Potential of a recombinant *Schistosoma bovis*-derived glutathione S-transferase to protect cattle against experimental and natural *S. mattheei* infection

J. DE BONT[1]*, J. VERCRUYSSE[1], J. M. GRZYCH[2], P. F. M. MEEUS[3] *and* A. CAPRON[2]

[1] *Department of Parasitology, Faculty of Veterinary Medicine, University of Gent, Salisburylaan 133, B-9820 Merelbeke, Belgium*
[2] *Centre d'Immunologie et de Biologie Parasitaire, Unité Mixte INSERM-U167, IFR No. 17 Institut Pasteur de Lille, 1 rue du Pr Calmette, 59019 Lille, France*
[3] *Department of Clinical Studies, Samora Machel School of Veterinary Medicine, University of Zambia, Box 32379, Lusaka, Zambia*

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SUMMARY

The potential of a recombinant *Schistosoma bovis*-derived glutathione S-transferase (rSb28GST) to protect cattle against *S. mattheei* infection was tested in Zambia. All animals were challenged 2 weeks after the second inoculation with either 0·250 mg rSb28GST in adjuvants (vaccinated calves, $n = 14$) or adjuvants alone (controls, $n = 14$). In a first experiment, 7 vaccinated and 7 control animals were exposed to 10000 *S. mattheei* cercariae percutaneously. All animals developed clinical schistosomiasis 7–8 weeks after challenge. At perfusion, 12 weeks after challenge, vaccinated and control groups had averages of 887 and 541 eggs per gramme (epg) faeces, 6515 and 5990 worms, and 4·2 and 3·4 million tissue eggs, respectively. These results indicate that the immunization protocol used did not protect these calves against the massive single experimental challenge. In a second experiment, another 2 groups ($n = 7$) of vaccinated and control animals were challenged naturally over a period of 9 months on a farm known to be endemic for *S. mattheei*. The natural infections were much lighter in intensity, as indicated by the mean faecal egg count (13 epg), worm count (139) and tissue egg count (294000) in non-vaccinated controls. In vaccinated calves, significant reductions in female worm burdens (50%), faecal egg counts (89%) and miracidial counts (93%) were recorded. Total tissue egg counts were also reduced by 42% in vaccinated animals. It therefore appears that the rSb28GST can provide significant protection in cattle against *S. mattheei* under conditions of low to moderate natural infection.

Key words: *Schistosoma mattheei*, vaccination, glutathione S-transferase, cattle.

INTRODUCTION

Cattle schistomiasis is widely distributed in Africa and Asia (Christensen, Mutani & Frandsen, 1983; Kumar & de Burbure, 1986). Although most infections occur under a subclinical form, characterized by high prevalence of low to moderate worm burdens in the cattle population (Dargie, 1980; Kassuku *et al*. 1986; De Bont *et al*. 1991, 1994), it has been established that schistosomiasis causes significant losses to cattle farms in endemic areas (Dargie, 1980; Pitchford & Visser, 1982; McCauley, Tayeb & Majid, 1983; McCauley, Majid & Tayeb, 1984). Curative drugs are now available but, for obvious practical and economical reasons, they are not suitable for mass treatment in domestic stock (Hussein, 1980).

Studies on immunity development against schistosomiasis in cattle have clearly indicated the existence of acquired resistance to infection, but differences appear in the form of protection acquired either experimentally or naturally. In experiments using a single primary infection followed by a challenge, Massoud & Nelson (1972) and Lawrence (1973*a*) observed no protection against challenge infection but significant reductions in the fecundity of reinfecting worms. In contrast, naturally infected animals exposed to continuous moderate challenge develop high levels of resistance, not only through a reduction of worm fecundity (Bushara *et al*. 1980; Majid *et al*. 1980*a*; De Bont *et al*. 1995*a*), but also through protection against reinfection (Bushara *et al*. 1983*a*, *b*; De Bont *et al*. 1995*a*, *b*) and decreased egg hatchability (De Bont, Vercruysse & Massuku, 1996).

