FIELD ASSESSMENT OF RECOMBINANT *SCHISTOSOMA JAPONICUM* 26 KDA GLUTATHIONE *S*-TRANSFERASE IN CHINESE WATER BUFFALOES

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Abstract. We have shown previously that anti-fecundity immunity can be induced experimentally against recombinant 26 kDa glutathione *S*-transferase (reSjc26GST) in Chinese water buffaloes (*Bos buffelus*), important reservoir hosts for *Schistosoma japonicum* in China. In the field study described here, we immunized buffaloes with reSjc26GST to induce protective immunity against *S. japonicum* and to evaluate its effectiveness in controlling schistosomiasis japonica. We selected two villages as test and control groups in inside-embankment areas endemic for schistosomiasis japonica. The buffaloes in the test village were vaccinated with reSjc26GST, whereas those in the control village were not. The indicators of the effect of the vaccine included the generation of specific IgG antibodies in the vaccinated buffaloes, changes in the prevalence and infection intensity in buffaloes and village children, changes in the density of infected snails, and changes in the infectivity of water bodies (assessed by sentinel mice) in transmission areas adjacent to both villages. Twenty months after vaccination, the infection rate of buffaloes in the test village was decreased by 60.4% (from an initial prevalence of 13.5% to 5.4%), and 67.9% when compared with that in the control village (initial prevalence of 16.7%). However, the infection rate in village children remained unchanged. The density of infected snails decreased by 71.4%, from 0.0049/ 0.11m^2 to 0.0014/0.11m^2 in the high transmission area outside the embankment in the test village. There was no change in the infectivity of the water body transmission areas between the test and control villages. The levels of specific antibodies to reSjc26GST showed a continuous increase after vaccination. These results indicate that protective immunity was induced and maintained in buffaloes after vaccination with reSjc26GST. The vaccine could thus play a significant role in reducing *S. japonicum* transmission caused by water buffaloes in the Lake region of China.

INTRODUCTION

Schistosomiasis is a major parasitic disease, which ranks second only to malaria in terms of human suffering in the tropics. In China, it is estimated that approximately one million people are currently infected in endemic areas situated along the middle and lower reaches of the Yangtze River. Bovines, especially water buffaloes, are the principal reservoir sources for S*chistosoma japonicum* infection in the Dongting Lake area. We have hypothesized that bovines are responsible for the persistence of human schistosome transmission in lake and marshland areas where endemic schistosomiasis remains uncontrolled (Guo *et al*, 2001). This is because of their large population, high infection rate, large number of eggs passed in stool, and their high contamination index. Buffaloes aged less than 3 years are the most important source because of their higher infection rates and heavier intensity of infection (He *et al*, 1991). Results from the 1995 nationwide sampling sur-

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vey indicate that 100,250 bovines are presently infected with *S. japonicum* (Chen, 1999). We predict that a reduction in schistosome infection in buffaloes following vaccination would play an important role in reducing local schistosomiasis transmission.

Recombinant 26kDa glutathione *S*-transferase (reSjc26GST) is recognized as a major anti-*S. japonicum* vaccine candidate. It has been shown to induce significant anti-fecundity immunity in vaccinated/challenged 8-month-old buffaloes bought from a non-endemic schistosomiasis area (Liu *et al*, 1997). During the period 1997-1999, we carried out a 20-month prospective study of protective immunity to *S. japonicum* induced in buffaloes and analyzed the effect on local transmission of schistosomiasis after vaccination with reSjc26GST. The results are reported here.

MATERIALS AND METHODS

Villages

Two Dongting Lake communities were selected as test (Shuangping) and control (Xuping) villages in Lijiapu Town, Jinshi City, Hunan Province. As buffaloes are major reservoir hosts for schistosomiasis japonica, praziquantel (30mg/kg body weight) chemotherapy is normally provided to them in September or October each year as part of the ongoing control program. High transmission foci occur outside the embankment adjacent to the villages, whereas there are no *Oncomelania* snails inside the embankment. Both Shuangping and Xuping belong to the inside-embankment type area endemic for schistosomiasis japonica along the Lishui Branch of Dongting Lake. These two villages are similar in terms of endemic status, topographic features, community life-style, production modes of the villagers, and procedures for herding domestic animals.

Baseline information

Infection status of buffaloes. In September 1997, the buffaloes in the test and control villages were registered and numbered. There were 96 buffaloes in the test village with a mean age of 6.5±3.5 (mean±SD) years, and 90 in the control village with a mean age of 5.8±3.2 years; there was no significant age difference between these two villages ($t=1.526$, $p>0.05$). Fecal samples (100g) were collected from all buffaloes in the test and control villages to detect miracidia using the egg-hatching method, and to determine prevalence and intensity of infection.

