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SUSCEPTIBILITY OF *AUSTRALORBIS TENAGOPHILUS* TO INFECTION WITH *SCHISTOSOMA MANSONI*

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SUMMARY

A. tenagophilus from Pindamonhangaba (São Paulo) proved insusceptible to infection with *S. mansoni* from Belo Horizonte (Minas Gerais) when individually exposed to 50 miracidia. Exposure to 1000 miracidia resulted in infection of 1 out of 54 specimens. On the other hand, *A. tenagophilus* from São José dos Campos (São Paulo), which was also insusceptible to infection with the Belo Horizonte strain, was easily infected with a sympatric strain of *S. mansoni*. The latter, however, was uninfective to *A. glabratus* from Belo Horizonte, which is highly susceptible to the local strain of *S. mansoni*.

These results point to the existence, at least in certain areas, of a physiological adjustment between the snail and the local strain of the parasite.

INTRODUCTION

The role of *Australorbis tenagophilus* as an actual transmitter of *Schistosoma mansoni* was not recognized until recently. The first reference to this species in connection with schistosomiasis was made by LUTZ [17]. After describing his *Planorbis confusus* (a synonym of *A. tenagophilus* [25]), he stated that it was "unable to transmit the parasite". In 1923, LUTZ [19] proposed the substitute name *P. immunis* for the preoccupied *P. confusus*, thus emphasizing his belief on the insusceptibility of this species [18].

The first focus of schistosomiasis transmitted by *A. tenagophilus* was found by ARANTES [3] in Santos, state of São Paulo, but infected snails were not recorded there until about 20 years later (MOURA [23]), in spite of repeated search. The identity of the transmitting host was ascertained by PARAENSE & DESLANDES [27, 28], who called it *A. nigricans* following LUTZ's nomenclature [17], according to which *Planorbis nigricans* was considered a senior synonym of *P. tenagophilus* (see PARAENSE [25]).

Additional foci of schistosomiasis mansoni transmitted by *A. tenagophilus* were subsequently recorded by other workers in the states of São Paulo, Rio de Janeiro and Guanabara (see Table I).

A consistent characteristic of such foci, as recorded up to 1956, was a very low snail infection rate. On the other hand, susceptibility experiments showed that this species was highly resistant to infection with *S. mansoni* [7, 20, 34, 36]. In fact, so far only two authors have recorded positive results in infection experiments: COUTINHO [13], who infected about 2.5% of 282 specimens from Santos and São Paulo city, and COELHO [8], who infected 0.5% of 1019 specimens from the states of Guanabara, Rio de Janeiro, Minas Gerais, São Paulo and Rio Grande do Sul.

In recent years, however, comparatively high infection rates have been recorded, as shown in Table I. MARTINS [21] observed a

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TABLE I

Natural infection rates of *Australorbis tenagophilus* with *Schistosoma mansoni*

