

ELISA FOR BOVINE SCHISTOSOMIASIS VACCINE EVALUATION: A PRELIMINARY REPORT

USO DO ELISA PARA AVALIAÇÃO DA RESPOSTA IMUNE EM BEZERROS VACINADOS CONTRA *S. bovis*: ESTUDO PRELIMINAR

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SUMMARY

Six calves were immunized with schistosomula of *Schistosoma bovis* irradiated at 3 or 20 Kilorad (Krad) and three calves were kept as controls. Twenty four weeks post immunization, three calves (one from the 20 Krad and two from the 3 Krad group) were challenged with normal cercaria of *S. bovis*. The immune response was monitored by agar gel immunodiffusion (AGID) and the Enzyme Linked Immunosorbent Assay (ELISA) using adult worm antigen. Using AGID, precipitin lines were observed only with sera from challenged animals. Using ELISA, the immune response of the vaccinated calves was first detected by 2-3 weeks, peaking by 6-8 weeks post vaccination. The immune response of the three challenged calves was elevated by 2 weeks post challenge, peaking at 8-10 weeks post challenge and remained high throughout the experimental period.

This study suggests that ELISA could be used for diagnosis of bovine schistosomiasis.

Key words: ELISA, *Schistosoma bovis*, vaccine.

RESUMO

Seis bezerros foram imunizados com esquistossomúide *Schistosoma bovis* irradiados com 3 ou 20 Kilorad (Krad) e três animais serviram de controles. Vinte e quatro semanas após a imunização três bezerros (um com 20Krad e dois do 3Krad grupo) foram desafiados com cercarias normais de *S. bovis*. A resposta imune foi medida pela prova de imunodifusão em gel de ágar (IDGA) e a prova imunoenzimática (ELISA) usando como antígeno o parasita adulto. Quando a prova do IDGA foi usada linhas de precipitação foram observadas somente no soro dos

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animais desafiados. Usando o ELISA nos animais vacinados a resposta foi detectada pela primeira vez entre a segunda e a terceira semana com pique máximo entre 6-8 semana após a vacinação. A resposta imune dos três animais desafiados estava elevada as duas semanas após o desafio com pique entre a 8-10ª semana e permaneceu alto durante todo período do experimento. Este estudo sugere que o ELISA pode ser utilizado para o diagnóstico da esquistossomíase bovina.

Palavras-chave: ELISA, *Schistosoma bovis*, vaccine.

INTRODUCTION

Schistosoma bovis causes a chronic debilitating infection in cattle and other domestic animals characterized by emaciation and poor subsequent reproductive performance (HUSSIEN, 1973; Mc CAULEY et al., 1984; ARADAIB et al., 1993). The diagnosis of human schistosomiasis has received special attention and different immunologic techniques including enzyme linked immunosorbent assay (ELISA) have been applied for the diagnosis of the disease (VOLLER et al., 1976; MOTT & DIXON, 1982; MATSUDA et al., 1984). However, little work has been done in relation to the species of veterinary importance, *S. bovis*. The previous methods included agar-gel immunodiffusion (AGID) (ARADAIB & ABBAS, 1985; ARADAIB, 1988; ARADAIB et al., 1993); indirect fluorescent antibody technique (HUSSIEN, 1972); complement fixation test (HUSSIEN et al., 1972); indirect hemagglutination (DUFFUS et al., 1975). All these are pilot studies only and none of these methods have been used for diagnosis on a practical scale. In the present study, *S. bovis* adult worm extract was employed as an antigen to monitor the immune response of zebu calves to *S. bovis* vaccine.

MATERIALS AND METHODS

Experimental Animals

Fifteen 6-8 months old zebu calves were purchased from Schistosomiasis free area and were checked by fecal examination. They were then divided into three groups (by lottery). Group 1 (six calves) were immunized against *S. bovis* by a subcutaneous injection of Schistosomula of *S. bovis* which had

been irradiated at 20 Krad. Group 2 (six calves) were immunized with intramuscular injection of *S. bovis* Schistosomula Irradiated at 3 Krad. Each calf received a dose of 10.000 irradiated Schistosomula. Group 3 (three calves) were kept as non immunized controls. Twenty four weeks after the first immunization, two calves from group 2 and a calf from group 1 were challenged with 10.000 normal cercariae of *S. bovis* administered percutaneously to the shaved tail. During the course of the experiment the animals were maintained indoors and were fed a ration of concentrate and hay with free access to water.

Serum Samples

Blood samples were collected from the jugular vein at weekly intervals. Sera were separated and divided into 0.5ml aliquotes and stored at -20°C until used.

