The Initial Epidemiological Studies in the Low Endemicity Schistosomiasis Area in Esteio, Rio Grande do Sul, the Southernmost Brazilian State, 1997 to 2000

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Nor Biomphalaria glabrata *neither* Schistosoma mansoni *were reported from Rio Grande do Sul, the southernmost Brazilian state before 1997. Their detection next to the Sinos River, Esteio, confirmed predictions of schistosomiasis expansion to the south. Parasitological examinations both in snails and fecal samples from the human population were performed from 1997 to 2000. The last 3 out of 5 surveys were performed after a preliminar serological screening procedure in a risk group identified at a population census. A total of 11 infected individuals were found infected and snails from 2 different sites were positive for* S. mansoni. *Samples from these 2 and other sites were identified as* B. glabrata. *Egg counts in feces were below 1 per gram in 6 out of 11 patients. Some socio-cultural perceptions of water contact activities next to the Sinos River may cause difficulties to control efforts, but they also may be partially acting against a very rapid increase in transmission intensity. The southernmost schistomiasis mansoni foci in Americas rise the alert for its ongoing expansion.*

Key words: *Schistosoma mansoni* - *Biomphalaria glabrata* - low endemic area - Rio Grande do Sul - Brazil

The establishment of a transmission focus of schistosomiasis in the southernmost Brazilian state, Rio Grande do Sul, was predicted many years before it became reality (Cunha-Neto 1972, Paraense & Corrêa 1987). *Biomphalaria glabrata* was detected for the first time in 1997 (Carvalho et al. 1998) after the diagnosis of what later was confirmed to be the first detected autochthonous human infection with *Schistosoma mansoni* in the region. A year later, the identification of infected snails in a pond next to the Sinos River was reported (Graeff-Teixeira et al. 1999). The initial epidemiological investigation in the current southern border of the geographic distribution of schistosomiasis in Americas is now reported.

MATERIALS AND METHODS

The municipality of Esteio (aproximately S29°50' to S29°54' and W51°12' to W51°10') is in the metropolitan area of Porto Alegre, the capital of Rio Grande do Sul (RGS) (Fig. 1). The Sinos River is one of 5 rivers forming the Guaiba Delta and Lake, that ultimately drains to the Patos Lake. Esteio has aproximately 80,000 inhabitants and 9 km2 (27%) of its territory has a rural environment, with 2 large irrigated rice plantations next to the river (Fig. 2).

Five coproparasitological surveys were performed as follows: in September-October of 1997, the first survey was conducted at "Vila Pedreira", a slum limited by a railway on the east, a free-way road on the west (BR-116) and an industrial plant to the north. This was the place where the index case was living. A year later, the diagnosis of another human infection and the very suspicious environmental situation lead to a survey in other 2 slums – the "Beco da Rua Carmen Miranda" and the "Valo Três Portos – Seu Remy". The next 3 surveys (December 1999, May and October 2000) were performed in a selected group of individuals, initially defined by the positive serological examination with IgM imunofluorescence reaction to *S. mansoni* gut antigens, as described elsewhere (manu-

Fig. 1: schematic map of Brazil showing Rio Grande do Sul (RGS) and the approximate location of Esteio. "N" indicates North.

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script in preparation), in September-October 1999. Several individuals were added to the group under survaillance whenever risk, defined as "any contact with the waters next to the Sinos River" was identified.

From every fecal sample, at least 2 slides were prepared by the method of Kato-Katz (KK). In the first survey, in October 1997, besides the KK slides, 1 slide was prepared with a commercial concentration kit (Total-Test). In the last 3 surveys, besides KK, aproximately 2 g was prepared according to the method of Ritchie (1948). From the sediment resulting from Ritchie's method, 2 slides were examined (September 1998) and all the sediment was ex-

