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Kinetics of antibody response of calves immunized with *Schistosoma mansoni* glutathione-S-transferase

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ABSTRACT

Six calves were immunized with *Schistosoma mansoni* glutathione-S-transferase (GST) antigen vaccine, expressed in *E. coli* (GST-coli) or yeast (GST-yeast). Six calves were injected with extracts of *E. coli* or yeast and kept as controls. Two experimentally infected calves were kept as source of infection and to provide positive antiserum to *Schistosoma bovis*. Kinetics of antibody response of the immunized calves was monitored by Western blots and enzyme-linked immunosorbent assay (ELISA). Using Western blots, the immunized calves produced specific IgG antibodies, which recognized *S. mansoni* GST (S.mGST) antigen, whereas the control calves did not. Using ELISA, antibodies to S.mGST antigen were detected in sera from S.mGST-coli and GST-yeast vaccinated calves, but not in sera from control calves or *S. bovis* experimentally infected calves. In addition, the ELISA failed to detect S.mGST specific antibodies in sera from immunized calves or their controls when *S. bovis* adult worm extract was used as an antigen. The results of this study indicated that S.mGST antigen has no diagnostic potential for detection of bovine schistosomosis, caused by *S. bovis* infection, in susceptible ruminants.

Key words: schistosome, cattle, immune response, glutathione-S-transferase

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Introduction

Schistosomosis is a chronic debilitating infection affecting both humans and animals by different species of schistosomes and hence the disease is of public health importance. Control of the disease by destruction of the snail intermediate host using mulloscicides is impractical (MAJID et al., 1980; BUSHARA et al., 1993). In previous studies we have conducted a series of immunization experiments against bovine schistosomosis using irradiated *S. bovis* vaccine, adult worm extracts, and soluble egg antigen vaccine (ARADAIB, 1988; ARADAIB et al., 1993; ARADAIB et al., 1995; ARADAIB and OSBURN, 1995a; ARADAIB and OSBURN, 1995b). A number of defined antigen vaccines have been developed for control of schistosomosis. Among the candidate antigen vaccines, the glutathione-S-transferase (GST) of *S. mansoni* (S.mGST) is a leading candidate schistosome vaccine that protected rodents and baboons against homologous infection (BALLOUL et al., 1987). *S. mansoni* GST (S.mGST) has a molecular weight of approximately 28 kilodalton (kD) and shares cross-reactive epitopes with other schistosome species including *S. hematobium*, *S. bovis* and *S. japonicum* (ARADAIB and OSBURN, 1995a). In our laboratory, significant research efforts have been directed toward improving diagnosis of bovine schistosomosis. In a previous report, *S. bovis* adult worm extract, whole egg antigen and cercarial antigen were evaluated for diagnosis of *S. bovis* infection in cattle (ARADAIB, 1988; ARADAIB et al., 1993; ARADAIB and OSBURN., 1995a; ARADAIB, 2001). In the present study, calves were immunized with S.mGST antigen expressed in *E. coli* (GST-coli) or yeast (GST-yeast). The kinetic of antibody responses of the immunized calves were evaluated using Western blotting and enzyme-linked immunosorbent assay (ELISA). The diagnostic potential of S.mGST antigen for detection of *S. bovis* infection in cattle was also evaluated.

Materials and methods

Infective materials. *Bulinus africanus*, the snail intermediate host of *S. bovis*, were collected from Elmoglad, a schistosomosis-endemic area in Western Sudan. The snails were screened to exclude already parasitized snails. The non-parasitized snails were infected with 3-5 miracidia obtained

from experimentally infected calves. Cercariae were collected from shedding snails for 6 hours, using a light source.