State	Locality	Examined snails	Infection rate (%)	Authors
Guanabara	Jacarepaguá	2,905	0.3	DEANE & cols. [14]
Guanabara	4 localities	22,152	0.01 to 2.56 *	ANDRADE & MARTINS [1]
Rio de Janeiro	Niterói	31,245	0.4 to 16.6	MARTINS [21]
São Paulo	Ana Dias	2,860	7.73	MOURA, in CODA & cols. [6]
São Paulo	Ana Dias	1,685	7.9 to 51.3	RAMOS & cols. [33]
São Paulo	Aparecida	2,242	1.42	PIZA & cols. [31]
São Paulo	Caçapava	4,671	0.32	PIZA & cols. [30]
São Paulo	Campinas	319	1.25	PIZA & RAMOS [29]
São Paulo	Guarujá	4,111	0.17	MOURA, in CODA & cols. [6]
São Paulo	Pindamonhangaba	498	0.2	CORRÊA & cols. [10]
São Paulo	Pindamonhangaba	21,137	0.19 to 48	PIZA & cols. [31]
São Paulo	Santos	1,172	0.85	MOURA [23]
São Paulo	Santos	8,654	0.07 to 3.35	COUTINHO [12]
São Paulo	Santos	46,445	0.35	MOURA [22]
São Paulo	Santos	500	0.8	RUIZ [35]
São Paulo	Santos	36,172	0.9	ANTUNES [2]
São Paulo	Santos	11,524	0.1	PARAENSE & DESLANDES [27]
São Paulo	Santos	2,099	0.81	CODA & cols. [6]
São Paulo	Santos	75,888	0.27	MOURA, in CODA & cols. [6]
São Paulo	Santos	5,397	0.53	PIZA & RAMOS [29]
São Paulo	São José dos Campos	4,007	0.03	PIZA & cols. [31]
São Paulo	São Vicente	32,829	0.09	MOURA, in CODA & cols. [6]
São Paulo	Taubaté	6,327	1.31	PIZA & cols. [32]
São Paulo	Tremembé	1,775	0.17	PIZA & cols. [32]
São Paulo	64 localities	142,840	0.06 to 4.62	CORRÊA & cols. [11]

* The snails from Manguinhos (2.6%) are *A. glabratus*.

whole infection rate of 0.5% in 31,245 snails from Niterói. Taking into account only the positive samples, the mean rate increased to 6.3%, and the sample rates showed a variation from 0.4 to 16.6%. PIZA & cols. [31] and RAMOS, PIZA & CAMARGO [33] recorded sample rates ranging from 0.19 to 48% at Pindamonhangaba, and from 7.9 to 51.3% at Ana Dias, respectively.

In an attempt to reproduce such high natural infection rates in the laboratory, the present work was undertaken.

MATERIAL AND METHODS

The snails used in the experiments were either reared in the laboratory or collected in the field. In the latter case they were kept under observation for 30 days before exposure to miracidia, so that only specimens free from natural infection were used.

Miracidia were collected, as described by CHAIA [5], either from feces of a chronic patient who contracted the infection on a single exposure to cercariae at Belo Hori-

zonte (BH strain of *S. mansoni*), or from feces of mice inoculated with the BH strain or with cercariae from 13 naturally infected *A. tenagophilus* from a breeding-place at São José dos Campos (SJC strain of *S. mansoni*).

Technical details concerning exposure of snails to miracidia and subsequent handling of the exposed specimens are given elsewhere (PARAENSE & CORRÊA [26]).

Four snail strains were used: *A. tenagophilus* from Fazenda Experimental of Instituto Agronômico, Pindamonhangaba (FE strain); *A. tenagophilus* from São José dos Campos (SJC strain); *A. glabratus* from Belo Horizonte (BH strain); and *A. glabratus* from Santa Luzia (SL strain).

EXPERIMENTS AND RESULTS

1. One hundred and twenty-five *A. tenagophilus* of the FE strain, 5 to 8 mm in diameter, collected in the field, were exposed each to 50 miracidia of the BH strain (human source). Nine died, 5, 7, 20, 24, 31, 34, 38, 41 and 42 days after exposure to miracidia, respectively, showing no sign of infection. The remaining 116 shed no cercariae up to the 60th day, when they proved negative by dissection.

Twenty laboratory-reared *A. glabratus* of the LS strain, 6 to 8 mm in diameter, each exposed to 5 miracidia of the BH strain (human source), were used as controls. One died on the 4th day without apparent infection; 2 died on the 7th and 10th days, respectively, with sporocysts in the head; 16 began to shed cercariae between the 28th and 35th days; and 1 remained negative for 60 days, showing no infection at dissection.

2. Fifty-four *A. tenagophilus* of the FE strain (24 collected in the field and 30 reared in the laboratory), 6 to 13 mm in diameter, were exposed each to about 1000 miracidia of the BH strain (human source). Seven specimens (2 from the field and 5 from the laboratory) died during the experiment (3, 19, 25, 30, 34, 36 and 46 days after exposure); the one that died on the 25th day was autolysed and the others were

TABLE II

Infection rates in two strains of *A. tenagophilus* and two of *A. glabratus* exposed to two Brazilian strains of *S. mansoni*.

