

TRANSMISSION OF *Schistosoma mansoni* UNDER EXPERIMENTAL CONDITIONS USING THE BOVINE - *Biomphalaria glabrata* - BOVINE MODEL

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SUMMARY

Three calves experimentally infected with *Schistosoma mansoni*, and passing viable eggs in feces, as well as 5 normal calves (coming from a non-endemic area for schistosomiasis) kept as controls, were maintained in an enclosure (850 m² in area). In this enclosure, a tank with water received 500 laboratory reared *Biomphalaria glabrata*. All the control calves were infected for a period ranging from 79 to 202 days after the beginning of the experiment, and afterwards presented viable *S. mansoni* eggs in feces. The mean worm recovery was 555. The snail population increased throughout the experimental period, showing a high number of *B. glabrata* infected with *S. mansoni* (42% on average). According to the present study, bovine has been suggested as having potentially a role in the maintenance of the life cycle of *S. mansoni*.

KEY WORDS: *Schistosoma mansoni*; Domesticated reservoirs; Bovine schistosomiasis; Experimental schistosomiasis.

INTRODUCTION

In the epidemiology of schistosomiasis *mansoni* it has been conclusively established that man is the main vertebrate reservoir in the maintenance of the disease in nature.

Several other vertebrate hosts pertaining to mammalian orders (Rodentia, Marsupialia, Carnivora, Primate, Insectivora, and Artiodactyla) have been described as bearing natural infection with *Schistosoma mansoni*. Rodentia is by far the order that presents the largest number of species described with natural infection.

Although several species have been described as being naturally infected with *S. mansoni*, only one focus maintained by baboons and *Biomphalaria* where, supposedly, man would not have any participation in snails infection, has been described (NELSON¹⁰).

Experimental research works carried out under semi-natural conditions showed that it is possible

to maintain the life cycle of *Schistosoma mansoni* using the aquatic rodent *Nectomys squamipes* and *Biomphalaria glabrata* (ANTUNES et al.²), and also other aquatic rodent the *Holochilus brasiliensis* and *B. glabrata* (CARVALHO et al.⁴; KAWAZOE⁷). However, the work by PITCHFORD & VISSER¹³ in Africa, utilizing the rodent *Mastomys natalensis* and *Biomphalaria*, showed that the life-cycle was uncompleted after an 8-month-observation period in a human-contact-free area.

Bovines have been already described as bearing natural infection or being susceptible to experimental infections (BARBOSA et al.³; PIVA & BARROS¹⁴; MAYAUDON & POWER⁸; SAEED et al.¹⁵; COELHO et al.⁶). On account of his large size, only one bovine is able to eliminate a fecal amount superior to that excreted by hundreds of rodents, daily.

Based on these observations, and considering

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that in Brazil bovines are transported to several regions without any control for *S. mansoni* infection, thus theoretically acting as disseminators of the disease, the present study was designed to determine the potential of those animals as possible maintainers for schistosomiasis mansoni, under semi-natural conditions.

MATERIAL AND METHODS

This study was carried out in an enclosed area of approximately 850 m² situated in Belo Horizonte, State of Minas Gerais, Brazil. The area was totally fenced off with barbed wire, and a shelter with a bunk was built in the center for feeding and housing the animals.

For maintenance of the planorbids and water supply for the calves, a 16m² tank was built with a variable depth of 15 to 55cm. The only source of water (without chemical treatment) was an artesian well.

Three calves previously infected with *S. mansoni* (Group I) and five sentinel calves (Group II) were housed for a period of 10 months, approximately. The population density was of one animal per 106m². The group of infected animals was composed of two Holstein calves and one crossbred calf (Holstein x Zebu), and the sentinel group (non infected) comprised three Holstein and two crossbred calves, aged 8-12 months, coming from a non-endemic area for schistosomiasis. The animals in Group I were transcutaneously infected in the abdominal region with a single dose of 25,000 cercariae of *S. mansoni* (LE-BH strain), following the technique by COELHO et al.⁶. Fecal exams (according to COELHO et al.⁶) and rectal mucosal scrapings (according to MODENA⁹ and PELLEGRINO et al.¹²) for all the sentinel animals, performed before the beginning of the experiment, were found to be negative for *S. mansoni*.

