

EXPERIMENTAL BOVINE SCHISTOSOMIASIS IN ZEBU CALVES

ESQUISTOSSOMOSE BOVINA EXPERIMENTAL EM BEZERROS ZEBUÍNOS

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SUMMARY

Five calves were each experimentally infected with 30,000 cercariae of *Schistosoma bovis* and three calves were kept as controls. *S. bovis* eggs first appeared in feces of the infected animals by week 5 post infection and all animals were shedding by week 6 post infection. Between week 7 and 9 post infection, where fecal egg counts were highest, the infected animals developed mucoid and then hemorrhagic diarrhoea and they became dull and depressed. Packed cell volumes and hemoglobin concentrations of the infected animals showed progressive reductions compared to the uninfected control calves. The animals were necropsied and perfused at week 12 post infection and tissue egg densities and worm burden were determined.

Key words: *Schistosoma bovis*, bovine schistosomiasis.

RESUMO

Cinco bezerros foram infectados experimentalmente com 30.000 cercarias de *Schistosoma bovis* e três bezerros foram usados como controle. Ovos de *S. bovis*

apareceram inicialmente nas fezes dos animais infectados pela 5ª semana após infecção e todos os animais estavam eliminando ovos na 6ª semana após infecção. Entre as 7ª e 9ª semanas após infecção, nas quais a quantidade fecal de ovos era mais alta, os animais infectados manifestaram diarreia com muco seguida por disenteria e os animais apresentando-se moribundos e depressivos. PCV e concentração da hemoglobina dos animais infectados demonstrou redução progressiva em comparação com os bezerros não infectados. Os animais foram necropsiados na 12ª semana para determinação da densidade de ovos nos tecidos e quantidade de parasitas.

Palavras-chave: *Schistosoma bovis*, schistosomose bovina.

INTRODUCTION

Schistosoma bovis, the cause of bovine schistosomiasis, and other trematode parasites constitute serious veterinary problem in many part of the world including the Sudan (HUSSIEN, 1968; BUSHARA et al., 1978; MAJID et al., 1980; SAAD et al., 1980; ARADAIB and ABBAS, 1985; ARADAIB, 1988; GORAISH et al., 1988 and ARADAIB et al., 1993). In cattle the disease is

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characterized by emaciation and poor subsequent reproductive performance (HUSSIEN, 1973; Mc CAULY et al., 1984; ARADAIB et al., 1994b). The prevalence of *S. bovis* infection is higher in younger animals as estimated by fecal egg counts; and monthly incidence rate is higher in the fall due to high infectivity of the snail intermediate host, the *Bulinus* spp, during that season (MAJID, 1980). Most of the studies conducted on schistosomiasis were directed towards the species of human importance and little has been done in relation to the species of veterinary importance, *S. bovis*. The present study was undertaken to provide some clinical, hematological and parasitological information on experimental bovine schistosomiasis using a relatively higher cercarial dose.

MATERIALS AND METHODS

Experimental Animals

Eight 6-to 8-months old zebu calves were purchased from an area known to be free from schistosomiasis. All the animals were found to be healthy and free from trematode infections after repeated fecal and clinical examinations. They were then divided randomly into two groups (by lottery). Five calves (group 1) 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. Three additional calves (group 2) were kept as uninfected controls. During the course of the experiment the animals were maintained together indoors and were fed a ration of concentrate and hay with water *ad libitum*. Twelve weeks post infection, the animals were necropsied and perfused for worm recoveries and tissue egg counts.

Infective Materials

Bulinus africanus, the snail intermediate host of *S. bovis*, were collected from Elmoglad, a schistosomiasis 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 fecal samples of experimentally-infected goats. Cercariae were collected, using a light source, from shedding snails for 6h.

Blood Samples

Blood samples were collected from the jugular vein in heparinized vacutainers for hematology. Immediately following collection of blood samples, packed cell volumes (PCV) were determined in a microhematocrit centrifuge and hemoglobin concentrations (Hb) by cyanmethemoglobin method.