Infection status of villagers. Villagers participating in this study were selected from all villagers aged 4-65 years in the test and control villages by stratified cluster sampling, and fecal samples were collected for parasitological assessment. The Kato-Katz method was used to detect stool eggs. Thick smear slides (41.7 mg/slide) were made from each stool (2 samples, 3 slides per sample) and the prevalence and intensity of infection for both villages determined. The numbers of subjects examined at baseline were 319 and 325 for the test and control villages, respectively. There was no significant difference in infection rates in various age groupings from either village, so to simplify follow-up examinations, children (aged 7-15 years) only were re-assessed at months 9 and 20.

Infection status of snails. Snails were surveyed by systematic sampling within the herding area of the transmission foci near the village residential quarters, for both villages, to calculate the density of live and positive snails. In the test village, 615 plots (0.11m^2/plot) were surveyed, and 500 were surveyed in the control village.

Determination of infectivity of water body using sentinel mice. During the first flooding season for each of the years 1997-1999, 50 mice were placed at sentinel stations in transmission foci in both villages for 2 hours each, for 3 consecutive days, and dissected 30 days later. The numbers of schistosome worms were recorded for each sentinel mouse, and then the infection rate and mean worm burden were calculated for each sentinel area.

Immunization schedule

Each buffalo from the test village was immunized subcutaneously on day 1 of the trial with 0.2 mg purified reSjc26GST (Liu *et al*, 1997) emulsified in an equal volume of Freund's com-

plete adjuvant (FCA), and then again at day 16 but with the antigen emulsified in Freund's incomplete adjuvant (FIA). At day 32, the buffaloes were immunized for a third time with 1.0 mg reSjc26GST/FCA. Buffaloes in the control village were not injected with anything.

Detection of anti-reSjc26GST antibodies

Venous blood (5 ml) for serum isolation was taken from the ears and/or necks of buffaloes on day 1 of the trial, before commencement of immunization, and at months 2, 5, 9, 12, 15 and 20 after immunization. All sera were stored at -70ºC for detection of anti-reSjc26GST IgG antibodies by indirect ELISA. Briefly, ELISA plates were coated with 1 µg/ml reSjc26GST (200 µl/well) and incubated at 4ºC overnight. All sera were diluted to 1:200 in PBS (pH7.4), and 100 µl of diluted serum was added to each well and incubated at 37ºC for 2 hours. Horse-radish peroxidase labeled anti-bovine IgG conjugate (Sigma St Louis, Mo, USA) was then added at a dilution of 1:28,000 and the plates incubated at 37ºC for 1 hour. O-phenylenediamine was used as substrate at a concentration of 0.4 mg/ml. Optical density (OD) values were determined on an automated model 450 microplate reader (Bio-Rad, Hercules, CA, USA) at 492 nm.

Effectiveness of vaccination

The effectiveness of vaccination was determined at months 9 and 20 after baseline, and the indicators included prevalence and intensity in buffaloes and schoolchildren (aged 7-15 years) in the test and control villages (determined as described above), infection rate in snails, and infectivity of water bodies (assessed by prevalence in sentinel mice) around the foci of transmission.

RESULTS

Anti-reSjc26GST antibodies

Fig 1 shows that the levels of anti-reSjc26GST serum antibodies, determined by indirect ELISA, produced in test buffaloes compared with controls during the course of the vaccine trial. The mean OD values of specific serum IgG antibodies in the test and control buffaloes were both below 0.2 before immunization, but those in test buffaloes were elevated significantly at month 2 after immunization and showed a continuous increase. The mean OD values of serum antibodies from test buffaloes were 2.15-fold (month 2), 2.20-fold (month 5), 2.38-fold (month 9), 4.38-fold (month 12), 6.76-fold (month 15) and 2.20-fold (month 20) higher than those in controls at the corresponding month. The Student's *t* test showed that the mean levels of anti-reSjc26GST antibodies in the test buffalo sera were significantly higher than those in controls at all time points ($t=11.129\sim28.128$; $p<0.001$).

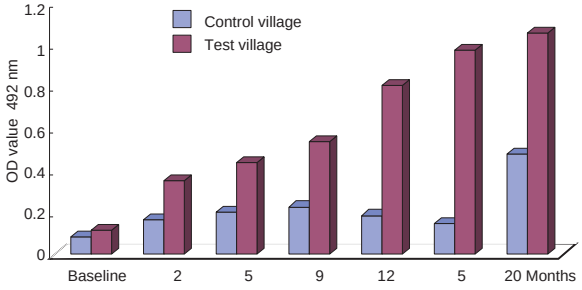

Fig 1–Levels of anti-Sjc26GST IgG antibodies in buffaloes from test and control villages at basline and at multiple time points following immunization with reSjc26GST.

