Effects of Bayluscide WP 70® on the Survival and Water-leaving Behaviour of *Biomphalaria straminea,* Snail Host of Schistosomiasis in Northeast Brazil

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The toxic and behavioural effects of niclosamide (Bayluscide WP 70®) on Biomphalaria straminea *from a highly endemic area of schistosomiasis in northeastern Brazil were investigated through laboratory bioassays. The LD_{50} and LD_{90} were 0.114 mg/l and 0.212 mg/l, respectively. Water-leaving behaviour occurred among 14% to 30% of the snails in the presence of sublethal doses of niclosamide and among 16% of the controls. It was concluded that both the relatively low susceptibility to niclosamide and water-leaving behaviour of local* B. straminea *may be responsible for the recolonization of transmission foci after mollusciciding. It was suggested that recently improved measures of snail control, such as controlled-release formulations of niclosamide and plant molluscicides should be considered in areas where snail control is recommended.*

Key words: schistosomiasis - snail control - molluscicide - Bayluscide® - *Biomphalaria straminea*

The sugar-cane zone of Pernambuco, northeastern Brazil, has long held high prevalence levels of schistosomiasis despite sucessive control campaigns based mainly on chemotherapy (Pieri 1995). Thus, it can be recommended that auxiliary measures such as snail control should be used in conjunction with chemotherapy to reduce transmission to manageable levels. However, a recent study in the area showed that *Biomphalaria straminea*, the local snail host, is capable of recolonizing the breeding places shortly after niclosamide application, and that monthly mollusciciding is necessary to reduce the snail population satisfactorily (Pieri et al. 1995).

As long-term, repeated application of niclosamide may not be cost-effective in such endemic areas, a laboratory investigation was carried out aiming to identify the factors responsible for recolonization of transmission sites by *B. straminea* after molluscicide treatment. Two possible factors are considered in the present paper: (i) low susceptibility of the local snail host species to niclosamide and (ii) occurrence of water-leaving behaviour.

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MATERIALS AND METHODS

The assay snails were collected from transmission sites in Camorim, a rural village situated in the municipality of São Lourenço da Mata, sugarcane zone of the State of Pernambuco. After being individually screened for cercarial emergence as described by Favre et al. (1995), groups of 50 negative snails measuring 5-8 mm in shell diameter were transferred to 5 l glass aquaria containing 4 l of dechlorinated filtered tap-water, fresh lettuce and a substrate of earth, calcium carbonate and oyster powder (Rozemberg et al. 1992). These snails were kept up to 24 hr for acclimatization in environmental chambers (FANEM, São Paulo, Model 347-CDG) set at 25º ± 1ºC, 12 hr light, 12 hr dark. Two assays were carried out, one for susceptibility and other for water-leaving behaviour. In the assays, groups of 10 snails were randomly transferred to 1 l beakers containing 0.8 l of either dechlorinated filtered tap-water (control groups) or varying doses of Bayluscide WP 70® (experimental groups), and left in the environmental chambers for 24 hr of exposure. Based on the median lethal dose (LD_{50}) of 0.06 mg/l obtained by Souza et al. (1992) for a laboratory colony of *B. straminea*, the following doses of niclosamide were used: (i) 0.04, 0.06, 0.08, 0.10, 0.12, 0.14, 0.16 and 0.18 mg/l for the susceptibility assay, and (ii) 0.01, 0,02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.10 and 0.11 mg/l for the behaviour assay. In the former assay, a lid of nylon mesh was placed at the water/air interface to prevent the snails from leaving the water, whereas in the latter the lid was placed 4 cm above the water-air interface to allow water-leaving behaviour. In the susceptibility assay 20 snails were tested in each dose, the same number being used as controls. In the behaviour assay at least 30 snails were used in each dose. After the exposure period the snails found out of the water in each dose of the behaviour assay were identified and counted for calculating the escape index (Jurberg et al. 1995). All snails were then rinsed and placed in individual 250 ml glass vials containing 100 ml of dechlorinated filtered tapwater and fresh lettuce, and allowed to recover in the environmental chambers for 24 hr before being checked for mortality. The LD_{50} and LD_{90} values, as well as their 95% confidence intervals, were determined through probit analysis (Finney 1971) of the mortality data from the susceptibility assay. Two-by-two contingency tables (Wilkinson 1990) were used to test the significance of differences in water-leaving behaviour and in mortality between the control group and each experimental group from the behaviour assay.

RESULTS

Table I and Fig. show the results of the susceptibility assay obtained through probit analysis. The LD_{50} and LD_{90} values and their 95% confidence intervals were 0.114 (0.099-0.131) mg/l and 0.212 (0.162-0.369) mg/l, respectively.