Fecal Egg Counts

After infection, weekly fecal samples were collected from the rectum and the fecal egg counts were determined as described by PITCHFORD and VISSER (1975).

Perfusion

Twelve weeks post infection, the infected animals were anaesthetized and slaughtered. Adult schistosomes were collected from mesenteric vessels of the infected animals by perfusion as described by BUSHARA et al., (1978). The worms were preserved in Rudabush solution and counted individually.

Tissue egg counts

Samples from the liver, small and large intestines were collected from the infected animals at the time of necropsy and were stored at -20 C until used. The tissue egg determination was made according to the digestion method of CHEEVER (1968).

Statistical Analysis

Means \pm standard deviations ($X \pm SD$) were calculated for parasitological parameters (fecal egg counts, tissue egg counts and worm recoveries). Means were also calculated for hematological parameters (PCV and Hb) using conventional statistical methods described by SCHWABE et al. (1977).

RESULTS

Clinical Observations

Control calves appeared normal and did not show any adverse clinical reaction throughout the experimental period. Infected animals developed mucoid and then hemorrhagic diarrhoea, dehydration, loss of appetite, emaciation, roughness of the skin and pale mucous membranes. The changes were observed around week 5 post infection and they coincided with the time of oviposition. The severity of the signs was highest between week 7 and 9 post infection, where the fecal egg counts were highest. The animals became dull and depressed at the time of slaughter. On necropsy, the liver was slightly congested and the cut surface was hard with fibrous strand and necrotic foci. The intestines were hyperemic with catarrhal exudate in the lumen. The hepatic and the mesenteric lymph nodes were enlarged and the gall bladder was distended with bile.

Hematology

Initially, haemoglobin concentrations (Hb) in gm/dl and packed cell volume (PCV) in percentage were similar in both groups. However, the infected animals showed a progressive reduction in their PCV values and Hb concentrations from around week 5 post infection (Table 1 and 2, respectively).

Parasitological findings

Eggs first appeared in feces of infected animals at week five post infection and all infected animals were shedding by week six post infection (Table 3). No eggs were detected in fecal samples from control calves. Tissue egg counts were highest in the small intestines followed by the large intestines and then the liver (Table 4). The result of worm recovery is presented (Table 5).

DISCUSSION

In the present study, zebu calves experimentally infected with *S.bovis* developed diarrhoea, anemia and emaciation. The animals became clinically ill 6 week after exposure to *S.bovis* cercariae. The disease increased in severity between week 7-9 post infection when the fecal egg

Table 1 - Mean packed cell volume (PCV) values in *S. bovis* infected and control calves.

weeks post infection	PCV	
	Infected	controls
zero	29.8	28.0
1 st	28.5	27.0
2 nd	29.0	29.0
3 rd	28.0	28.5
4 th	28.2	29.2
5 th	24.1	28.4
6 th	22.8	29.1
7 th	20.4	29.2
8 th	20.8	28.4
9 th	19.2	27.2
10	21.4	27.8
11 th	21.8	28.2
12 th	21.8	28.5

Table 2 - Mean hemoglobin concentrations (Hb) in *S. bovis* infected and control calves.

weeks post infection	Hemoglobin concentrations (Hb)	
	Infected	controls
zero	9.3	9.4
1 st	8.42	9.2
2 nd	9.14	9.4
3 rd	8.7	8.7
4 th	8.4	8.5
5 th	7.5	9.1
6 th	7.5	9.2
7 th	6.8	9.3
8 th	6.7	9.3
9 th	6.4	8.8
9 th	7.0	9.0
10 th	7.2	9.1
11 th	7.2	9.1
12 th	7.5	9.2

Table 3 - Mean ± SD Fecal egg counts (Eggs per gram of feces) of calves experimentally infected with *S. bovis*.

calf number	weeks post infection							
	5th	6th	7th	8th	9th	10th	11th	12th
530	02	30	383	505	387	321	56	32
513	01	12	63	109	64	55	71	43
369	03	37	247	195	220	135	149	187
280	15	152	297	215	309	87	12	27
zero	01	29	428	411	209	264	353	235

Mean + SD	4	52	284	287	238	172	128	105
	±6	±56	±142	±164	±121	±115	±135	±99

Table 4 - Mean ± SD Tissue egg counts by type of tissue (Eggs per gram of tissue) of calves experimentally infected with *S. bovis*.