Initial investigations towards immunological control of cattle schistosomiasis focused on the use of homologous schistosomular vaccines attenuated by irradiation (reviewed by Taylor, 1987). In the Sudan, one such vaccine was shown to induce significant reductions in *Schistosoma bovis* infection rates, both in the laboratory (Bushara *et al*. 1978) and in the field (Majid *et al*. 1980*b*). More recent investigations have

* Corresponding author: Department of Parasitology, Faculty of Veterinary Medicine, University of Gent, Salisburylaan 133, B-9820 Merelbeke, Belgium. Tel: +32 9 264 74 03. Fax: +32 9 264 74 96. E-mail: Jean.DeBont@rug.ac.be.

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been aimed at the identification of defined, schistosome-derived protective antigens that are easier to use on a large scale than live vaccines. One such class of molecules, originally isolated from *S. mansoni* (Balloul *et al*. 1987) and later identified as a glutathione S-transferase (GST) (Taylor *et al*. 1988) has shown protective capacities in various animal models experimentally infected with *S. mansoni* (Capron *et al*. 1994). Interestingly, immunity induced by vaccination with the recombinant GST induced either a reduction in parasite burden or a depression of the female worm fecundity, depending on the animal model and the immunization protocol used (Boulanger *et al*. 1991; Xu *et al*. 1993).

In the only vaccination trial with a GST reported in cattle, inoculation of naive calves with the native GSTs purified from *S. bovis* (SbGST) followed by a single experimental infection with a local strain of *S. bovis* induced a highly significant reduction of faecal and tissue egg counts but had no effect on the worm burden (Bushara *et al*. 1993).

The present study was carried out to test the potential of the recombinant *S. bovis*-derived GST (rSB28GST) to protect cattle against *S. mattheei* infection. In a first trial a similar experimental design as for the trial with *S. bovis* was used. In a second experiment, the vaccine was tested in calves exposed to moderate natural challenge.

MATERIALS AND METHODS

Animals

Twenty-eight castrated male calves (Friesian) aged 4 to 6 months were obtained from a schistosome-free farm near Lusaka in central Zambia. The animals were transported to the School of Veterinary Medicine where they were fed on a balanced ration of roughage and concentrates and had free access to clean water. They were divided according to live weight into 4 equal groups of 7 calves each: 2 groups for the experimental infection trial (EV and EC) and another 2 for the natural infection trial (NV and NC). One calf of group EC died from pyelonephritis before the end of the immunization period.

Immunization and infection schedules

The rSb28GST was prepared as previously described (Trottein *et al*. 1992) from a Salamanca strain of *S. bovis*. Animals of groups EV and NV received 2 intramuscular injections of 0·25 mg of the rSb28GST in PBS emulsified in an equal volume of Freund's Complete Adjuvant (FCA, Sigma), at 3-week intervals. The 2 other groups of non-vaccinated controls (EC and NC) also received 2 injections, but with PBS emulsified in FCA only. All calves were then challenged with *S. mattheei*, either experimentally or naturally, 2 weeks after the second vaccination. Animals involved in the experimental infection trial (groups EV and EC) were exposed, for 30 min on the skin of their shaved tails (Bushara *et al*. 1980), to 10000 cercariae of a local strain of *S. mattheei* (see De Bont *et al*. 1995*a*, *b*) maintained in *Bulinus globosus* snails. They were perfused for worm recovery 12 weeks after challenge. The calves involved in the natural infection trial (groups NV and NC) were transported to a nearby farm where *S. mattheei* is endemic, and mixed with the farm herd. All the grazing animals were led twice a day to a drinking pond which harboured *B. globosus* snails. The calves were removed from the farm 9 months later and immediately perfused.

Parasitological methods

The development of infections was monitored weekly in the animals infected experimentally and every fortnight in those stationed at the farm. Each time the body weight was measured, faeces were collected from the rectum and blood sampled from the jugular vein for determination of the packed cell volume (PCV) value. Faecal egg counts were determined using a modification (De Bont *et al*. 1995*a*) of the concentration technique of Lawrence (1970). Miracidial counts in 50 g of faeces (De Bont *et al*. 1991) were used to measure faecal excretion of viable eggs at perfusion. Techniques for worm recovery by perfusion and for tissue egg counting were as described previously (De Bont *et al*. 1995*a*).