Parasites

Infection of the snail intermediate hosts with miracidia and collection of cercariae were carried out as described by BUSHRA et al. (1978). Cercariae were irradiated by gamma irradiation using (Co)⁶⁰ source, at a dose rate of 1.9 Krad per minute. Cercariae were then transformed into schistosomula using the method of JAMES & TAYLOR (1976).

Antigen Preparation

Three-six months old Nubian goats were experimentally infected with 3.000 cercariae of *Schistosoma bovis* administered percutaneously to the shaved flank region. Ten weeks after the infection, the goats were slaughter and perfused for collection of adult worms. The worms were washed 10 times in physiological saline and placed in a glass homogenizer together with twice their volume of phosphate buffered saline (pH = 7.2) and sonicated with intermittent cooling in ice until no distinct worms were left (usually 2-3 minutes). The homogenate was then centrifuge at 12.000rpm for an hour in a cold centrifuge. The supernatant was taken off immediately using Pasteur pipettes and stored frozen at -20°C. The protein concentration of the antigen was 20mg/ml.

Agar-gel Immunodiffusion (AGID)

AGID was carried out basically as described by OUCHTERLONEY (1958) with modification of ARADAIB et al., (1993). Briefly, 15ml of 1.5% Noble agar^a in barbitone buffer (2.76gm barbitone, 15.44gm

barbitone sodium, 0.1gm NaN_3 and one liter distilled water) pH 8.2, was poured into a Petri dish. A pattern consisting of one central and six peripheral wells was cut into the agar. The diameter of the well is approximately 0.5cm. Each well was filled twice with 0.025ml of reactant fluid. The plate was incubated at 37°C for 48 hours. The antigen was used at a protein concentration of 10mg/ml.

Enzyme linked Immunosorbent Assay (ELISA)

ELISA was carried out according to the procedure described by VOLLER et al., (1976). The assay was performed using adult worm antigen at a protein concentration of 5µg/ml in 0.05M carbonate bicarbonate buffer (pH 9.6). All reagents were used at 0.1ml volumes. Optimal reagent dilutions were determined by checker board titration. The conjugate was horseradish peroxidase labeled rabbit antiovine IgG^b. The substrate was orthophenylene diamine (OPD) in 0.1M phosphate-citrate buffer (pH=5) and 30% hydrogen peroxide (2mg OPD, 10ml distilled water, 10ml citrate-phosphate buffer and 0.01ml 30% hydrogen peroxide). The reaction was stopped by 0.025ml (8N H_2SO_4) when the absorbance value of the positive reference serum was approximately 1.00. This was usually obtained 10-15 minutes after the addition of the substrate. The plate was read at 492nm in a micro-ELISA reader^c. Each serum was tested in duplicate and the results were expressed as mean ELISA absorbance values.

RESULTS

By AGID test no precipitin lines were observed with sera from vaccinated or control calves. However, challenged animals produced precipitin lines of varying intensity. Refilling the wells with serum or antigen increased the sensitivity of the test. In the ELISA, the immune response of the 20 Krad group was detectable by 2-3 weeks post vaccination peaking by 6-8 weeks post vaccination. The antibody response of the 3 Krad group was detectable by 2 weeks, peaking by 6 weeks post vaccination. The antibody response of the challenged calves was elevated by 2 weeks, peaking by 8-10 weeks post challenge and remained high through out the experimental period (Figure-1).

DISCUSSION

Irradiation-attenuated *S. bovis* schistosomular vaccine sensitized the host to schistosome

antigens without acquisition of patent infection (BUSHARA et al., 1978; TAYLOR & BICKLE, 1986). In experimental infection of calves with *S. bovis*, parasitological diagnosis by finding egg in fecal samples is possible only after 6-8 weeks post infection (SAAD et al., 1980; DARGIE, 1980; ARADAIB et al., 1993) and by 7-8 weeks post infection in mice (MURARE, 1983). These observations render serologic diagnosis an interesting alternative. In the present study no precipitin line was formed with sera from vaccinated or control calves. Challenged calves with patent infection produced precipitin lines of varying intensity depending on the level of the infection. Incubation at 37°C for 48 hours and refilling the wells were necessary for the development of the precipitin line. The serological response of the 3 Krad group was higher than the 20 Krad group. This was probably due to either the different route of injection or the effect of irradiation on the property of the schistosomular antigens. The immune response of the challenged calves was detectable by 2 weeks, peaking by 8-10 weeks post challenge. The peak is probably due to the patent infection or death of adult worms which provide more antigenic material around that time (SAAD et al., 1980).