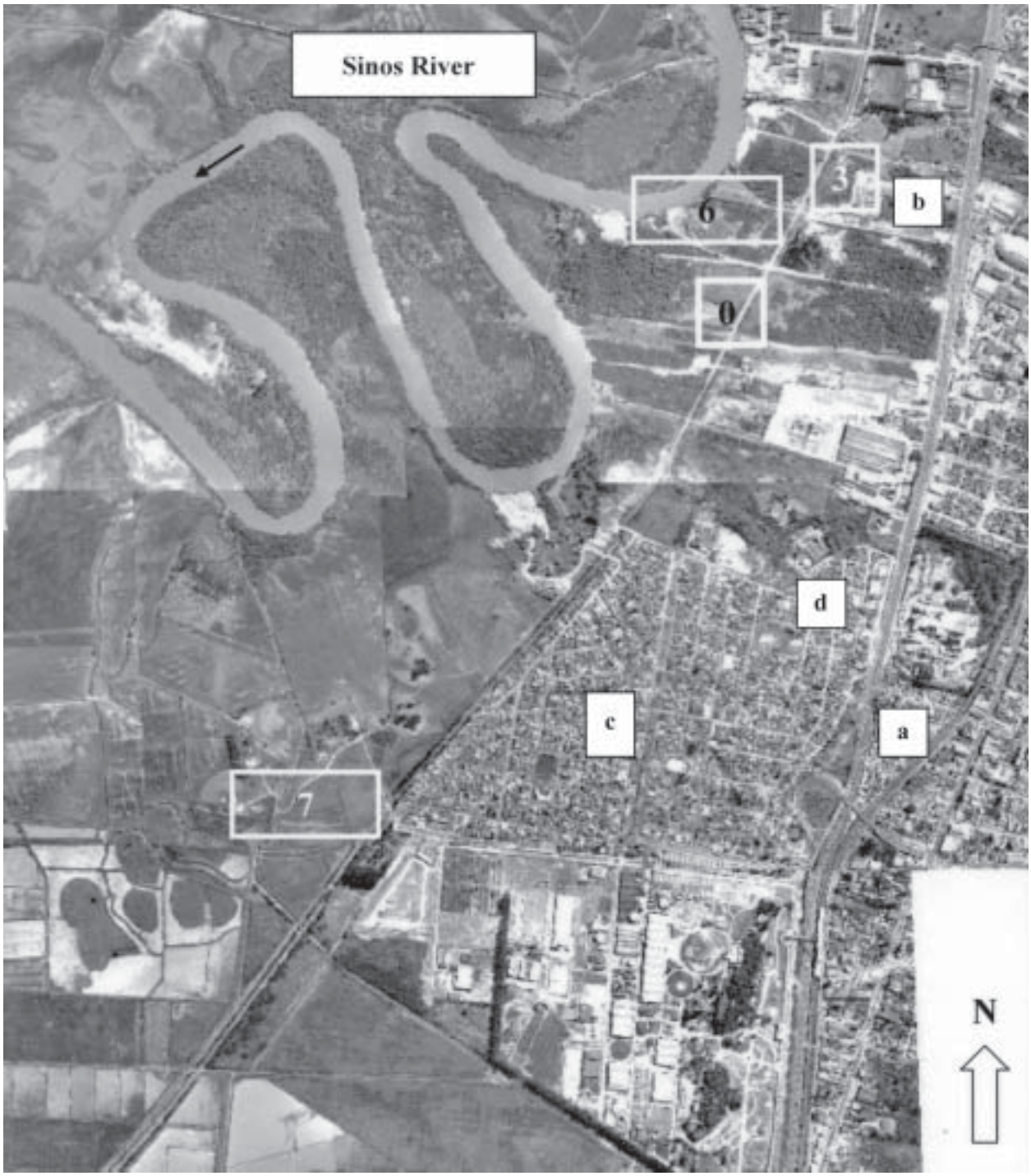

Fig. 2: partial aerial view of the municipality of Esteio, Rio Grande do Sul, Brazil, showing the confirmed transmission foci of schistosomiasis (identified with the number of individuals reporting contact with waters): 6: Banhado do Azeite; 3: "Valo Três Portos", 0: "Casa dos Trilhos"; a fourth unconfirmed transmission focus is 7: "Fazenda Kroeff". Three main urban aglomerates are in the vicinity of the foci: a: Vila Pedreira; b: Valo Três Portos; c: Novo Esteio. A small slum called "Beco da Carmen Miranda" is also indicated; d: the direction of the flow in the Sinos River is indicated by an arrow. Photo provided by Feema, Esteio.

amined in the surveys held in December, 1999 and May and October, 2000.

Data were recovered from the public health services epidemiological investigation registers and from several successive interviews with the infected individuals.

Snails were individually pressed between 2 glass plates and bifurcated cercarias were searched and measured under a stereomicroscope. Initially 9 mice were infected by intra-peritonial injection of 80 cercaria and after 7 weeks, identification of adult *S. mansoni* was possible, as already previously reported (Graeff-Teixeira et al, 1999). Samples of the snails were sent for identification at the Laboratório de Helmintíases Intestinais, Centro de Pesquisas René Rachou-Fiocruz, Belo Horizonte.