Experimental animals. Fourteen 6-8-month-old calves were purchased and after repeated clinical and parasitological examinations to exclude the possibility of schistosome infection they were randomly divided into 3 groups. Six calves (Group-1) were immunized with GST-yeast or GST-coli. Six calves (Group-2) were injected with extracts of *E. coli* or yeast. Two calves (Group 3) were experimentally infected with 300 cercariae per kg body weight (a total dose of 30,000 *S. bovis* cercariae per animal) administered percutaneously to the shaved tail. The S.mGST vaccine was administered subcutaneously at a dose rate of 50 mg/ml. Alum was used as an adjuvant at the same dose of the vaccine. Details for description of S.mGST protein antigen were described previously (BALLOUL et al., 1987). Two weeks after the first immunization each calf was boosted with the same initial dose (each animal received a total dose of 100 mg). The animals were maintained indoors and were fed a ration of concentrate and hay and had free access to water.

Serum samples. Sera were collected at weeks 0, 2, 3, 4, 7, 10, 12 and 15 (where 0 represents the first day of immunization). Positive reference sera were obtained from a rabbit experimentally inoculated with S.mGST antigen). Negative reference sera were obtained from 3 non-infected calves obtained from a schistosomosis-free area and checked by faecal examination (ARADAIB and OSBURN, 1995a).

Serological techniques. Western blot and ELISA were carried out as described previously (ARADAIB, 1988). ELISA (Mean ± sd) absorbance values and differences between means were calculated by conventional statistical procedure described by SCHWABE et al. (1977).

Results

Using Western blots, sera from GST-coil and GST-yeast immunized calves recognized a protein band at a molecular weight of 28-kilodalton at week 3-post vaccination, whereas sera from control calves or *S. bovis* experimentally infected calves did not (Fig. 1). In the ELISA, using homologous antigen, the antibody response of GST-coil vaccinated calves

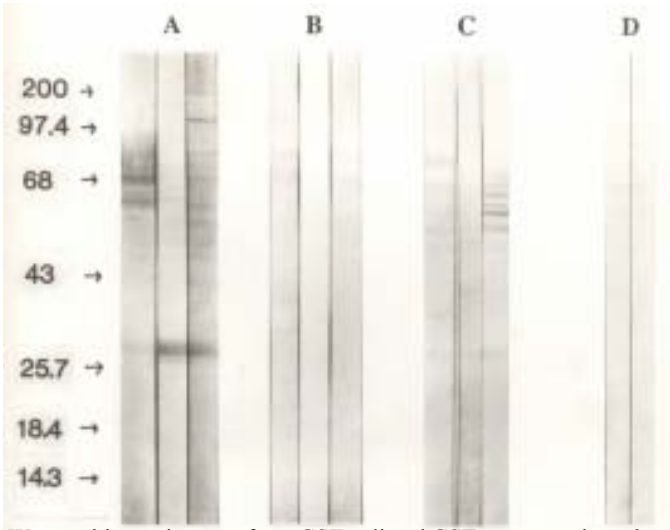

Fig. 1. Western blots using sera from GST-coli and GST-yeast vaccinated and control calves bled at week 3 post vaccination. Molecular weight marker proteins are indicated with sizes in KDa. A = coli-GST vaccinated calves; B = coli control calves; C= GST-yeast vaccinated calves; D = *S. bovis* experimentally infected calves.

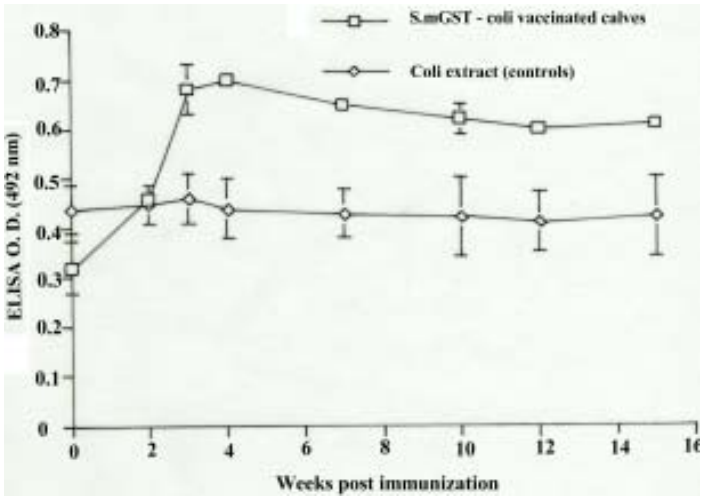

Fig. 2. ELISA O.D. (492 nm) values of S.mGST - coli vaccinated calves and their controls using S.mGST-coli antigen