Two months before the introduction of bovines in the enclosure, 500 *B. glabrata* (BH strain) reared at the laboratory were placed in the tank.

One month after the introduction of bovines in the experimental area, the population density of the planorbids was monthly assessed through individual markings of the snails, using a technique by COELHO et al.⁵. The snails were randomly col-

lected in different locations in the tank, about one meter distant from one another. All the captured snails were counted and shell diameters were noted. Those with diameter larger than 5mm were marked. The marking was done by perforating two holes of about 2mm in diameter on the anterior part of the shell, on each side, with a stylet.

After marking, the snails were immediately sent back to the aquatic environment. One week later, the collection was repeated, and the diameter of all the captured snails, as well as the number of those ones marked were recorded. All individuals were immediately reintroduced into the location where they had been captured.

The evaluation of population density was done using the formula:

$$\text{Population density} = \frac{A \times C}{B}$$

Where

A = total of captured snails (marked and unmarked)

B = total of recaptured snails (marked)

C = total of marked snails introduced into the habitat after marking.

Simultaneously, planorbids were individually examined every month under artificial light to observe if cercariae were being shed.

Faecal examinations and rectal mucosal scrapings were weekly conducted by day 35 after introduction of control animals in the experimental area. After the first detection of eggs in the feces in the sentinel animals, two calves were sacrificed on days 60, and three other ones on days 90. Perfusion of viscera for recovery of helminths was performed according to a technique described by COELHO et al.⁶, and determination of eggs in tissues was achieved using a quantitative oogram method (PELLEGRINO & FARIA¹¹).

RESULTS

All the sentinels were infected between 79 and 202 days after the first shedding of eggs by the infected animals producing viable eggs, and the development of adult helminths was compatible with the results observed in other susceptible animals (Table 1, 2 and 3).

Table 1

First detection of *Schistosoma mansoni* eggs in feces of infected calves, under experimental field conditions

Animal N°	Breed	First detection of eggs in feces (days)
21	Holstein (red x white)	79
22	Holstein (black x white)	117
23	Holstein (red x white)	186
24	Crossbred (Holstein x Zebu)	96
25	Crossbred (Holstein x Zebu)	202

Table 2

Recovery of adult helminths in calves infected with *Schistosoma mansoni* under experimental field conditions, using the technique of portal system perfusion

Animal N°	Days after detection of infection through fecal examination	Total of helminths recovered										Total
		Liver					Mesentery					
		Ms	Fs	Ma	T	%	Ms	Fs	Ma	T	%	
21	60	291	89	12	404	49.0	303	92	17	429	51.0	833
22	90	269	106	09	393	40.0	281	294	06	587	60.0	980
23	90	209	97	08	322	44.0	301	94	10	415	56.0	737
24	60	24	06	04	38	46.0	28	08	04	44	54.0	82
25	90	41	07	05	58	41.0	55	13	09	86	59.0	144

X = 555

Ms = Males Fs = Females Ma = Mated T = Total

Table 3

Quantitative oogram samples of liver and intestines of calves infected with *Schistosoma mansoni*, under experimental field conditions

Animal N°	Days after first detection of eggs	Viable eggs per gram of tissue	
		Liver	Intestine
21	60	301	297
22	90	205	244
23	90	249	122
24	60	113	114
25	90	46	57

Although neither the date of infection nor the number of cercariae to which the sentinels were exposed were known, it was possible to note that on average, the recovery of helminths and oograms were higher in the Holstein calves than in the Holstein X Zebu.

In relation to the population density of *B. glabrata*, it was observed that between October and December there was an increase in population

from 1,161 to 2,804 planorbids, then a decrease in January, when the population dropped to 1,372 mollusks. After that, the population began to increase again, reaching a total of 3,304 in May (Table 4)

One month after introduction of positive bovines, planorbids began to shed cercariae. In the presence of bovines in the fenced area, the rate of infection of the snails reached 42% on average (Table 4).

After the sacrifice of all the bovines, the water of the tank was removed and the snails collected. The snails with diameter larger than 3mm were eliminated and the smaller ones were put back in the tank after addition of a new amount of water. The young snails grew and reproduced well. The population density reached similar levels to that observed in previous experiment (Table 4). Snails were collected and examined for cercariae at monthly intervals for one year, and no snails were found to be positive for *S. mansoni* cercariae. The results obtained proved that the only source of *S. mansoni* miracidia were the bovine feces, under those experimental conditions. Furthermore, the possibility of contamination by miracidia from feces of naturally infected rodents was excluded.