Tables II and III show the results of the behaviour assay. Water-leaving behaviour occurred in all of the sublethal doses of niclosamide tested and also in the control group. The escape index varied from 12% at 0.01 mg/l to 30% at 0.09 mg/l. There was no significant differences in water-leaving behaviour between the control group and each

TABLE I

Susceptibility of *Biomphalaria straminea* from Camorim to niclosamide, evaluated through probit analysis. Twenty snails were assayed in each dose. No snail died in the control group

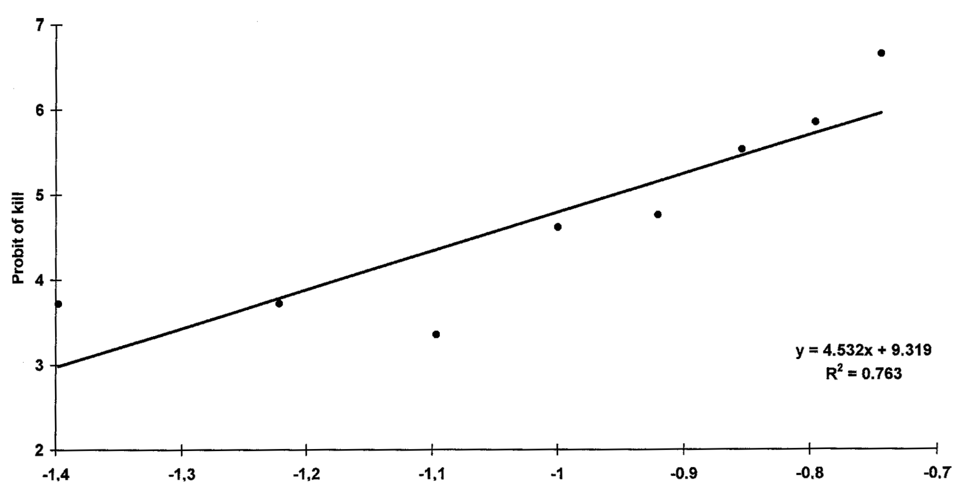
Dose (mg/l)	% mortality	Log dose	Probit
0.04	10	-1.398	3.717
0.06	10	-1.222	3.717
0.08	5	-1.097	3.353
0.10	35	-1.000	4.614
0.12	40	-0.921	4.746
0.14	70	-0.854	5.525
0.16	80	-0.796	5.843
0.18	95	-0.745	6.647

TABLE II

Occurrence of water-leaving behaviour in *Biomphalaria straminea* from Camorim

Dose (mg/l)	Snails assayed	Snails alive out of the water	Escape index (%)	χ^2
Control	50	8	16	—
0.01	50	7	14	1.00 NS
0.02	50	11	22	0.61 NS
0.03	50	6	12	0.77 NS
0.04	50	8	16	0.78 NS
0.05	50	8	16	0.78 NS
0.06	50	7	14	1.00 NS
0.07	30	8	26	0.38 NS
0.08	50	7	14	1.00 NS
0.09	30	9	30	0.23 NS
0.10	50	7	14	1.00 NS
0.11	30	6	20	0.87 NS

The escape index (Jurberg et al. 1995) was calculated as the percentage of snails assayed which were alive out of the water. χ^2: chi-squared values; NS: not significant at the 5% level (p>0.05).



Relation between probit of kill of *Biomphalaia straminea* from Camorim and dose of niclosamide, showing probit regression line, linear equation and regression coefficient (R^2).

TABLE III

Mortality among *Biomphalaria straminea* snails from Camorim in relation to water-leaving behaviour

Dose	Snails out of the water			Snails in the water			N	% of total
(mg/l)	Total	Dead	%	Total	Dead	%		dead
Control	8	0	0	42	0	0	50	0
0.01	7	0	0	43	1	2.3	50	2
0.02	12	1	8.3	38	0	0	50	2
0.03	6	0	0	44	0	0	50	0
0.04	8	0	0	42	2	4.8	50	4
0.05	8	0	0	42	3	7.1	50	6
0.06	7	0	0	43	6	14	50	12
0.07	8	0	0	22	0	0	30	0
0.08	7	0	0	43	9	21.8	50	18
0.09	9	0	0	21	1	4.8	30	3
0.10	7	0	0	43	18	41.8	50	36
0.11	8	2	25	22	13	59.1	30	50
Total	95	3	3.2	445	53	11.9	540	10.9

N: number of snails assayed in each dose of niclosamide.

of the experimental groups. Of the 490 snails exposed to any dose of niclosamide, 87 (17.8%) were found out of the water, of which only 3 (3.4%) were dead at the end of the recovery period. In contrast, of the 403 snails remaining in the water from the experimental groups, 53 (13.2%) were dead. Mortality was significantly less among the snails which left the water than among those which remained in the water during the exposure period (χ^2 = 5.73; P<0.05).