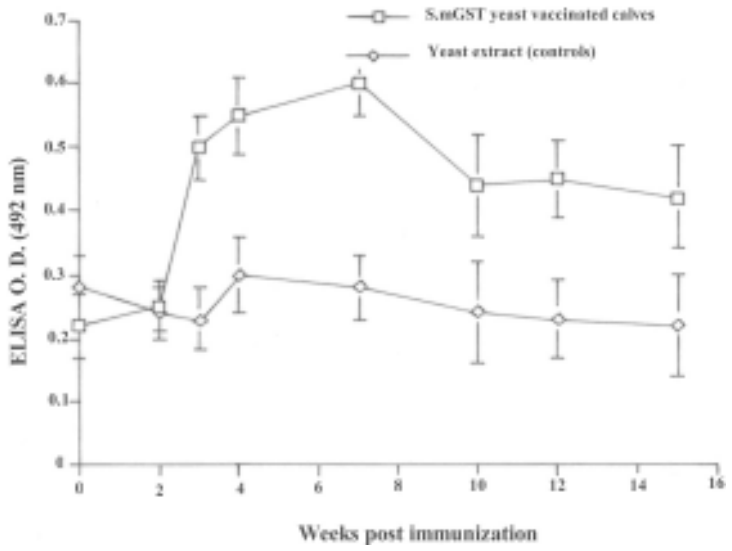

Fig. 3. ELISA O.D. (492 nm) values of S.mGST - yeast vaccinated calves and their controls using S.mGST yeast antigen

was first detected by week 2, peaking by week 3 post-vaccination (Fig. 2). The immune response of GST-yeast vaccinated calves was first detected by week 3, peaking by week 7-post vaccination (Fig. 3). The immune response of the vaccinated calves remained higher than the controls throughout the 16-week experimental period. There was no significant difference in the ELISA (mean ± sd) absorbance values between sera from S.mGST vaccinated calves (2.2 ± 0.25) or their controls (2.3 ± 0.32) when *S. bovis* adult worm antigen was used. In addition, there was no significant difference in the ELISA (mean ± sd) between sera from experimentally infected calves (2.8 ± 0.32) and non-infected controls (2.5 ± 0.45) when S.mGST was used as an antigen.

Discussion

Schistosoma bovis, the cause of bovine schistosomosis, is one of the major veterinary problems in the Sudan (BUSHARA et al., 1979; MAJID et al.,

1980; ARADAIB, 1988). In cattle, the economic importance of the disease is mainly attributed to mortality in young calves, liver condemnations, reduced productivity and poor subsequent reproductive performance (ARADAIB, 1988; ARADAIB, 2001). The majority of research activities on the diagnosis of schistosomosis are directed towards species of medical importance and little is known in relation to species of veterinary importance, *S. bovis*. Current diagnosis of *S. bovis* infection by traditional methods includes evaluation of clinical signs, pathological lesions, parasitological and serological techniques. As clinical signs caused by *S. bovis* are indistinguishable from those produced by other trematode parasites, confirmation of *S. bovis* infection under field condition by these methods is unreliable (ARADAIB et al., 1993). At necropsy, the presence of adult worms of the parasite in the mesenteric vessels, or demonstration of parasite eggs in crushed smears from infected tissues, represents the most accurate diagnosis. Parasitological diagnosis by finding eggs in a faecal sample or biopsy specimen will remain the only definitive diagnostic method for detection of an active *S. bovis* infection in a living individual. The major thrust of the current research conducted in our laboratory is directed towards improvement of the existing techniques used for diagnosis of *S. bovis* infection in cattle. The development of a rapid, sensitive, specific and inexpensive method for diagnosis of the disease would greatly facilitate clinical disease investigations, epidemiological investigation, and treatment of the infected animals and would also enhance vaccination and control programs (ARADAIB, 2001). A major problem in the application of serological techniques for diagnosis of bovine schistosomosis is the availability of suitable and specific antigens that could be used to avoid, or to minimize, false positive results due to cross reactions with other helminth infections (ARADAIB and OSBURN, 1995a; ARADAIB and OSBURN, 1995b). Most investigators have used crude or partially purified antigens for detection of *S. bovis* infection (reviewed by ARADAIB, 2001). Application of soluble egg antigens (SEA) had commonly been reported to yield better results than adult worm antigens (AWA), irrespective of their purity (ARADAIB and OSBURN, 1995a). The serological techniques, despite their advantage in detecting *S. bovis* infected individuals, are complicated by cross-reactions between other trematode parasites and within different species of