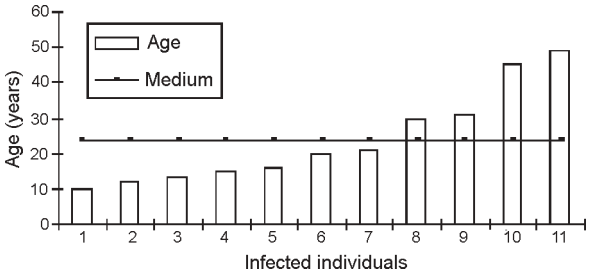

Fig. 3: age distribution of the 11 infected individuals (medium = 23 years) in the schistosomiasis transmission foci in Esteio, Rio Grande do Sul, Southern Brazil.

RESULTS

A total of 11 male individuals were found infected in the 5 surveys (Table I) and 7 were less than the medium age of 23 years-old (Fig. 3). Their occupation and activity in the transmission foci is shown in Table II and Fig. 2 shows the number of individuals in each of their usual place of water contact. Except for 2 individuals, all spent their leisure time next to the river.

Clinical presentation is shown in Table III. Most of the individuals were assymptomatic at the time of diagnosis and in the follow-up up to 2003. Two individuals (VCS and JBC) were found infected 33 and 24 months after initial treatment and were re-retreated. In both cases there is a reasonably high possibility of re-infection, althought treatment failure can not be ruled out. Two of the symptomatic individuals were diagnosed at clinical laboratories after examination at public health service (JCLS) or at a private medical service (JBC). The epidemiological investigation of JCLS led to the identification of 2 relatives sharing the risky behavior of the index case, with diagnosis confirmed by parasitological examination (CLS and JLM).

TABLE I

Low endemicity schistosomiasis area in Esteio, Rio Grande do Sul, Southern Brazil, 1997 to 2000. Results from parasitological surveys (stool examinations)

Date	Total target population	Total examined	Positive for other enteroparasites	Positive for *Schistosoma mansoni* [a] (%)
October 1997	1483	783	639	1 (0.0013)
September 1998	306	279	41	1 (0.0036)
December 1999	23 [b]	18	3	0
May 2000	41 [b]	16	8	2 (0.13)
October 2000	54 [b]	27	10	2 (0.075)

a: other 5 individuals found infected after clinical or epidemiological investigations are not included in this table; *b*: besides the initial 23 individuals screened by IgM-IF, other individuals were successively included in the targeted population as a closer and repetitive contact disclosed a risky behavior.

TABLE II

Low endemicity schistosomiasis area in Esteio, Rio Grande do Sul, Southern Brazil, 1997 to 2000. Occupations, activities, and locations where infected individuals have had their main water contact

Initials	Age	Occupation	Water contact	Locations
OJSP	31	Not defined	Swimming + transit (leisure)	Banhado do Azeite, Fazenda Kroeff
VCS	45	Not defined	Transit (leisure)	Banhado do Azeite, Fazenda Kroeff
JCLS	13	Student	Swimming (leisure)	Banhado do Azeite, Fazenda Kroeff
CLS	15	Student	Swimming (leisure)	Banhado do Azeite, Fazenda Kroeff
JLM	21	Not defined	Swimming (leisure)	Banhado do Azeite, Fazenda Kroeff
AAG	16	Student	Transit (leisure + work [a])	Valo Três Portos
MJF	30	Shopkeeper	Transit (leisure)	Banhado do Azeite
MMF	20	Electrician	Swimming (leisure)	Fazenda Kroeff
JBC	12	Student	Swimming + transit (leisure)	Valo Três Portos
WLJr	10	Student	Transit (leisure)	Valo Três Portos
JHM	49	Peasant	Swimming + transit (leisure + work [b])	Fazenda Kroeff

a: conducting horses or cattle to feed on grassy margins of Banhado do Azeite; *b*: employee at a rice irrigated plantation (Fazenda Kroeff)

TABLE III

Schistosomiasis in Esteio, Rio Grande do Sul, 1997 to 2000. Clinical manifestations at the time of diagnosis