Data comparison

Non-parametric tests were used in the data analysis because even after log-transformation the data were not normally distributed. The purpose of the vaccination trials was to evaluate whether there had been a reduction in parasitological parameters following vaccination, and therefore one-sided Mann–Whitney tests were performed on faecal egg, miracidia, tissue egg and worm counts. For the worm count data, the number of female worms was considered as the most important parameter. The number of adult female worms obtained from perfusion should equate with the number of worm pairs in the host. It is only worm pairs that produce eggs, and it is these eggs which result in the pathological effects of schistosome infection. However, male worm burdens (which include immature males) were also considered to see whether there were sex-related differences in the effect of the vaccine.

RESULTS

Experimental infections

The faecal egg excretion started 6 weeks after challenge in 3 of the experimentally infected calves. By 7 weeks post-challenge, all calves were found positive on faecal examination. Mean faecal egg

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Table 1. Faecal egg counts, adult worm recoveries and tissue egg counts in calves vaccinated with the rSb28GST (Group EV) and in controls receiving adjuvant alone (Group EC)

(Animals were perfused 12 weeks after challenge with 10000 *Schistosoma mattheei* cercariae. Results are expressed as mean ± S.D.)

	EC Controls ($n = 5$)	EV Vaccinated ($n = 7$)
Faecal egg counts	541·7 ± 240·7	887·1 ± 497·9
Worm recoveries		
Male	3092·6 ± 435·1	3262·9 ± 1105·8
Female	2897·2 ± 675·5	3252·4 ± 1112·4
Total	5989·8 ± 538·3	6515·3 ± 2199·4
Tissue egg counts ($\times 10^3$)		
Liver	133·4 ± 41·1	209·0 ± 121·5
Small intestine	2619·5 ± 2711·5	3212·8 ± 3688·9
Large intestine	644·8 ± 559·7	815·0 ± 648·3
Total	3397·7 ± 3261·3	4236·8 ± 1625·3

counts in both groups of vaccinated and non-vaccinated animals increased rapidly up to week 10 and stabilized around the values recorded at perfusion (Table 1). Differences in average egg output between two groups were not statistically significant at any time-point, probably because of large individual variations within groups. Beginning 7–8 weeks after infection, all animals developed clinical schistosomiasis characterized by diarrhoae, sometimes haemorrhagic, and general weakness. One control calf died at week 12, apparently from acute rumenitis, and could not be perfused. The mean PCV values in vaccinated and control animals before immunization were 24·1 and 25·0 %, respectively. In spite of the visible loss of blood through the guts, PCV values remained fairly stable throughout the experiment (ranges 24·1–28·0 and 22·8–27·8, respectively), and no significant difference between groups was observed ($P < 0{\cdot}344$). Similarly, there was no significant difference between groups in the average daily weight gain, either before challenge (157 and 175 g/day, respectively) or later (210 and 230 g/day, respectively).

The data collected from the perfusion are summarized in Table 1. The highest individual faecal egg count recorded then was 1620 epg faeces (Group EV). As could be expected from the egg counts, the numbers of miracidia in the 2 pooled faecal samples were too massive to be counted. The average total worm recoveries in the vaccinated and control groups were 65·2 and 59·9 % of the cercarial dose, respectively. Whilst faecal egg counts, worm burdens and total tissue egg count were higher in the vaccinated group than in controls, differences were not significant (P always $> 0{\cdot}117$). Diffuse, sometimes severe, haemorrhagic lesions were present in the mucosa of the intestines in all animals.

Natural infections

Natural infections were much lighter in intensity. Faecal egg excretion was first detected at week 14 post-challenge. Counts remained too low (maximum 30 epg) and irregular to allow any statistical comparison of the 2 groups during the grazing period. Except for 1 control calf, all animals were shown positive on faecal egg examination at least once before perfusion. No clinical signs of schistosomiasis were observed and the original mean PCV values in groups NV (27·6 %) and NC (28·7 %) remained stable throughout the experiment. The average gains in body weight during the 9 months spent on the farm were 78·3 and 79 kg in groups NV and NC, respectively.

The data collected at the perfusion are shown in Table 2. Vaccinated calves had significantly lower faecal egg counts than control animals ($P < 0{\cdot}018$) and the excretion of viable eggs, as determined by miracidial counts, was significantly reduced by 93 % ($P < 0{\cdot}0009$). Although female worm burdens did vary within each group, the vaccinated group had significantly lower numbers ($P < 0{\cdot}048$) with a mean reduction of 50 %. In addition, the variance to mean ratio, an indication of how much variation in female worm burden there was between the animals in the different groups, was much lower in vaccinated animals (1·7), compared to controls (16·2). There was also a reduction in the numbers of male worms in vaccinated calves of 40 %, but this reduction was not significant ($P < 0{\cdot}125$). Total tissue egg counts were reduced by 42 % in vaccinated animals but the difference was not significant ($P < 0{\cdot}159$). No macroscopical lesions attributable to schistosomiasis were observed at post-mortem.