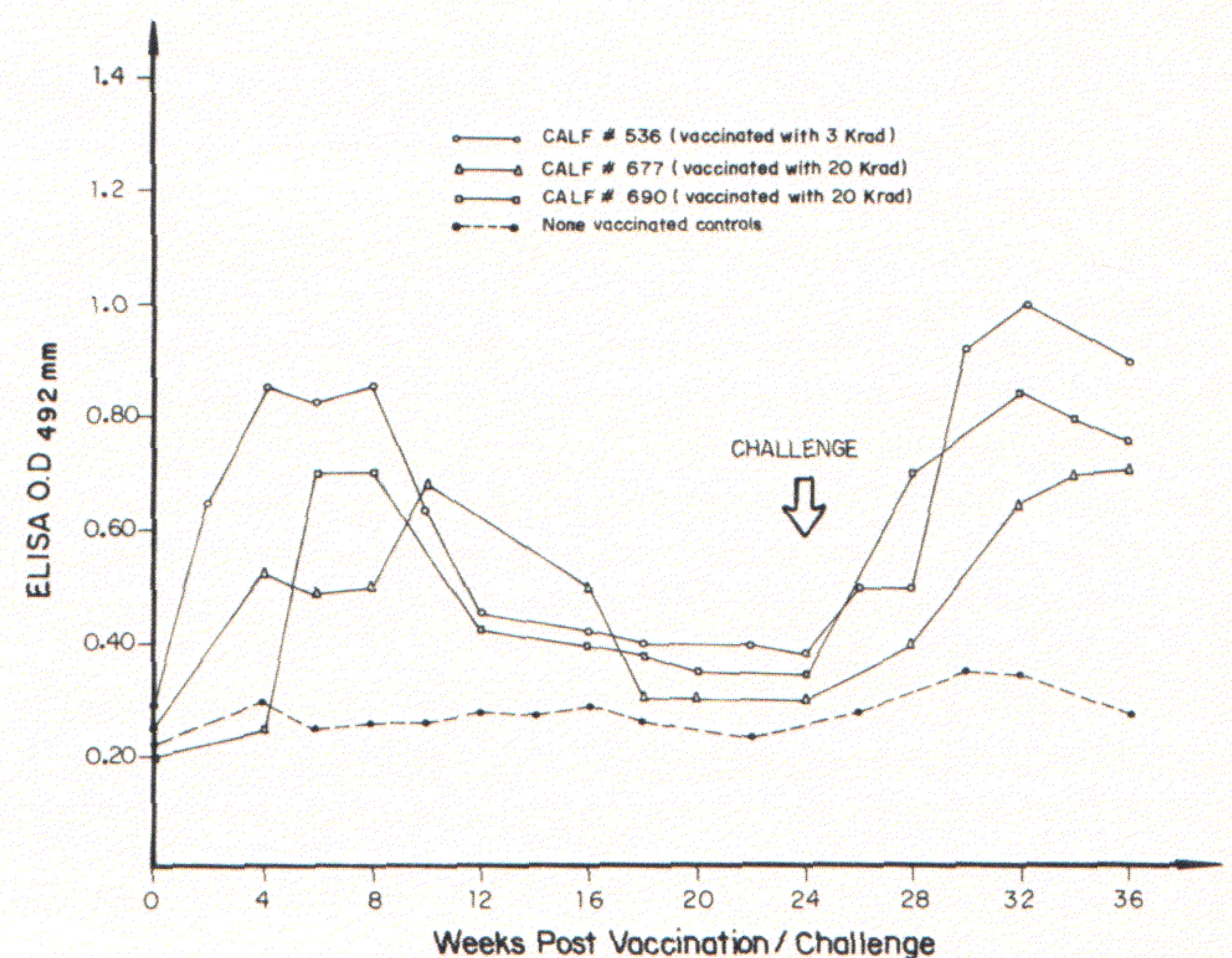


Figure 1 - The ELISA O.D. (492) values in *Schistosoma bovis* vaccinated, challenged and control calves.

In conclusion, these investigations have shown that although AGID is far too insensitive, ELISA can be used to monitor the serological responses of calves to *S. bovis* vaccine. Investigations are in progress to determine whether ELISA antibody titers in vaccinated calves correlate with resistance as judged

by field challenge. Studies are also under way to examine the diagnostic potential of ELISA in natural *S. bovis* infection.

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SOURCES AND MANUFACTURE

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