Individuals	Date diagnosis	Eggs/g feces	Clinical manifestations
OJSP	Jan 97	nd [a]	Abdominal pain, fever, jaundice, diarrhea
VCS	Oct 97 and May 00 [c]	269 / nd [a]	Ichting in submersed skin / asymptomatic
JCLS	Oct 98	< 1 [b]	Facial and palpebral edema
CLS	Oct 98	< 1	Diarrhea with colicky pain, sonolence, itching, and puntiform dermitis in arms and legs
JLM	Oct 98	< 1	Asymptomatic
AAG	Oct 98	< 1	Asymptomatic
MJF	May 00	40	Diarrhea, abdominal pain
MMF	May 00	16	Asymptomatic
JBC	Jul 00 and Jul 03 [c]	nd [a]/ 24	Fever, abdominal pain, dizziness, itching in the elbows, muscular aches, anorexia, loss of weight
WLJr	Jul 00	< 1	Asymptomatic
JHM	Nov 00	< 1	Asymptomatic

a: counting not done; *b*: less than one egg per gram of feces; *c*: reinfection or treatment failure

Samples of snails from BA and VTP were identified as *B. glabrata* and cercaria of *S. mansoni* were found in snails from 3 areas: BA, Casa dos Trilhos (2 out of 11 snails) and VTP (10 out of 19 snails) (Table IV and Fig. 2). "Casa dos Trilhos" means a house next to the railway. There was a small and very shallow and seasonal pond, next to the house, where the snails were once collected and only examined for cercaria. Inhabitants of the house were transient workers at the farm and their were lost to contact soon afterwards.

TABLE IV

Low endemicity schistosomiasis area in Esteio, Rio Grande do Sul, Southern Brazil, 1997 to 2000. Results from parasitological examination of snails

Local	Number of snails examined	Number of infected snails	Prevalence (%)
Banhado do Azeite	3806	22	0.57
Fazenda Kroeff	24	0	0
Valo Três Portos	22	0	0
Casa dos Trilhos	11	2	18.20
Brasilit	6	0	0
Colméia	4	0	0
Lagoa Meio	4	0	0
Estrada de Ferro	4	0	0
Lansul	1	0	0

DISCUSSION

The expansion of schistosomiasis to southern parts of South America has been a concern for many years, exemplified by the long-term vigilance along the Rivers Uruguay and Paraná by the argentinian group from the Universidad Nacional del Nordeste at Corrientes (Borda & Rea 1997). Migration and movement of people from endemic areas produces the oppoturnity for establishment of new foci, especially at big projects like the construction of roads and dams (Southgate et al. 2001). This is not exactly the case in Esteio, but the focus is next to a major road (BR-116) linking Southern to Northeastern Brazil, where the endemic schistomiasis area is situated. On the eastern margin of BR-116 there are several large industries and to go fishing and resting at the Sinos River has been a popular destination for some local workers as well as for the truck drivers, especially at lunch interval. The area next to Banhado do Azeite has already been the site of a "hunting and fishing club" and although today it does not have any infrastructure like toilets, in sunny weekends the place may be crowded with visitors spending the day and the usual sites for defecation are the bushes (see Fig. 4) next to the pond where *B. glabrata* was found in huge numbers (Graeff-Teixeira et al. 1999). The distribution of infected snails along the margins next to the bushes and the River supports the ranking of BA as a very important transmission site (Fig. 4).

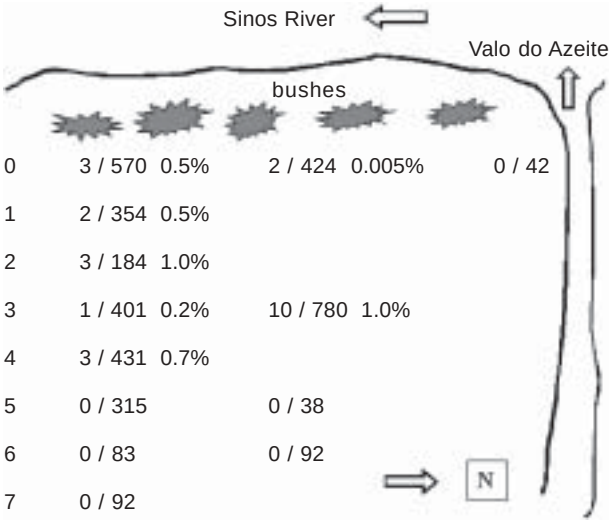

Fig. 4: schematic drawing of the Banhado do Azeite, showing the Sinos River (above) and its northern border, "Valo do Azeite", a stream delivering industrial partially treated waste water but still rich in organic debris, producing a very productive fishing point in the River. The southern border (to the left) is a small road and the eastern border (not shown) is a railway. The numbers represent the number of infected snails and total number of snails collected, with prevalence in parentesis (%).

