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SCHISTOSOMA MANSONI: EXPERIMENTAL BOVINE SCHISTOSOMIASIS (*)

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SUMMARY

Six calves infected transcutaneously with 30,000 **Schistosoma mansoni** cercariae (LE strain) were sacrificed and perfused for worms. Perfusion gave a high rate of worm recovery; examination of tissue samples from the liver and intestinal wall revealed numerous viable eggs. The oogram performed with these eggs was similar to that for eggs obtained from infected albino mice (same **S. mansoni** strain). Anatomo-pathological studies revealed granulomas and other pathological signs similar to those found in man. The eggs from feces of those animals produced miracidia infective to **Biomphalaria glabrata** (41% infected). Cercariae shed by these snails produced normal infection in mice. Thus, the life cycle of **S. mansoni** was completed under laboratory conditions, using experimentally infected bovines and laboratory-reared **B. glabrata.** Epidemiological surveys of feces from bovines bred in an endemic schistosomiasis area of the State of Minas Gerais, Brazil, showed a low natural infection rate (less than 3%). Nevertheless, under certain conditions, the problem of bovine schistosomiasis could prove to be a serious matter. At one small farm, 4 out of 8 calves were found to be passing viable eggs in stools.

INTRODUCTION

Natural schistosomiasis mansoni in calves (in an endemic area of the State of Pernambuco, Brazil) was reported for the first time by BARBOSA et al.[1], who found only non-viable eggs.

In 1969, SAEED et al.[5], using Puerto Rican strain of **Schistosoma mansoni,** infected some calves and detected eggs of a viable appearance in the feces of these animals.

The purpose of the present study was to elucidate some aspects of bovine schistosomiasis, such as the degree of worm development, egg production, potential of fecal eggs to produce miracidia infective to **Biomphalaria glabrata,** pathological effects of schistosomiasis infection, and, finally, stool surveys in order to obtain preliminary information about natural infection in cattle from endemic schistosomiasis areas.

MATERIALS AND METHODS

Six male calves produced by crossbreeding Zebu and Holstein cattle, a breed maintained for milk production, and often found in several endemic schistosomiasis areas, were infected with about 30,000 **S. mansoni** cercariae (LE strain). The calves were kept in dorsal decubitus, and the cercarial suspension was dropped over their abdomens. The animals were given 5 ml of heparin solution (*), intravenously, just before sacrifice.

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Perfusion of the viscera for worm recovery was carried out by means of physiological salt solution (NaCl 0.85%) injected in mesenteric and liver vessels. The saline flowed through a polyethylene tube inserted in a small plastic barrel. This barrel was placed 1 meter above the visceral material. About 10 liters of saline were needed to perfuse all the contents of the visceral blood system of each calf.

Worms in the pre-postural phase were classified by the schistogram method, described by BARBOSA et al.[2]. Eggs from tissues were classified by the oogram method (PELLEGRINO & FARIA[4]).

The present report presents a new method to detect **S. mansoni** eggs through bovine stool examination. Basically this new method consists in several passages of bovine feces through a mesh of gauze (folded in 4 parts), and mixed in cold water (5°C), in order to prevent miracidia eclosion. The supernatant was discarded, and some more cold water was added several times, until the water was clear.

Infection of **Biomphalaria glabrata** snails (using miracidia hatched from fecal bovine eggs) was carried out by the PELLEGRINO & KATZ's method[3].

Anatomo-pathological examination was performed with different samples of visceral tissue.

RESULTS

a) Schistogram after experimental infection

The schistogram of calves showed a developmental pattern very similar to that obtained with mice infections by BARBOSA et al.[2] (Table I).

TABLE I

Perfusion on the 16th day after infection — worm recovery and development pattern according to schistogram (BARBOSA et al., 1968)

Animals	Total of worm recovery	Schistogram: % worm/evolutive stage				
		1st stage	2nd stage	3rd stage	4th stage	5th stage
Calf (1)	3.843	2%	16%	47%	31%	4%
Calf (2)	2.084	3%	14%	51%	28%	4%

b) Total worm recovery

Mean and standard error of worm recovery was 2,705 ± 1,361. This mean represents 9% of the number of cercariae used in transcutaneous infection (Tables I and II).

TABLE II

Worm recovery by perfusion of the remaining calves

Animals	Days after cercariae infection	Total of worm recovery
Calf (3)	38	2.197
Calf (4)	46	2.256
Calf (5)	60	4.797
Calf (6)	94	1.050

c) Quantitative oogram (viable eggs)

The quantitative oogram performed with tissue samples from liver and intestine of calves is summarized in Table III.

TABLE III

Quantitative oogram (PELLEGRINO and FARIA, 1965) performed with tissue samples from liver and intestine of calves

Animals	Days after infection	Viable eggs per gram of tissue	
		Liver	Intestine
Calf (4)	46	45	35
Calf (5)	60	968	750
Calf (6)	94	789	732

Based on the high proportion of viable eggs, it was inferred that a normal egg laying occurred.

d) Anatomo-pathological findings

1. Post-mortem macroscopic aspects

Ascites at discrete levels was observed in calves number (5) and (6), with infections of longer duration. The livers of calves number (4), (5), and (6) were brown in color and contained several granulomas in the form of small white spots. Calves number (4), (5), and (6) also had moderate splenomegaly.

2. Microscopic lesions

2.1 — **Liver** — The structure of the liver was relatively well preserved. However, a disse-

minated inflammatory focus was detected along the length of the portal space. This process began with vasculitis, changing to thrombosis with total fibrosis of vessels and adjacent areas.

