2892	−48%	<0.05
Per 24 h per female	33.8±18.9	36.2±23.6	−7%	NS
Adult worms				
Males	84.4±28.3	142.1±44.8	−41%	<0.05
Females	86.0±23.9	136.6±41.8	−37%	<0.05
Total	170.4±39.5	278.6±79.3	−39%	<0.05
Tissue eggs				
Liver (%)	16±14	28±30		
Small intestine (%)	39±26	46±29		
Large intestine (%)	46±28	27±23		
Total (×10³)	434±368	527±332	−18%	NS
Per female	4571±2688	3875±2199	+18%	NS
Intra-uterine eggs	16.1±4.3	19.1±3.4	−16%	NS

[a] Data are expressed as mean±S.D.
[b] Protection (%)=$(A-B)/B\times 100$, where A is the rSb28GST-immunised group average and B the control group average.
[c] Expressed as the mean difference between the start and the end of the experiment.

Control animals received adjuvant alone. Four weeks after the boost, sheep were anaesthetised then infected percutaneously with a single dose of 2000 cercariae of a local strain of *S. bovis*. Perfusion took place 20 weeks later. Statistical comparisons were made by the non-parametric Mann–Whitney test.

Clinical and parasitological data are summarised in Table 1. One animal died before the end of the experiment in each group (weeks 16 and 18, respectively). Packed cell volume values were negatively correlated with the intensities of faecal egg excretion. Decreases of body weights and of PCV were significantly lower in the vaccinated group. Mean worm burdens and faecal egg excretion were significantly reduced. In contrast, the mean number of eggs trapped in the tissues or counted in the uterus of each female schistosome on the day of perfusion were not affected by the immunisation schedule. Thus, the mean numbers of faecal or tissue eggs per female were comparable in both groups. Individual levels of protection were somewhat heterogeneous. Figure 1 shows that, in terms of faecal egg excretion, six vaccinated sheep had protection levels ranging from 47% to 68%, whereas two animals displayed levels of egg excretion close to the control group average.

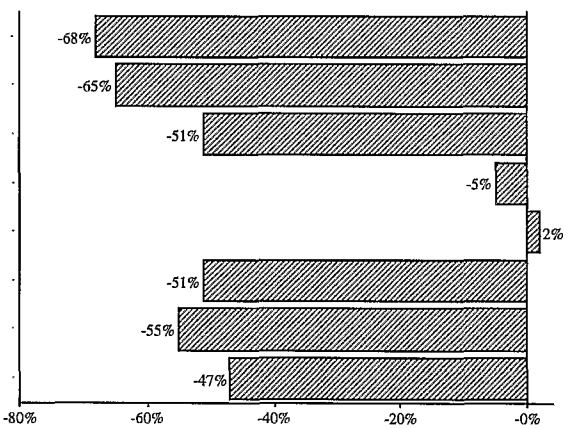

Fig. 1. Individual levels of reduction of faecal egg excretion in rSb28GST-immunised sheep compared to the control average.

The dual goal of this experiment was first to

Table 2
Compared protective effects of the rSb28GST in two species of small ruminants

	Goats[a]	Sheep
Female worm burden	−46%[b]	−37%[b]
Tissue eggs	−35% (NS)	−18% (NS)
Tissue eggs per female	−7% (NS)	+18% (NS)
Faecal egg excretion 24 h	−30% (NS)	−48%[b]
Faecal egg excretion per 24 h per female	+29% (NS)	−7% (NS)

[a] Data from Boulanger et al. [5].
[b] $P<0.05$; NS: not significant.

check in sheep the vaccine capacity of the rSb28GST already demonstrated in goats [5], and second to assess the expression of vaccine-induced immunity. Table 2 compares the protective effects on parasitological parameters obtained in both species. At a somewhat lower level, a similar worm burden reduction was obtained in sheep. In contrast, a sharp effect on faecal egg excretion was obtained in sheep, whereas it did not reach significance in goats. Taken as a whole, one can assume that both species reacted roughly in the same manner. The immunological peculiarities of goats and sheep when facing parasites [7] required this confirmation.

The lack of modification of faecal, tissue and intra-uterine eggs per female schistosome is a strong argument in favour of an immune target being the worm capacity to establish an adult population in its definitive host, rather than its egg-laying productivity. Both male and female worms were equally affected, suggesting that paired schistosomes, not migrating larvae, are likely to be the real targets. These data confirm that small ruminants do not develop the same protective mechanisms as other animal models of schistosomosis [8]. For instance, vaccination with the *Schistosoma mansoni*-derived 28GST of Patas monkeys challenged with *Schistosoma haematobium* clearly targeted worm fecundity [9]. The contrast is especially striking with the results of a trial performed on calves [4], showing that vaccination with native SbGST induced significant reductions in faecal egg counts and in tissue egg densities, although adult worm counts were not modified. However, rSb28GST was able to affect both parameters when calves were subjected to natural infection with *Schistosoma matthei* [10]. It should also been kept in mind that, unlike cattle [11], the development of a naturally acquired resistance does not seem to occur in sheep following repeated exposures to the parasite in the field [12], suggesting that the two host species do not develop the same mechanisms when subjected to natural schistosome infection. Basic studies on their immune responses to experimental challenge are under investigation and would be of great interest in the context of comparative immunology.

Taken together with the results derived from experiments carried out in cattle against *S. bovis* and against *S. matthei*, the results presented here suggest that the recombinant molecule rSb28GST can be proposed as a relevant tool to control the important economic impact of schistosomes affecting ruminants in Africa. In the more general framework of trematodosis, they confirm previous observations showing that preventive immunisation of sheep and cattle with *Fasciola hepatica*-derived GST also results in significant worm burden reductions [13].

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