DISCUSSION

Following the identification of the schistosome-derived GSTs as a promising vaccine candidate, several vaccination experiments were undertaken in various animal models experimentally infected with *S. mansoni*, *S. japonicum* or *S. bovis* (Capron *et al.* 1992*a*, 1994; Bashir *et al.* 1994; Boulanger *et al.* 1994). Vaccination with the 28GST was shown to affect either worm viability, female worm fecundity or egg hatchability, depending on the parasite/animal model and immunization protocol used (Boulanger *et al.* 1991, 1994; Xu *et al.* 1991). This is clearly illustrated by the different results obtained in the 2 reported trials using the *S. bovis*-derived GST in ruminants. Significant reductions of faecal and tissue egg counts but not worm burden were observed using the native GST in cattle (Bushara *et al.* 1993), whereas immunization with the recombinant protein in goats affected worm viability but not fecundity (Boulanger *et al.* 1994). Such diversity in the type of protective effect suggests that the

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Table 2. Faecal egg and miracidial counts, worm recoveries and tissue egg counts in calves vaccinated with the rSb28GST (Group NV) and in controls receiving adjuvant alone (Group NC)

(Animals were perfused after natural exposure to *Schistosoma mattheei* challenge for a period of 9 months. Results are expressed as mean ± S.D.)

	NC Controls ($n = 7$)	NV Vaccinated ($n = 7$)	% Reduction*
Faecal egg counts	12·9	1·4	89
Miracidial counts (50 g faeces)	187·3 ± 168·2	12·9 ± 8·6	93
Worm recoveries			
Male	90·6 ± 63·7	54·4 ± 39·8	40
Female	48·1 ± 28·0	24·3 ± 6·4	50
Sex ratio	1·86 ± 0·65	2·09 ± 1·14	
Total	138·7 ± 87·8	78·7 ± 44·6	43
Tissue egg counts ($\times 10^3$)			
Liver	14·6 ± 9·8	6·6 ± 2·5	55
Small intestine	110·1 ± 97·7	70·8 ± 45·4	36
Large intestine	168·9 ± 160·8	94·2 ± 45·4	44
Total	293·6 ± 217·3	171·6 ± 73·6	42

* % Reduction = $(B-A)/A \times 100$, where A is the average of the control group and B the average of the immunized group.

induced immune response may act against multiple but not exclusive targets (Boulanger *et al*. 1994). Investigation of the immune effector mechanisms underlying the effects of immunization is now part of on-going research aimed at optimizing protection levels (Capron *et al*. 1992*b*). Meanwhile, immunization tests should be continued to explore the different factors which may affect the protecting capacities of the vaccine. The experiment described here is the first using the potential recombinant vaccine as it should eventually be applied on a large scale; this is in recipients exposed under field conditions to a natural challenge. For the purpose of comparison, the now classical protocol of a single experimental infection was used in other animals.

Our results show that vaccination with the recombinant Sb28GST could significantly affect the course of schistosome infections in cattle naturally exposed to *S. mattheei*. After 9 months of natural challenge, reductions by 50 % in female worm burdens and by 42 % in overall accumulation of eggs in tissues were found in vaccinated calves as compared to controls. This protection of susceptible animals would reduce their chance of developing acute schistosomiasis, which is mainly caused by the passage of large numbers of eggs through the intestinal wall (Hussein, 1971; Lawrence, 1977*a*). Furthermore, the spectacular decreases in faecal egg counts (89 %) and miracidial counts (93 %) observed in vaccinated animals suggests that vaccination could significantly reduce the contamination of the environment with eggs, which is largely caused by young animals (Christensen *et al*. 1983; De Bont *et al*. 1991, 1994, 1996). It has been postulated that such a decrease in egg excretion would reduce transmission (Capron *et al*. 1994). However, this aspect remains controversial (Dunne, Hagan & Abath, 1995): owing to the ability of schistosomes to multiply in their intermediate host, the number of infected snails at the transmission site does not need to be necessarily high to ensure significant levels of transmission. Studies of transmission levels in pools visited by vaccinated cattle could test the validity of this hypothesis. The decrease in female worm burden in vaccinated calves after 9 months of natural challenge was found to be only just significant ($P < 0{\cdot}048$), which could possibly be attributed to the small number of animals and the large individual differences in worm counts within each group. These variations can either be due to individual differences in susceptibility and/or exposure to infection. However, much lower variation in female worm burdens was observed in vaccinated animals as compared to controls. This is perhaps an indication that the higher susceptibility to infection observed in some controls is immune related, and can be reduced by vaccinating with the rSb28GST. There was no significant difference between the reductions in male and female worms. However, the numbers of male worms were reduced by 40 %, as opposed to 50 % in females. Clearly, more data are required to verify whether this could be indicative of sex-related differences in the effect of vaccination.