Gastropode snails, with the exception of *B. glabrata*, have been identified in the southernmost Brazilian state, Rio Grande do Sul (Cunha-Neto 1972. Teles et al. 1981, Paraense & Correa 1991). Only in 1997 the main intermediate host for *S. mansoni* was detected in the municipality of Esteio, after the diagnosis and indications from the first autochthonous infected individual. In early 1970s, the infection of a child from a family coming from the endemic area in Southeastern Brazil to work in a dam construtions site in the municipality of São Valentim, northern RGS, was not considered autochthonous by the health authorities (Louzada 1973). Cunha-Neto (1972) reported only the presence of apparently non-infected *B. peregrina* in Vila Alegre, where the child was living with her family.

No *B.glabrata* has been found on the left margin of Sinos River up to aproximately 500 m upstream from Banhado do Azeite (BA), while they were found aproximately 300 m downstream at a site where water was pumped out from the river into irrigation channels for a rice plantation. The role of BA as a recreational site, its huge number of snails and the distribution of snails up and downstream the Sinos River are indications of the importance of BA not only as a transmission focus, but also a source of dissemination of snails along lower Sinos River and Guaiba Lake.

In March 1998, the second autochthonous infected individual (VCS) indicated what he described as "the best place to collect snails...". He was proud to give this information not mentioned by OJSC, the index case diagnosed in 1997. For OJSC, like for many other infected individuals, the diagnosis of "the disease of the snails" was a shameful situation and he did denied informations when he was initially interviewed . This is one aspect of the shame associated with the activities of fishing and walking around the uninhabited areas next to Sinos River, sometimes the path or hiding site for fugitives and meeting point for drug dealers. This conception adds serious obstacles to adherence to profilatic recomendations and cooperative participation in serological or parasitological surveys in the area.

A relatively large population (aproximately 6000 inhabitants) was living immediately next to the transmission foci (Fig. 2) but only a number of them could be considered of risk for schistosomiasis, having leisure or rarely professional activities in water bodies in the area. This was clearly shown in the population census in April-May 1999. There are some people that used to come from several municipalities in the metropolitan area for fishing at BA. Therefore, the definition of a population at risk should not be restricted to the geographic area around the transmission foci and a fairly more important role has the active search for infected individuals. Although Esteio has most of its 9 km^2 of rural area with rice plantation farms, the most important transmission focus was apparently not located in those areas, but in BA, as described above. The importance of the rice plantation area (Fazenda Kroeff) is still to be fully investigated.

Low prevalence and low egg burden in feces is expected to be low in areas either under advanced control of transmission (Alarcon-de-Noya et al. 1997) or with recent introduction (Mouchet et al. 1988, Carvalho et al. 1989, Vargas et al. 1990) as it is the case in Esteio. This focus in Southern Brazil is distinguished by an extremely low prevalence (less than 1%) at a range of egg counts in feces where current diagnostic methods have not been extensively tested for sensitivity. In ultra-low endemicity areas, like Esteio, molecular diagnostic methods, like serology (Kanamura et al. 2002) or nucleic acid detection (Pontes et al. 2002), may also face special difficulties not yet fully accessed (for a review on serology, see Doenhoff et al. 2004).

The diagnostic difficulties in a low endemicity area led to the proposal and implementation of successive screening procedures: first, the identification of a population at risk through a census; second, serology preceding intensive and repeated fecal examinations in the selected group. An investigation was undertaken in 2002-2003, in Esteio, testing SEA-ELISA and it will the subject of another report, as well as extensive evaluation of other antibody-, antigen-or nucleic acids-detection systems are planned.

After introduction in Esteio, schistosomiasis transmission appears to remain stable and the event is clearly different from dramatic situations like the one reported by Talla et al. (1990), in Richard-Toll, Senegal, where a rapidly increasing prevalence, from 0.8% to 71% in 2 years, followed the detection of the first cases. The temperate climate with well defined winters, the relatively high education level of the population and the shameful perception of activities next to the River may be some of the factors preventing a high intensity of transmission in Esteio. Water contact activities are not frequent in the local population, being an exception for some groups (swimming for youngs, fishing for adults and working for peasants at the rice plantation) and restricted to summer time. These socio-ecological characteristics may also explain the fairly equal age distribution of infection among children and adults, to be confirmed with more extensive surveys.