Snail strain	Schistosome strain	No. miracidia	Exposed snails	Examined snails	No. Positive	% Positive
A. tenagophilus FE	BH	50	125	125	—	—
A. tenagophilus FE	BH	1,000	54	53	1	1.9
A. tenagophilus SJC	SJC	100	20	16	15	93.7
A. tenagophilus SJC	SJC	50	10	9	9	100.0
A. tenagophilus SJC	SJC	10	85	82	54	65.8
A. tenagophilus SJC	BH	20	100	95	—	—
A. tenagophilus SJC	BH	100	20	20	—	—
A. glabratus BH	SJC	5	45	45	—	—
A. glabratus BH	SJC	100	15	15	—	—
A. glabratus SL	BH	5	20	20	18	90.0
A. glabratus BH	BH	5	54	50	47	94.0

BH: from Belo Horizonte
FE: from Fazenda Experimental, Pindamonhangaba
SJC: from São José dos Campos
SL: from Santa Luzia

negative. Of the remaining 47 specimens, 46 did not shed cercariae and were negative at dissection on the 60th day, and the last one eliminated numerous cercariae 35 days after exposure and died three days later. The only positive snail had been reared in the laboratory and exposed to miracidia at a size of 11 mm.

As controls for this experiment, 54 laboratory-reared *A. glabratus* of the BH strain, 8 to 12 mm in diameter, were exposed each to 5 miracidia of the BH strain (mouse source). The results were as follows: 4 were found autolysed; 2 died on the 21st day, with immature sporocysts; 45 began to shed cercariae from the 28th to the 45th day; and 3 remained negative and were dissected on the 60th day.

3. Twenty laboratory-reared *A. tenagophilus* of the SJC strain, 9 to 13 mm in diameter, were exposed each to 100 miracidia of the SJC strain. Four specimens were found autolysed and the remaining 16 showed the following results: 5 died between the 28th and 47th days, with immature sporocysts; 10 shed the first cercariae between the 35th and 54th days; and 1 remained negative till the 42nd day, when it died and was dissected.

As controls, we used 45 *A. glabratus* of the BH strain (30 from the field and 15 from the laboratory), 8 to 13 mm in diameter, each of which was exposed to 5 miracidia of the SJC strain. Four specimens died, 10, 20, 32 and 65 days after exposure, respectively, showing no sign of infection. The remaining 41 were observed for 60 days. As they shed no cercariae, they were dissected and proved free from infection.

4. Ten laboratory-reared *A. tenagophilus* of the SJC strain, 8 mm in diameter, were exposed each to 50 miracidia of the sympatric strain. One was autolysed on the 5th day, and the remaining nine eliminated the first cercariae between the 30th and 40th days.

5. Eighty-five *A. tenagophilus* of the SJC strain (75 from the field and 10 from the laboratory), 6 to 10 mm in diameter, were exposed each to 10 miracidia of the sympatric strain. Three were found autolysed; 2 died negative on the 20th day; 2 died with immature sporocysts on the 16th and 25th days; 25 shed the first cercariae between the 30th and 35th days; and of the remaining 53, dissected on the 35th day, 20 showed immature sporocysts and 33 were negative.

6. One hundred *A. tenagophilus* of the SJC strain, 6-10 mm in diameter, collected in the field, were exposed each to 20 miracidia of the BH strain (mouse source). Five specimens were found autolysed and the remaining 95, examined by dissection between the 30th and 35th days after exposure, showed no sign of infection.

7. Twenty laboratory-reared *A. tenagophilus* of the SJC strain, 6 to 8 mm in diameter, were exposed each to 100 miracidia of the BH strain (human source). They remained negative for 35 days, showing no infection at dissection.