DISCUSSION

Susceptibility - The LD_{50} and LD_{90} values of niclosamide obtained for *B. straminea* from Camorim are more than twice those reported for both *B. straminea* and *B. glabrata* from the laboratory (Souza et al. 1992) , as well as for *B. glabrata* from the field (Souza et al. 1982, Souza & Mendes 1991), tested under similar conditions. This strongly suggest that *B. straminea* from the present study area is relatively less susceptible to niclosamide than snail hosts from southern endemic areas in Brazil. It is interesting to note that a laboratory colony of *B. straminea* also originated from Pernambuco was shown to be significantly less susceptible to the plant molluscicide *Euphorbia splendens* than a laboratory colony of *B. glabrata* from the same state (Baptista et al. 1994).

Differing susceptibility of snail hosts to molluscicides are probably due to differences in natural tolerance among geographic isolates rather than to selection of resistant strains as a result of continuous treatment of breeding places. Although some resistance to niclosamide can be induced under extreme conditions of genetic selection (Sullivan et al. 1984), there is no evidence of that occurring after years of continuous use in the field (Sturrock 1995). As low susceptibility to molluscicide may require higher doses of the product, it is advisable that snail populations be assayed, and lethal doses determined, before being subjected to control campaigns.

Behaviour - Sublethal doses of niclosamide had no repellency effect on *B. straminea*, as water-leaving behaviour occurred at a similar proportion in the control and the experimental groups. These results are in constrast with those obtained for *B. glabrata* by Jurberg et al. (1995), who found a repellency range between 0.01 mg/l and 0.03 mg/l of niclosamide, and no water-leaving behaviour in the control group. Since the present study followed the same procedures as that by the above authors, it can be concluded that *B. straminea* is more liable to leave the water than *B. glabrata* irrespective of the presence of sublethal doses of niclosamide.

Water-leaving behaviour apparently increased the survival of *B. straminea* exposed to sublethal doses of niclosamide. Thus, mortality was 3.7 times less among the snails found out of the water at the end of the exposure period than among those found in the water. This is not unexpected, as leaving the water prevents prolonged contact with molluscicide. Increased survival due to water-leaving behaviour was also reported for *B. glabrata* exposed to the plant molluscicide *Phytolacca dodecandra* (Jurberg et al. 1988).

B. straminea from northeastern Brazil is known to be highly resistant to desiccation, provided there are favourable micro-habitat conditions out of the water (Olivier & Barbosa 1956). Thus, it is likely that some snails may escape mollusciciding by leaving the water and survive long enough to recolonize the transmission foci after the toxic effects are over.

The epidemiological importance of water-leaving behaviour by snail hosts of schistosomiasis in relation to molluscicides has been well emphasized (Pieri & Thomas 1986, 1992, Jurberg et al. 1988, 1995, Dussart 1991, Green et al. 1992, Dannemann & Pieri 1993). However, the occurrence of other patterns of protective behaviour which could hamper the chemical control of snail hosts should also be considered. For instance, burrowing behaviour by *B. glabrata* has been implicated in the recolonization of breeding places after molluscicide application (Paraense et al. 1954, Souza & Mendes 1991). Although *B. straminea* is apparently unable of active burrowing, it can penetrate a few centimetres in the soft substrate in the course of normal locomotor activity (Olivier & Barbosa 1956) and eventually escape molluscicide action.

Snail control - Recolonization of breeding places soon after niclosamide application clearly indicates that snail control needs to be permanent. As the continuous application of niclosamide in the sugar-cane areas of Pernambuco may not be cost-effective (Pieri et al. 1995), decisions about undertaking snail control in such areas must be preceded by an evaluation of the local epidemiological situation. Thus, repeated mollusciciding can be recommended if the following conditions are met in the target area: (i) prevalence among school-aged children are over 50% despite chemotherapy; (ii) water contact remains intense irrespective of educational strategies; (iii) there is no perspective of improved sanitation and safe water supply in the short term. Unfortunately, several rural villages in the sugar-cane zone of Pernambuco currently meet the above conditions (Pieri 1995).

Probably due to some equivocal beliefs about molluscicides (Webbe & Jordan 1993), research on snail control has been regrettably underrated. Luckily, there are recent attempts to improve snail control through controlled-release formulations (Emara 1994) and plant molluscicides (Baptista et al. 1994). Both alternatives are worth considering in the highly endemic areas of schistosomiasis in northeastern Brazil, particularly those subjected to recolonization by snail hosts after the application of conventional molluscicides.

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