Despite a decrease of 50 % in female worm burdens in vaccinated calves, total tissue egg counts in these animals were only reduced by 42 %, which suggests a lack of vaccine-induced effect on fecundity. In trials using single experimental challenges, all adult

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females recovered at perfusion may be assumed to have started egg production at the same time, and the total tissue egg count per adult female worm may therefore be used as indicator of worm fecundity. In our trial based on daily exposure to natural challenge, the duration of egg production of each female worm is unknown and it is not possible to measure the effect of vaccination on worm fecundity. In calves experimentally exposed to between 5000 and 45000 *S. mattheei* cercariae, Lawrence (1973*b*) observed a significant reduction in total egg output as parasite numbers increased. A vaccine-induced reduction in worm burden could therefore lead to an increase in female worm fertility. However, the existence of density dependence in schistosome egg production remains a matter for debate (Gryseels & de Vlas, 1996). In contrast with tissue egg counts, faecal egg counts in vaccinated animals were significantly lower than in controls. This cannot readily be explained and shows the possible complexity of the relationship between the two parameters. However, faecal egg counts were all very close to the lower sensitivity limit of the counting technique used and the percentage of reduction should therefore be interpreted with caution.

Because of the low faecal egg outputs in natural infections, it was not possible to compare egg and miracidial counts in the same samples, and test the existence of a vaccine-induced decline in egg viability. Such a decline was demonstrated in vaccination trials against *S. mansoni* (Boulanger *et al.* 1991; Xu *et al.* 1993). However, a comparison in our experiment of the levels of reduction in numbers of adult females (50%), tissue egg counts (42%) and miracidial counts (93%) suggests that such a decline also occurred. An age-specific decrease in hatchability of eggs was demonstrated in *S. mattheei* infections in cattle (De Bont *et al.* 1996) and *S. mansoni* human infections (Upatham, Sturrock & Cook, 1976).

The results from the experimental infections are in marked contrast with those obtained under the conditions of natural challenge. A possible explanation for the latter results is that the sudden development of massive infections leading to the 'acute intestinal syndrome' described by Lawrence (1977*a*) simply nullified by any immunological response putatively induced by vaccination. Using similar infection protocols with *S. bovis*, Bushara *et al.* (1993) recovered 34% of the parasites in control calves, and Aradaib *et al.* (1995*a*) only 10%. In the present experiments, the mean recovery of parasites from control calves was 60%. This is similar to the mean 55–64% recoveries recorded by Van Wyk, Heitmann & Van Rensburg (1975) and Lawrence (1977*b*) and confirms the high susceptibility of cattle to *S. mattheei* infection. Testing the potential of the *S. bovis* whole egg antigen to protect calves against an homologous experimental infection with 20000 cercariae, Aradaib *et al.* (1995*b*) recovered more worms from vaccinated calves than from non-vaccinated controls. Our study indicates that their immunization protocol could have produced different results if animals had been exposed to a moderate natural challenge.

In conclusion rSb28GST can provide significant protection against *S. mattheei* in cattle under conditions of low to moderate natural infection. The different results obtained in our vaccination trial after either experimental or natural exposure raise questions as to the validity of using single, relatively heavy experimental infections to study the immunological response against schistosomiasis.

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REFERENCES

ARADAIB, I. E., ABBAS, B., RIEMANN, H. P. & OSBURN, B. I. (1995*a*). Experimental bovine schistosomiasis in zebu calves. *Ciencia Rural* **25**, 99–103.