One of the most frequent activities of infected individuals was swimming, but not necessarily as a purely recreational activity. Some individuals, like OJSP, VCS, and JHM, immersed their whole body in channels in order to collect snails to use them as a bait. This does not mean necessarily collecting *Biomphalaria* sp., since *Pomacea* sp. is used as bait by recreational fishermen in Southern Brazil. There is also an apparent association of a lower age with swimming acitivity. Older individuals usually only transiently crossed shallow channels and swamps on their way to the margins of the Sinos River.

The low magnitude of the schistosomiasis in Esteio should not prevent the continuity of measures for eradication of transmission already in place. There is always a potential risk that attention from some health policy makers is driven away from low endemicity areas, what may contribute to the expansion of schistomiasis.

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REFERENCES

Alarcón-de-Noya B, Cesari IM, Losada S, Colmenares C, Balzán C, Hoebeke J, Noya O 1997. Evaluation of alkaline phosphatase immunoassay and comparison with other diagnostic methods in areas of low transmission of schistosomiasis. *Acta Trop 66*: 69-78.

Borda CE, Rea MJF 1997. Susceptibilidad de *Biomphalaria tenagophila* de las cuencas de los ríos Paraná y Uruguay a *Schistosoma mansoni*. *Rev Panam Salud Publica 1*: 167-173.

Carvalho OS, Nunes IM, Caldeira RL 1998. First report of *Biomphalaria glabrata* in the state of Rio Grande do Sul, Brazil. *Mem Inst Oswaldo Cruz 93*: 39-40.

Cunha-Neto AG 1972. *Biomphalaria straminea* em Porto Alegre, Rio Grande do Sul, Brasil. *Atas Soc Biol Rio de Janeiro 15*: 151.

Doenhoff MJ, Chiodini PL, Hamilton JV 2004. Specific and sensitive diagnosis of schistosome infection: can it be done with antibodies? *Trends Parasitol 20*: 35-39.

Graeff-Teixeira C, Anjos CB, Oliveira VC, Velloso CEP, Fonseca MBS, Valar C, Moraes C, Garrido C, Amaral RS 1999. Identification of a transmission focus of *Schistosoma mansoni* in the southernmost Brazilian state, Rio Grande do Sul. *Mem Inst Oswaldo Cruz 94*: 9-10.

Kanamura HY, Silva RM, Chiodelli SG, Glasser CM, Dias LCS 2002. IgM-immunofluorescence test as a diagnostic tool for epidemiologic studies of schistosomiasis in low endemic areas. *Mem Inst Oswaldo Cruz 97*: 485-489.

Louzada JLZ 1973. Esquistossomose mansônica (primeiro caso autóctone no Rio Grande do Sul). *Rev Bras Med 30*: 533-535.

Mouchet F, Develoux M, Balla-Magassa M, Sellin B 1988. Etude epidemiologique dufoyer a *Schistosoma mansoni* de Bana (Niger). *Med Trop 48*: 209-213.

Paraense WL, Corrêa LR 1987. Probable extension of schistosomiasis mansoni to southernmost Brazil. *Mem Inst Oswaldo Cruz 82*: 577.

Pontes LA, Dias-Neto E, Rabello A 2002. Detection by polymerase chain reaction of *Schistosoma mansoni* DNA in human serum and feces. *Am J Trop Med Hyg 66*: 157-162.

Ritchie L 1948. An ether sedimentation technique for routine stool examinations. *Bull US Army Med Depart 8*: 326.

Southgate VR, Tchuem-Tchuenté L-A, Sène M, De Clercq D, Théron A, Jourdane J, Webster BL, Rollinson D, Gryseels B, Vercruysse J 2001. Studies on the biology of Schistosomiasis with emphasis on the Senegal River Basin. *Mem Inst Oswaldo Cruz 96*: 75-78.

Talla I, Kongs A, Verle P, Belot J, Sarr S, Coll AM 1990. Outbreak of intestinal schistosomiasis in the Senegal River Basin. *Ann Soc belge Mèd Trop 70*: 173-180.

Teles HMS, Pereira PAC, Richinitti LMZ 1981. Distribuição de *Bimphalaria* (Gastropoda, Planorbidae) nos Estados do Rio Grande do Sul e Santa Catarina, Brasil. *Rev Saúde Púb 25*: 350-352.

Vargas M, Malek EA, Perez JG 1990. Schistosomiasis mansoni in the Dominican Republic; prevalence and intensity in various urban and rural communities, 1982-1987. *Trop Med Parasitol 41*: 415-418.