Table 1
Prevalence of *S. japonicum* in buffaloes before and after immunization with reSjc26GST.

Time after immunization (months)	Test village		Control village		p-value
	No. examined	Infection (%)	No. examined	Infection (%)	
Baseline	96	13.5	90	10.0	>0.05
9	74	17.6	75	36.8	<0.01
20	56	5.4	60	16.7	<0.05

Schistosome infection in buffaloes

Table 1 shows the schistosome prevalence in buffaloes from the test and control villages before and after immunization. Before immunization, the infection rates of buffaloes in test and control villages were 13.5% (n=96) and 10.0% (n=90), respectively, with no significant difference ($p>0.05$). Twenty months after immunization, the infection rate in buffaloes in the test village was 5.4% (n=56), 67.8% lower than that in the control village (16.7%, n=60). There was no difference ($p>0.05$) in the intensity of infection (mean eggs per gram ±SD) in the egg-positive buffaloes in the test (baseline, 0.48±0.29; 9 months, 0.32±0.09; 20 months, 0.19±0.07) and control (baseline, 0.60±0.14; 9 months, 0.32±0.15; 20 months, 0.20±0.09) villages.

Schistosome infection in villagers

Table 2 shows the changes in the infection rates of test and control villagers before and after immunization. Before immunization, the infection rates of villagers in the test and control villages were 10.0% (n=319) and 10.2% (n=325), respectively. At months 9 and 20 after immunization, the infection rates in children in the test village were 10.5% (n=105) and 9.2% (n=98), respectively, and those in the control village were 10.9% (n=110) and 11.1% (n=99), respectively, showing no significant difference between the two villages ($p>0.05$). Similarly, there was no difference ($p>0.05$) in the intensity of infection (mean eggs per gram±SD) in the egg-positive children (Table 2).

Changes in infected snails

The density of infected snails in the test village decreased by 71.4% from 0.0049/0.11m^2 before immunization to 0.0014/0.11m^2 20 months after immunization, whereas that in the control village increased by 7.5% (Table 3).

Changes in infectivity of water body

The infection rates of sentinel mice in the test and control villages were 14.3% and 16.7%, respectively, before immunization. At month 20 after immunization, the infection rates increased to 91.2% and 83.3%, respectively (Table 4), but there was no significant difference ($p>0.05$) between the two villages.

Table 2
Human prevalence and infection intensity before and after immunization of buffaloes with reSjc26GST.

Time after immunization (months)	Test village				Control village			
	No. examined	Infection (%)	EPG[a] Mean	± SD	No. examined	Infection (%)	EPG[a] Mean	± SD
Baseline	319	10.0	44.8	127.3	325	10.2	16.2	1.7
9	105	10.5	82.9	148.9	110	10.9	81.3	8.9
20	98	9.2	01.3	92.2	99	11.1	105.5	11.7

[a]Eggs per gram feces among egg-positive patients.

Table 3
Density of infected snails in high transmission zones around the test and control villages.

Time after immunization (months)	Test village			Control village		
	No. plots	Density[a] (0.11m^2)	Density[b] (0.11m^2)	No. plots	Density[a] (0.11m^2)	Density[b] (0.11m^2)
Baseline	615	0.18	0.0049	500	0.17	0.0040
9	630	0.25	0.0016	870	0.26	0.0034
20	700	0.28	0.0014	700	0.29	0.0043

[a]Density of live snails; [b]Density of infected snails.

Table 4
Infectivity of water with schistosomes in high transmission zones around test and control villages.

Time after immunization (months)	Test village				Control village			
	No. mice	Infection %	Worms collected Mean±SD		No. mice	Infection %	Worms collected Mean±SD	
Baseline	35	14.3	0.60	1.7	36	16.7	0.75	1.9
9	50	58.0	3.20	4.4	48	10.2	0.21	0.7
20	34	91.2	9.15	6.7	48	83.3	3.80	3.0