ARADAIB, I. E., OMER, O. H., ABBAS, B. B., BUSHARA, H. O., ELMALIK, K. H., SAAD, A. M., OSBURN, B. I. & TAYLOR, M. G. (1995*b*). *Schistosoma bovis* whole egg antigen did not protect Zebu calves against experimental schistosomosis. *Preventive Veterinary Medicine* **21**, 339–345.

BALLOUL, J. M., SONDERMEYER, P., DREYER, D., CAPRON, M., GRZYCH, J. M., PIERCE, R. J., CARVALLO, D., LECOCQ, J. P. & CAPRON, A. (1987). Molecular cloning of a protective antigen of schistosomes. *Nature, London* **326**, 149–153.

BASHIR, M., BICKLE, Q., BUSHARA, H., COOK, L., SHI FUHUI, DIAN HE, HUGGINS, M., LIN JIAOJIAO, MALIK, K., MOLONEY, A., MUKHTAR, M., YEH PING, XU SHOUTAI, TAYLOR, M. & SHI YAOCHUAN (1994). Evaluation of defined antigen vaccines against *Schistosoma bovis* and *S. japonicum* in bovines. *Tropical and Geographical Medicine* **46**, 255–258.

BOULANGER, D., REID, G. D., STURROCK, R. F., WOLOWCZUK, I., BALLOUL, J. M., GREZEL, D., PIERCE, R. J., OTIENO, M. F., GUERRET, S., GRIMAUD, J. A., BUTTERWORTH, A. E. & CAPRON, A. (1991). Immunization of mice and baboons with the recombinant Sm28GST affects both worm viability and fecundity after experimental infection with *Schistosoma mansoni*. *Parasite Immunology* **13**, 473–490.

BOULANGER, D., TROTTEIN, F., MAUNY, F., BREMOND, P., COURET, D., PIERCE, R. J., KADRI, I. S., GODIN, C., SELLIN, E., LECOCQ, J. P. & CAPRON, A. (1994). Vaccination of goats against the trematode *Schistosoma bovis* with a recombinant homologous schistosome-

derived glutathione S-transferase. *Parasite Immunology* **16**, 399–406.

BUSHARA, H. O., HUSSEIN, M. F., SAAD, A. M., TAYLOR, M. G., DARGIE, J. D., MARSHALL, T. F. DE C. & NELSON, G. S. (1978). Immunization of calves against *Schistosoma bovis* using irradiated cercariae or schistosomula of *S. bovis*. *Parasitology* **77**, 303–311.

BUSHARA, H. O., MAJID, A. A., SAAD, A. M., HUSSEIN, M. F., TAYLOR, M. G., DARGIE, J. D., MARSHALL, T. F. DE C. & NELSON, G. S. (1980). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. II. Experimental demonstration of naturally acquired resistance to *Schistosoma bovis*. *American Journal of Tropical Medicine and Hygiene* **29**, 442–451.

BUSHARA, H. O., MAJID, B. Y. A., MAJID, A. A., KHITMA, I., GAMEEL, A. A., KARIB, E. A., HUSSEIN, M. F. & TAYLOR, M. G. (1983*a*). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. V. The effect of praziquantel therapy on naturally acquired resistance to *Schistosoma bovis*. *American Journal of Tropical Medicine and Hygiene* **32**, 1370–1374.

BUSHARA, H. O., GAMEEL, A. A., MAJID, B. Y. A., KHITMA, I., HAROUN, E. M., KARIB, E. A., HUSSEIN, M. F. & TAYLOR, M. G. (1983*b*). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. VI. Demonstration of resistance to *Schistosoma bovis* challenge after a single exposure to normal cercariae or to transplanted adult worms. *American Journal of Tropical Medicine and Hygiene* **32**, 1375–1380.

BUSHARA, H. O., BASHIR, M. E. N., MALIK, K. H. E., MUKHTAR, M. M., TROTTEIN, F., CAPRON, A. & TAYLOR, M. G. (1993). Suppression of *Schistosoma bovis* egg production in cattle by vaccination with either glutathione S-transferase or keyhole limpet haemocyanin. *Parasite Immunology* **15**, 383–390.