Tissue egg count	Mean ± SD
Liver	245 ± 215
Small intestines	1133 ± 428
Large intestines	758 ± 516

Table 5 - Mean \pm SD Worm recoveries of *S. bovis* infected calves at the time of necropsy.

Worm recoveries	Mean \pm SD
Males	2152 \pm 508
Females	848 \pm 295
Total	3000 \pm 703

counts were highest. Subsequently, the disease subsided slowly as fecal egg counts declined. Initially, HB concentrations and PCV values were similar in the infected animals and the controls. However, infected calves showed a progressive reductions in their HB concentrations and PCV values from around week 5 post infection compared to the uninfected controls. Similar findings were reported by (SAAD et al., 1980) and were attributed to excessive loss of red blood cells from the circulation caused by the exit of the eggs from the mesenteric vessels into the lumen of the intestines, with consequent development of hemorrhagic diarrhoea (SAAD et al., 1980).

In previous studies, parasitological diagnosis of *S. bovis* infection by demonstration of schistosome eggs in fecal samples was possible only by 6-8 week post-infection (SAAD et al., 1980; DARGIE, 1980; ARADAIB et al., 1993; and ARADAIB et al., 1994a). However, in the present study, detectable egg excretion in fecal samples from infected calves started at week 5 and all infected animals were shedding by week 6 post infection. This is due to the higher cercarial infective dose used in this study. Higher cercarial infective dose was used in this study as the animals may be exposed to similar cercarial dose under field condition, specially during the rainy season of the year (MAJID, 1980). Fecal egg counts were highest between week 7-9 post infection, followed by a marked reduction in fecal egg excretion. Suppression of *S. bovis* egg production in chronic infected calves is well documented in repeated controlled experiments and this accounts for natural acquired immunity to the parasite (ARADAIB and OSBURN, 1994d). The natural immunity to *S. bovis* infection usually develops as a result of schistosome deaths and egg retention in tissues (SAAD et al, 1980). The immune response plays an important role in reducing the fecundity of female schistosomes (SAAD et al., 1980; ARADAIB et al., 1993). This study indicated that the pathogenicity of primary *S. bovis* infection is mainly due to egg production by female schistosomes. The severity of the infection is directly related to the infective cercarial dose which determines worm burden and subsequently, fecal and tissue egg counts compared to other pathogenicity studies (DARGIE, 1980;

SAAD, 1980). This study also provides useful information on understanding the nature of a future Schistosome vaccine which can be used for control of bovine schistosomiasis (ARADAIB, 1994c). In our laboratory, attempts to vaccinate calves against *S. bovis* infection using adult worm extracts or egg antigen were largely unsuccessful (ARADAIB, 1992; ARADAIB et al., 1993; ARADAIB et al., 1994a; and AARADAIB et al., 1994c). A vaccine which can induce antibodies that have a lethal effect on both worm viability and fecundity will be advantageous in limiting the extend of pathology and reducing the level of transmission of the disease.