DISCUSSION

Glutathione *S*-transferases (GSTs), shown to exist as 26 and 28 kDa molecules each consisting of several isoforms, play a very important role in the life activities of schistosomes (Tiu *et al*, 1988; O'Leary *et al*, 1988; 1992; Wright *et al*, 1991; Trottein *et al*, 1992). Accordingly, there has been considerable interest focused on the GSTs as potential components of anti-schistosome vaccines (Mitchell, 1989). Indeed, following promising pre-clinical assessment, human clinical trials (phase I and II) have been undertaken recently using *Schistosoma haematobium* GST (Sh28GST; (Bilhvax) (Capron *et al*, 2001). Protective immunity is induced in mice after vaccination with reSjc26GST followed by challenge infection with Chinese *S. japonicum* cercariae, resulting in a 44.4% reduction in worm numbers (Liu *et al*, 1991). Furthermore, we have previously reported a 22.3% reduction in worm burden, 40% reduction in the egg-hatching capacity of *S. japonicum* eggs into viable miracidia and 47.9%~56.8% reduction in eggs deposited in the livers and intestines of 8-month-old buffaloes vaccinated with reSjc26GST (Lou *et al*, 1996; Liu *et al*, 1997). The results demonstrated that protective immunity against challenge infection could be induced in buffaloes after vaccination with reSjc26GST coupled with a significant anti-fecundity effect. We also reported a similar effect in pigs (Liu *et al*, 1995).

We report here the results of a 20-month field trial on buffaloes using reSjc26GST as a vaccine. Significant levels of anti-Sjc26GST IgG antibodies were generated and maintained in the vaccinated animals over the course of the trial. The study confirmed that protective immunity was induced in the vaccinated buffaloes, with infection being reduced by 60.4% at 20 months following vaccination in the test village. Prevalence declined by 67.8% during this time, compared with the control village buffaloes.

The infectivity of lake water was assessed in this study by the use of sentinel mice. A new method to detect cercariae using a special membrane (C-6) suspended in water at potential transmission sites has been reported by Chinese scientists, but the C-6 membrane needs further development before being useful in lake areas where wave action tends to wash away adherent cercariae (Li *et al*, 2000a). The infection rate in sentinel mice in this study increased significantly during the course of the trial in both the test and control villages. These data do not exactly reflect the true situation of local transmission. This is because the spread of cercariae is dependent on water flow and wind direction and this is particularly the case during times of flooding (Huang *et al*, 1992; Mao, 1992). Field studies on *S. japonicum* have revealed that mice could be infected even if there were no evidence of *Oncomelania* snail colonies in the immediate vicinity (Zhou *et al*, 1991). The density of infected snails decreased by 71.4% 20 months after the buffaloes were vaccinated in the test village, but the density of infected snails was maintained at a stable level in the control village. It has been reported that the density of positive snails is associated with the total amount of infectious source in a given endemic area (Zhou *et al*, 1991). In our study, a decrease, therefore, in the density of positive snails in the test village may be attributable to the efficacy of the reSjc26GST vaccine,

which reduced the infection rate and hence the total egg output of the vaccinated buffaloes.

Previous studies have demonstrated that prevalence and morbidity related *to S. japonicum* in humans decline over time after implementation of successful praziquantel chemotherapy (Ross *et al*, 1998; 2001). However, it is difficult to block transmission of schistosomiasis in endemic areas, especially in the lake and marshland region of China (Li *et al*, 2000b). This is because the life cycle of *S. japonicum* is maintained by both human and non-human reservoirs, especially water buffaloes. Cattle and water buffaloes are of great importance in contaminating the environment by discharging schistosome eggs in their feces. In our study, the infection rate of buffaloes in the vaccinated group declined, although there was no difference of infection intensity between two groups. These results, nevertheless, confirm that vaccination of buffaloes with reSjc26GST can mirror an effect similar to the use of bovine chemotherapy in the current control program for schistosomiasis in the Dongting Lake region (Ye *et al*, 1991). It should be noted that the number of buffaloes available for examination decreased over the 20-month period in both the test (by 42%) and the control villages (by 33%). This is because some buffaloes were sold, others were lost from the villages and some died or were sacrificed during a relatively long period of observation. In addition, the local residents purchased buffaloes from other villages in both schistosomiasis-endemic and non-endemic areas. These newcomer buffaloes made up at least 10% of the total number at the end of the study. As these animals were not vaccinated or drug-treated, they probably provided a new source of schistosome infection, thus possibly influencing the results of the study.

In summary, although schistosome infection in buffaloes was substantially reduced by using the reSjc26GST vaccine, the approach based on vaccinating buffaloes only did not lead to a reduction in human infection in the test village. It is probable that the duration of this study was too short to observe an effect on human prevalence. Nevertheless, future control of schistosomiasis in an area such as the Dongting Lake will probably require an integrated approach. Chemotherapy of bovines and humans at-risk might be used as a first approach to effect a rapid decline in the transmission source. Then, the bovine vaccine would be used as a complementary intervention to reduce transmission further, possibly leading to elimination in schistosomiasis-endemic areas.

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