CAPRON, A., DESSAINT, J. P., CAPRON, M. & PIERCE, R. J. (1992*a*). Schistosomiasis: from effector and regulation mechanisms in rodents to vaccine strategies in humans. *Immunological Investigations* **21**, 409–422.

CAPRON, A., DESSAINT, J. P., CAPRON, M. & PIERCE, R. J. (1992*b*). Vaccine strategies against schistosomiasis. *Memorias do Instituto Oswaldo Cruz* **87** (Suppl. IV), 19–27.

CAPRON, A., RIVEAU, G., GRZYCH, J. M., BOULANGER, D., CAPRON, M. & PIERCE, R. (1994). Development of a vaccine strategy against human and bovine schistosomiasis. *Tropical and Geographical Medicine* **46**, 242–246.

CHRISTENSEN, N. Ø., MUTANI, A. & FRANDSEN, F. (1983). A review of the biology and transmission ecology of African bovine species of the genus *Schistosoma*. *Zeitschrift für Parasitenkunde* **69**, 551–570.

DARGIE, J. D. (1980). The pathogenesis of *Schistosoma bovis* infection in Sudanese cattle. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 560–562.

DE BONT, J., VERCRUYSSE, J. & MASSUKU, M. (1996). Variations in *Schistosoma mattheei* egg morphology and viability according to age of infection in cattle. *Journal of Helminthology* **70**, 265–267.

DE BONT, J., VERCRUYSSE, J., SABBE, F., SOUTHGATE, V. R. & ROLLINSON, D. (1995*a*). *Schistosoma mattheei* infections in cattle: changes associated with season and age. *Veterinary Parasitology* **57**, 299–307.

DE BONT, J., VERCRUYSSE, J., SABBE, F. & YSEBAERT, M. T. (1995*b*). Observations on worm population dynamics in calves naturally infected with *Schistosoma mattheei*. *Parasitology* **111**, 485–491.

DE BONT, J., VERCRUYSSE, J., SOUTHGATE, V. R., ROLLINSON, D. & KAUKAS, A. (1994). Cattle schistosomiasis in Zambia. *Journal of Helminthology* **68**, 295–299.

DE BONT, J., VERCRUYSSE, J., VAN AKEN, D., SOUTHGATE, V. R., ROLLINSON, D. & MONCRIEFF, C. (1991). The epidemiology of *Schistosoma spindale* Montgomery, 1906 in cattle in Sri Lanka. *Parasitology* **102**, 237–241.

DUNNE, D. W., HAGAN, P. & ABATH, F. G. C. (1995). Prospects for immunological control of schistosomiasis. *Lancet* **345**, 1488–1492.

GRYSEELS, B. & DE VLAS, S. J. (1996). Worm burdens in schistosome infections. *Parasitology Today* **12**, 115–119.

HUSSEIN, M. F. (1971). The pathology of experimental schistosomiasis in calves. *Research in Veterinary Sciences* **12**, 246–252.

HUSSEIN, M. F. (1980). Prospects for the control of *Schistosoma bovis* infection in Sudanese cattle. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **74**, 559–560.

KASSUKU, A. A., CHRISTENSEN, N. Ø., MONRAD, J., NANSEN, P. & KNUDSEN, J. (1986). Epidemiological studies on *Schistosoma bovis* in Iringa Region, Tanzania. *Acta Tropica* **43**, 153–163.

KUMAR, V. & DE BURBURE, G. (1986). Schistosomes of animals and man in Asia. *Helminthological Abstracts* (Series A) **55**, 469–480.

LAWRENCE, J. A. (1970). Examination of ruminant faeces for schistosome eggs. *Rhodesian Veterinary Journal* **1**, 49–52.

LAWRENCE, J. A. (1973*a*). *Schistosoma mattheei* in cattle: the host–parasite relationship. *Research in Veterinary Science* **14**, 400–402.

LAWRENCE, J. A. (1973*b*). *Schistosoma mattheei* in cattle: variations in parasite egg production. *Research in Veterinary Science* **14**, 402–404.

LAWRENCE, J. A. (1977*a*). *Schistosoma mattheei* infestation in the ox: the intestinal syndrome. *Journal of the South African Veterinary Association* **48**, 55–58.

LAWRENCE, J. A. (1977*b*). *Schistosoma mattheei* in the ox; observations on the parasite. *Veterinary Parasitology* **3**, 291–303.