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REFERENCES

- ARADAIB, I.E., and ABBAS, B. A retrospective study of diseases diagnosed at the University of Khartoum Veterinary Medical Teaching Hospital. *Sudan J Veterinary Science and Animal Husbandry*, v. 2, p. 55-66, 1985.
- ARADAIB, I.E. Serologic Studies on bovine schistosomiasis. Thesis (Master in Veterinary Medicine), University of Khartoum, Sudan, 1988.
- ARADAIB, I.E., 1992. Evaluation of *Schistosoma bovis* adult worm extracts for vaccination of calves. Thesis (Master of Preventive Veterinary Medicine), University of California at Davis, USA. 1992.
- ARADAIB, I.E., ABBAS, B., BUSHARA, H.O. et al. Evaluation of *Schistosoma bovis* adult worm extract for vaccination of calves. *Preventive Veterinary Medicine*, v. 16, p. 77-84, 1993.
- ARADAIB, I.E., OMER, O.H., ABBAS, B. et al. *Schistosoma bovis* whole-egg antigen did not protect zebu calves against experimental schistosomiasis. *Preventive Veterinary Medicine* (In press), 1994a.
- ARADAIB, I.E., ABBAS, B., Osburn, B.I. et al. ELISA for bovine Schistosomiasis vaccine: A preliminary study. *Ciencia Rural* v. 24. n. 3, 1994b.
- ARADAIB, I.E., BOLANGER, D., ABBAS, B., Osburn, B.I. et al, (1994c). Evaluation of a recombinant *Schistosoma mansoni* 28-KD protien for vaccination of calves against *S. bovis*. *Vaccine* (In press), 1994.
- ARADAIB, I.E., OSBURN, B.I. Vaccination of cattle against bovine schistosomiasis: current status and future prospects: A review. *Preventive Veterinary Medicine* (In press), 1994d.

- BUSHARA, H.O., HUSSIEN, M.F., SAAD, A.M. et al. Immunization of calves against *Schistosoma bovis* using irradiated cercariae or schistosomula of *S.bovis*. *Parasitology*, v. 77, p. 303-313, 1978.
- CHEEVER, A.W., 1968. Conditions affecting the accuracy of potassium hydroxide digestion techniques for counting *Schistosoma mansoni* eggs in tissues. *Bulletin of the World Health Organization*, v. 39, p. 328-331, 1968.
- DARGIE, J.D. The pathogenesis of *Schistosoma bovis* infection in Sudanese cattle. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, v. 74, p. 560-562, 1980.
- GORAISH, I. A, ADELSALAM, E.B., TARTOUR, G., ABBAS, B., ARADAIB I.E. The effect of Levamisole (L.Tetramizole) treatment on the susceptibility to *Fasciola gigantica* infection in goats. *Revue de Elv Med Vet Pay Trop*, v. 41, p. 283-287, 1988.
- HUSSIEN, M.F. The pathology of natural and experimental bovine Schistosomiasis. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, v. 62, p. 9-12, 1968.
- HUSSIEN M.F. Animal schistosomiasis in Africa: A review of *Schistosoma bovis* and *S. matthei*. *Veterinary Bulletin*, v. 43, p. 341-347, 1973.
- MAJID, A.A., BUSHARA H.O., SAAD, A.M. et al. Field testing of an irradiated *Schistosoma bovis* vaccine. *American J of Tropical Medicine and Hygiene*, v. 29, p. 452-455, 1980.
- Mc CAULEY, E.H., MAJID, A.A., TAYEB, A. Economic evaluation of the production impact of bovine schistosomiasis and vaccination in the Sudan. *Preventive veterinary Medicine*, v. 6, p. 735-754, 1984.
- PITCHFORD, R.J., VISSER, P.S. A simple and rapid technique for quantitative estimation of helminth eggs in human and animal excreta with special reference to shistosome spp. *Transaction of the Royal Society of Tropical Medicine and Hygiene*, v. 69, p. 318-322, 1975.
- SAAD, A.M., HUSSIEN, M.F., Dargie, J.D. et al. *Schistosoma bovis* in calves: The development and clinical pathology of primary infection. *Research in Veterinary Science*, v. 28, p. 105-111, 1980.
- SCHWABE, C.W., RIEMANN, H.P, and FRANTI, C.E. Epidemiology in Veterinary Practice. Philadelphia: Lea and Febiger. 303, 1977.