schistosomes. To address these problems, in the present study we have validated the potential of ELISA for the diagnosis of *S. bovis* infection using *S. mansoni* glutathione S transferase (S.mGST) as a defined protein antigen.

Using Western blots, sera from GST-coli and GST-yeast-immunized calves recognized a protein band at a molecular weight of 28-kilodalton at week 3 post-vaccination, whereas sera from control calves or *S. bovis* experimentally-infected calves did not. In the ELISA, using homologous antigen, the antibody response of GST-coli vaccinated calves was first detected by week 2, peaking by week 3-post vaccination. The immune response of GST-yeast vaccinated calves was first detected by week 3, peaking by week 7 post-vaccination. The immune response of the vaccinated calves remained higher than the controls throughout the experimental period. There was no significant difference in (mean ± sd) the ELISA absorbance values between sera from SmGST-vaccinated calves or their controls when *S. bovis* adult worm antigen was used. In addition, there was no significant difference in the ELISA between sera from experimentally infected calves and non-infected controls when *S.mGST* was used as an antigen. This is probably due either to antigenic competition or insufficiency of S.mGST present in the adult worm antigen.

In conclusion, S.mGST used in this study induced specific IgG antibodies in immunized calves, which can be detected by Western blot and ELISA. However, the specific S.mGST antibodies did not recognize *S. bovis* GST (S.bGST) protein antigen. The structural variations within the immunological-essential region of schistosome GST were thought to be responsible for these interspecies variations (TROTTIEN et al., 1992). Therefore, S.mGST has no diagnostic potential as an antigen for detection of *S. bovis* infection in susceptible ruminants.

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ARADAIB, I., R. OMER, A. MAJID: Kinetika tvorbe protutijela u teladi imunizirane glutation-S-transferazom metilja *Schistosoma mansoni*. Vet. arhiv 73, 17-25, 2003.

SAŽETAK

Šestero teladi bilo je imunizirano antigenom pripravljenim od glutation-S-transferaze (GST) metilja *Schistosoma mansoni*, koji je bio proizveden u bakteriji *E. coli* (GST-coli) ili kvascu (GST-kvasac). Kontrolnih šestero teladi dobilo je ekstrakt bakterije *E. coli* ili kvasca. Dva pokusno invadirana teleta držana su kao izvor invazije te za dobivanje antiseruma za metilj *Schistosoma bovis*. Kinetika tvorbe protutijela u cijepljene teladi bila je praćena postupkom Western blot i imunoenzimnim testom. Pomoću postupka Western blot u imunizirane teladi ustanovljena su protutijela IgG specifična za antigen *S. mansoni* (S.mGST), koja nisu dokazana u kontrolne teladi. Imunoenzimnim testom bila su dokazana protutijela za antigen S.mGST u uzorcima seruma teladi imunizirane antigenom S.mGST-coli i GST-kvasac, ali ne i u serumima kontrolne teladi i teladi pokusno invadirane metiljom *S. bovis*. Osim toga, imunoenzimnim testom nisu dokazana protutijela specifična za S.mGST u serumima imunizirane i kontrolne teladi kad je kao antigen bio rabljen ekstrakt adulta metilja *S. bovis*. Rezultati istraživanja su pokazali da S.mGST kao antigen nije prikladan za dijagnosticiranje shistosomoze goveda uzrokovane metiljom *S. bovis* u prijemljivih preživača.

Ključne riječi: shistosomoza, govedo, imunosni odgovor, glutation-S-transferaza