MAJID, A. A., MARSHALL, T. F. DE C., HUSSEIN, M. F., BUSHARA, H. O., TAYLOR, M. G., NELSON, G. S. & DARGIE, J. D. (1980*a*). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. I. Epizootiological observations on *Schistosoma bovis* in the White Nile province. *American Journal of Tropical Medicine and Hygiene* **29**, 435–441.

MAJID, A. A., BUSHARA, H. O., SAAD, A. M., HUSSEIN, M. F., TAYLOR, M. G., DARGIE, J. D., MARSHALL, T. F. DE C. & NELSON, G. S. (1980*b*). Observations on cattle schistosomiasis in the Sudan, a study in comparative medicine. III. Field testing of an irradiated *Schistosoma bovis* vaccine. *American Journal of Tropical Medicine and Hygiene* **29**, 452–455.

MASSOUD, J. & NELSON, G. S. (1972). Studies on heterologous immunity in schistosomiasis. 6. Observations on cross-immunity to *Ornithobilharzia turkestanicum*, *Schistosoma bovis*, *S. mansoni*, and *S.*

haematobium in mice, sheep, and cattle in Iran. *Bulletin of the World Health Organization* **47**, 591–600.

McCAULEY, E. H., TAYEB, A. & MAJID, A. A. (1983). Owner survey of schistosomiasis mortality in Sudanese cattle. *Tropical Animal Health and Production* **15**, 227–233.

McCAULEY, E. H., MAJID, A. A. & TAYEB, A. (1984). Economic evaluation of the production impact of bovine schistosomiasis and vaccination in the Sudan. *Preventive Veterinary Medicine* **2**, 735–754.

PITCHFORD, R. J. & VISSER, P. S. (1982). *Schistosoma mattheei* Veglia & Le Roux, 1929, egg output from cattle in a highly endemic area in the Eastern Transvaal. *Onderstepoort Journal of Veterinary Research* **49**, 233–235.

TAYLOR, M. G. (1987). Schistosomes of domestic animals: *Schistosoma bovis* and other animal forms. In *Immunology*, *Immunoprophylaxis and Immunotherapy of Parasitic Infections* (ed. Soulsby, E. J. L.), vol. 2, pp. 49–90. CRC Press, Boca Raton.

TAYLOR, J. B., VIDAL, A., TORPIER, G., MEYER, D. J., ROITSCH, C., BALLOUL, J. M., SOUTHAN, C., SONDERMEYER, P., PEMBLE, S., LECOCQ, J. P., CAPRON, A. & KETTERER, B. (1988). The glutathione transferase activity and tissue distribution of a cloned Mr28K protective antigen of *Schistosoma mansoni*. *EMBO Journal* **7**, 465–472.

TROTTEIN, F., GODIN, C., PIERCE, R. J., SELLIN, B., TAYLOR, M. G., GORILLOT, I., SAMPAIO, S. M., LECOCQ, J. P. & CAPRON, A. (1992). Inter-species variation of schistosome 28-kDa glutathione S-transferases. *Molecular and Biochemical Parasitology* **54**, 63–72.

UPATHAM, E. S., STURROCK, R. F. & COOK, J. A. (1976). Studies on the hatchability of *Schistosoma mansoni* eggs from a naturally infected human community on St Lucia, West Indies. *Parasitology* **73**, 253–264.

VAN WYK, J. A., HEITMANN, L. P. & VAN RENSBURG, L. J. (1975). Studies on schistosomiasis. 7. A comparison of various methods for the infestation of sheep with *Schistosoma mattheei*. *Onderstepoort Journal of Veterinary Research* **42**, 71–74.

XU, C. B., VERWAERDE, C., GRZYCH, J. M., FONTAINE, J. & CAPRON, A. (1991). A monoclonal antibody blocking the *Schistosoma mansoni* 28-kDa glutathione S-transferase activity reduces female worm fecundity and egg viability. *European Journal of Immunology* **21**, 1801–1807.

XU, C. B., VERWAERDE, C., GRAS-MASSE, H., FONTAINE, J., BOSSUS, M., TROTTEIN, F., WOLOWCZUK, I., TARTAR, A. & CAPRON, A. (1993). *Schistosoma mansoni* 28-kDa glutathione S-transferase and immunity against parasite fecundity and egg viability. *Journal of Immunology* **150**, 940–949.