DISCUSSION

For the first time, results demonstrated the maintenance of the extrahuman cycle under experimental field conditions, the bovine acting as final host.

The completion of life-cycle demonstrated the possibility of bovines to act as definitive hosts in the process of maintenance and diffusion of *S. mansoni*, independently of the human population, the main host. This fact became more consistent when it was noted that the snails infection rate was extremely high (Table 4), showing positivity throughout the experimental period. Furthermore, the sentinels prepatent period was relatively short, in some cases close to that determined in the ex-

perimental infection. Even though the number of cercariae to which the animals were exposed remained unknown, a high number of adult worms were recovered (Table 2).

These observations lead us to believe that in certain areas bovines may become involved in the transmission of *S. mansoni*.

In reference to the hypothesis raised by ANTUNES¹ as to the change in the profile of schistosomiasis as a zoonosis, considering the data available up to now, we believe that any generalization involving bovine in this sense would be premature. Every biocenosis must be distinctly considered in relation to the interaction among the different components of the epidemiological model.

It is possible that this phenomenon may occur in certain areas of high density of vertebrate hosts, specifically *H. brasiliensis* or *Nectomys s. squamipes*, rodents of semi-aquatic habitats, together with high density of intermediate hosts of good susceptibility, such as *B. glabrata*, as observed by ANTUNES et al.¹, CARVALHO⁴, and KAWAZOE⁷. As far as the bovine species is concerned, it should be emphasized that under the conditions in which this study was carried out the density was approximately one animal per 100m², which facilitates the transmission process. Furthermore, since the source of water was a tank with variable depth (15-55cm), the bovine was able to enter and sometimes to stay in the location. As a result, the amount of feces

Table 4

Evaluation of population density of *Biomphalaria glabrata* kept under experimental field conditions, using the method by Coelho et al.⁵

Month	Marked snails N°	Captured snails			Estimated population	% snails shedding cercariae
		Marked & Marked Recaptured				
		Unmarked	N°	%		
October	200	257	46	17	1.161	11%
November	250	382	49	13	1.949	29%
December	250	471	42	9	2.804	53%
January	200	254	37	15	1.372	31%
February	250	392	47	12	2.085	47%
March	200	589	42	7	2.805	52%
April	250	405	30	7	2.275	65%
May	300	749	68	9	3.304	49%

in the tank was relatively abundant, especially in the shallower parts.

The present data, as well as descriptions of natural infection in bovines (BARBOSA et al.³, PIVA & BARROS¹³, MAYADOUN & POWER⁸, COELHO et al.⁶), did not exclude the fact that infected humans are the main source of infection for the intermediate host.

Nevertheless, for the time being, bovines could play a secondary role in the maintenance and spreading of schistosomiasis. In future, a possible better adaptation of *S. mansoni* in host cattle, by selective process, could pose additional problems to the control of schistosomiasis in endemic areas. Finally, the transportation of infected bovines to non-endemic areas, but with the presence of susceptible *Biomphalaria* snails, could spread the disease.

RESUMO

Transmissão do *Schistosoma mansoni* em condições experimentais, usando o modelo bovino - *Biomphalaria glabrata* - bovino

Três bezerros, experimentalmente infectados com *Schistosoma mansoni* e passando ovos viáveis nas fezes, bem como 5 bezerros normais, oriundos de área não endêmica para esquistossomose e usados como controles, foram mantidos em uma área fechada de 850 m². Nesta área, foram colocados em um tanque com água 500 caramujos *Biomphalaria glabrata* criados em laboratório. Todos os bezerros controles foram infectados por um período de 79 a 202 dias após o começo do experimento e, mais tarde, apresentaram ovos viáveis de *S. mansoni* nas fezes. A recuperação média de vermes foi de 538. A população de moluscos aumentou durante o período experimental, mostrando um número significativo de *B. glabrata* infectados com *S. mansoni* (42% em média).

De acordo com o resultado do presente trabalho, que foi conduzido em condições experimentais, foi sugerido que bovinos tem, potencialmente, um papel na manutenção do ciclo vital do *S. mansoni*.

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