8. A group of 45 and another one of 15 laboratory-reared *A. glabratus* of the BH strain, 9 to 12 mm in diameter, were exposed to 5 and 100 miracidia of the SJC strain per snail, respectively. They were dissected 35 days after exposure, showing no sign of infection.

DISCUSSION

According to previously reported experimental evidence, there were no grounds for considering *A. tenagophilus* a suitable host for *S. mansoni*. In fact, not only were most attempts at infecting that snail unsuccessful [7, 20, 34, 36], but also very low infection rates were obtained in the few successful experiments [8, 13]. Such a resistance to infection depends on a cellular reaction to the invading miracidia, well studied by COELHO [7] and similar to that observed by NEWTON [24], BROOKS [4] and COELHO & BARBOSA [9] in other species. An explanation, however, was required for the high natural infection rates recorded by MARTINS [21], PIZA & cols. [31] and RAMOS, PIZA & CAMARGO [33] in some samples of snails.

The FE strain of *A. tenagophilus* is derived from the same population in which an

infection rate of 48% was observed by PIZA & cols. [31]. As shown in experiments 1 and 2, this strain was hardly infected with the BH strain of *S. mansoni* when exposed to 1000 miracidia per snail. On the other hand, exposure of each specimen of the SJC strain of *A. tenagophilus* to 100 and 50 miracidia of the sympatric strain of *S. mansoni* resulted in a surprisingly high infection rate. Accordingly, we reduced the number of miracidia to 10 per snail and, even so, a high proportion of specimens got infected. These results point to a physiological adjustment between the snail and the local strain of the parasite, which will explain the natural occurrence of the above-mentioned high infection rates. The likelihood of this hypothesis is reinforced by the failure of the SJC miracidia to infect the BH strain of *A. glabratus* and of the BH miracidia to infect the SJC strain of *A. tenagophilus*.

In a previous study (PARAENSE & CORRÊA [26]), differences in susceptibility of populations of *A. glabratus* to a single strain of *S. mansoni* were observed, indicating that the differences were related to the genotype of the snail populations. In other words, the observed variation in infection rate was due to differences in susceptibility of the snail populations. The present study shows that, in addition to such a variation in susceptibility of the snails, there is another variable governing the infection rate of snail populations, which is the infectiousness of the parasite strain.

The existence of physiological differences in parasites of different areas, postulated by FILES & CRAM [16] and FILES [15], is thus confirmed by the foregoing experiments.

Some delay in the development of the parasite was observed in many positive snails. This should be accounted for the present study having been carried out from March to September, which includes the colder period of the year. As previously observed in *A. glabratus* [26], some infected specimens of *A. tenagophilus* showed no parasites in the internal organs, the development of sporocysts taking place in the tissues of the head, foot and mantle collar.

Studies along the lines of the present one are needed for a better understanding of the host-parasite relationship, chiefly as regards the evolution of adaptation between their respective genotypes, and the mechanism by which new vectors and territories are conquered by the parasite.

RESUMO

Suscetibilidade do Australorbis tenagophilus *à infecção pelo* Schistosoma mansoni.

A. tenagophilus de Pindamonganhaba (São Paulo) mostrou-se insuscetível à infecção por *S. mansoni* de Belo Horizonte (Minas Gerais) quando exposto individualmente a 50 miracídios. A exposição a 1000 miracídios resultou na infecção de 1 entre 54 espécimes. Por outro lado, *A. tenagophilus* de São José dos Campos (São Paulo), que também se mostrou insuscetível à infecção com a cêpa de Belo Horizonte, infectou-se fàcilmente com uma cêpa simpátrica de *S. mansoni*. Esta última, entretanto, não foi infectante para *A. glabratus* de Belo Horizonte, o qual é altamente suscetível à cêpa local do *S. mansoni*.

Êsses resultados sugerem a existência, pelo menos em certas áreas, de uma adaptação fisiológica entre o molusco e a cêpa local do parasito.

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