The inflammatory reaction was clearly granulomatous showing giant cells of Langhans and foreign body types, intense fibroplasia, and eosinophil infiltration. In some areas, proliferate conjunctive tissue in portal spaces tended to circumscribe the lobular structure.

2.2 — **Intestine** (rectum) — The essential structure of the organ was preserved but many granulomas, associated with schistosome eggs, were seen in the submucosa and muscular wall.

2.3 — **Lungs** — Moderate endarteritis of the bronchial arteries with clear eosinophilic reaction in the walls was seen. Part of an adult male worm was found in a lung vessel of one of the samples.

e) **Examination of feces**

Viable eggs were found in the feces of calves (3), (4), (5), and (6) in all examinations made from the 48th day of infection and onwards.

f) **Infection of snails**

One-hundred seventy **B. glabrata** were divided into groups of 30-40 specimens and infected with miracidia hatched from fecal schistosome eggs from calves. Sixty days after infection, the molluscs were examined, and 30 out of 80 surviving specimens (41.3%) were shedding cercariae.

g) **Infectivity of** S, mansoni cercariae obtained **from** B. glabrata **(infected with miracidia from fecal eggs from calves) to mice**

After transcutaneously infecting 18 mice with 150 cercariae per mouse, the mean worm recovery rate was 59 ± 20, the percentage worm recovery was 39%, and intestinal tissue contained 30,311 viable eggs per gram.

h) **Preliminary survey of natural infections in cattle from schistosomiasis endemic areas**

The feces of 217 cattle (adults and immatures), and all reared in areas endemic for human schistosomiasis, were examined for schistosome eggs. Six calves (2.8%) were found to be excreting viable **S. mansoni** eggs. In one small farm, four out of the eight calves examined were excreting eggs. At this farm, the cattle graze in a swamp, traversed by a stream containing a large number of naturally infected **B. glabrata.**

DISCUSSION

The experimental infection of calves showed a remarkable susceptibility of these hosts. The results showed a high rate of worm recovery (% mean recovery = 9) in transcutaneous infection. The schistogram performed on days 16 after infection showed a close relation to that observed with worms obtained from albino mice. In the same way, the oogram carried out with visceral material from infected calves also revealed a strong similarity to that performed with infected mice.

The eggs found in the feces of calves were able to produce miracidia infective to **B. glabrata.**

The anatomo-pathological findings showed lesions with macroscopic and microscopic aspects analogous to those detected in man and in some other vertebrate hosts. On the other hand, a preliminary epidemiological survey — undertaken in a high endemic schistosomiasis area — showed a low rate of bovine shedding **S. mansoni** eggs in feces. Nevertheless, under some special conditions, bovine schistosomiasis could be a serious public health problem, as the calves examined were infected. Because of the high temperature in this place, the cattle for instance, at the small form where 50% of frequently bathe in contamined water. Bovine feces were often seen in the water or along the margins of water collection. Because cattle produce up to 5 kg of feces per day, they could also be the major source of infection for snails.

The fact that the veterinarians do not detect bovine schistosomiasis could be tentatively explained by two hypotheses. The first is connected with the kind of parasitological methods used for the examination of feces. These methods were based on flotation techniques, since in schistosomiasis endemic areas the helminths with economic importance produced eggs with

low density. Thus, the high density of **S. mansoni** eggs explains the low efficacy of the methods used to detect these eggs. The second one is related to the lack of information about infection of animals sacrificed at slaughterhouses. This could be explained by the difference of age of the animals used (adult), whereas in our preliminary epidemiological observations young animals were the most susceptible to schistosomiasis infection.

Finally, there are still some basic questions that need to be considered and answered: What is the impact of bovine schistosomiasis in the epidemiology of schistosomiasis, with special reference to the role played by domestic reservoirs in the transmission process? What is the real importance of the pathology of schistosomiasis for the economy of cattle breeding? Further epidemiological studies are, thus, warranted in order to clear up these fundamental questions.

RESUMO

Schistosoma mansoni: **esquistossomose experimental em bovinos**

Seis bezerros, infectados pela pele com 30 000 cercárias de **Schistosoma mansoni**, foram sacrificados e perfundidos para contagem de vermes. A perfusão revelou alta taxa de recuperação de vermes, e o exame de amostras de tecidos do fígado e da parede intestinal mostrou grande quantidade de ovos viáveis. O oograma feito com estes ovos foi semelhante ao executado com ovos obtidos de camundongos albinos infectados com a mesma cepa de **S. mansoni.** Exames anátomo-patológicos revelaram granulomas e outros sinais patológicos, semelhantes aos encontrados no homem.

Os ovos provenientes das fezes dos animais produziram miracídios infectantes para **Biomphalaria glabrata** (41% de infecção). As cercárias que emergiram destes caramujos produziram infecção normal em camundongos. Deste modo, o ciclo vital do **S. mansoni** foi obtido, sob condições de laboratório, com bovinos experimentalmente infectados e **B. glabrata** criadas em laboratório.

Levantamentos epidemiológicos feitos por exame de fezes de bovinos de zona endêmica esquistossomótica (Estado de Minas Gerais, Brasil), revelaram taxa de infecção natural baixa (menos de 3%).

Entretanto, sob condições especiais, a questão da esquistossomose em bovinos poderia constituir-se um problema de Saúde Pública. Exemplificando, em uma pequena fazenda, 4 entre 8 bezerros criados foram detectados eliminando ovos viáveis pelas fezes.

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