

Relationship between *Biomphalaria* snail occurrence, schistosomiasis incidence, and treated sewage coverage in municipalities of Espírito Santo, Brazil

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Abstract

Schistosomiasis remains an important public health problem in Espírito Santo, Brazil, particularly in municipalities characterized by greater socioeconomic vulnerability, frequent contact with freshwater bodies, and inadequate sanitation coverage. This study aimed to investigate the associations between the occurrence of snails of the genus *Biomphalaria*, the historical occurrence of *Schistosoma mansoni* at the municipal level, sanitation indicators, and schistosomiasis positivity across municipalities in the state of Espírito Santo (ES). This longitudinal ecological study used secondary data from 2011 to 2019 and included 2,766 investigated cases in ES. Descriptive analyses, analysis of variance, Pearson's chi-square test, and multiple linear regression were performed. An overall decreasing trend in the number of cases was observed throughout the study period. *Biomphalaria* species richness was not significantly associated with the schistosomiasis test positivity rate. In contrast, the presence of *B. glabrata* and a history of *S. mansoni* occurrence in the municipality were significantly associated with higher schistosomiasis positivity rates. Conversely, the occurrence of *B. straminea* and *B. tenagophila* was negatively associated with schistosomiasis positivity, whereas sewage treatment coverage, total population size, and the proportion of the population living in urban areas were not associated with the positivity rate after adjustment for the effects of the other variables. These findings indicate that the presence of *Biomphalaria* alone does not explain the municipal distribution of schistosomiasis, while highlighting the epidemiological relevance of *B. glabrata* compared with other congeneric species recorded in Espírito Santo, as well

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as the importance of previous *S. mansoni* occurrence. These results reinforce the need to integrate epidemiological surveillance, malacological surveillance, expansion of sanitation services, and molecular methods for *Biomphalaria* species identification to improve the identification of areas at risk and strengthen schistosomiasis control strategies in Espírito Santo.

KEYWORDS: Schistosomiasis; Epidemiology; Malacology; *Biomphalaria*; *Schistosoma mansoni*.

Introduction

Schistosomiasis is among the neglected tropical diseases with the greatest impact on global public health, with its distribution concentrated in regions where poverty and inadequate sanitation policies constrain both disease prevention and control (GRIMES *et al.*, 2014). In Brazil, areas with the highest endemic burden overlap with regions characterized by limited environmental surveillance and poor basic sanitation coverage, indicating that the persistence of transmission is largely determined by socioenvironmental factors (GAZZINELLI *et al.*, 2006; SANTOS *et al.*, 2023). This pattern shows that municipalities with higher schistosomiasis incidence tend to exhibit the greatest levels of social and environmental vulnerability, making the disease a sensitive indicator of territorial inequality and shortcomings in public sanitation infrastructure policies (GAZZINELLI *et al.*, 2006; OLIVEIRA *et al.*, 2013).

Transmission of *Schistosoma mansoni*, the etiological agent of schistosomiasis, occurs through a biological cycle closely associated with inadequate sanitation conditions (GRIMES *et al.*, 2015). The cycle begins with the excretion of parasite eggs in the feces of infected individuals, which may reach freshwater bodies as a consequence of absent or inadequate sewage collection and treatment. In these environments, snails of the genus *Biomphalaria*, which serve as intermediate hosts, become infected

and subsequently release cercariae, the free-swimming infective larvae that actively penetrate human skin during contact with contaminated water (GRIMES *et al.*, 2014; GRIMES *et al.*, 2015). Inadequate access to treated water further exacerbates this scenario by increasing reliance on natural water bodies for daily activities, thereby increasing the frequency of exposure to infective stages (GRIMES *et al.*, 2015). Accordingly, basic sanitation indicators represent important environmental determinants of the persistence of schistosomiasis transmission at the municipal level.

In Brazil, schistosomiasis is heterogeneously distributed, with a higher prevalence in states such as Minas Gerais, São Paulo, and Espírito Santo, where climatic conditions favor the proliferation of *Biomphalaria* snails and the maintenance of disease transmission (CARNEIRO *et al.*, 2025). However, disease persistence does not depend solely on environmental conditions. Evidence indicates that improvements in sanitation infrastructure and hygiene practices are associated with reductions in infection and disease rates (CRUZ *et al.*, 2025). Conversely, social inequalities, inadequate urban infrastructure, the accumulation of solid waste, and open sewage contribute to the persistence of infectious and parasitic diseases, particularly in rural communities and informal settlements, where access to public sanitation services is often limited (NAHAS *et al.*, 2019; SOUZA *et al.*, 2020).

Espírito Santo is among the Brazilian states with the highest schistosomiasis burden. In an epidemiological study conducted in southeastern Brazil between 2013 and 2023, the state had the second-highest cumulative prevalence of the disease (OLIVEIRA *et al.*, 2025). According to the Epidemiological Bulletin issued by the Espírito Santo State Health Department, this pattern is related to the interaction of climatic and geographic factors, such as altitude and precipitation, together with proximity to endemic states and deficiencies in basic sanitation (ESPÍRITO SANTO, 2023). To

investigate the factors underlying the high prevalence of schistosomiasis in Afonso Cláudio, an endemic municipality in Espírito Santo, a previous study assessed variables including age, income, educational level, place of residence, and the level of knowledge about the disease among affected individuals. The findings revealed a widespread lack of awareness of community-based educational initiatives, as well as inadequate sanitation infrastructure, particularly in rural areas, where the predominance of rudimentary pit latrines was shown to be ineffective in preventing contamination of water resources and the consequent maintenance of parasite transmission (FREITAS *et al.*, 2022). These findings reinforce the endemic nature of schistosomiasis in Espírito Santo, where transmission dynamics result from interactions among environmental, social, and structural factors that vary among municipalities.

Previous studies have examined schistosomiasis diagnosis and occurrence in relation to socioeconomic and health-related variables, including the Human Development Index (HDI), as well as the spatial and environmental distribution of reported cases, particularly in Minas Gerais and northeastern Brazil (SOUZA *et al.*, 2022). However, ecological studies investigating the association between basic sanitation indicators and schistosomiasis detection in Espírito Santo remain scarce, particularly those incorporating ecological characteristics of both the parasite and its intermediate host (SANTOS *et al.*, 2023). Therefore, this study aimed to investigate the association between municipal sanitation indicators, ecological characteristics of the biological groups involved in the natural transmission cycle of schistosomiasis, and disease detection in Espírito Santo from 2011 to 2019 through an ecological time-series study based on publicly available data. We hypothesized that the distribution of the etiological agent and its *Biomphalaria* host species is significantly associated with schistosomiasis

incidence across municipalities in Espírito Santo, even after controlling for population size and municipal sewage treatment coverage.

Methods

This longitudinal ecological study was conducted at the municipal level using secondary data on *Schistosoma mansoni*, the occurrence of snails of the genus *Biomphalaria*, and municipal-level indicators related to schistosomiasis in the state of Espírito Santo, Brazil, from 2011 to 2019, comprising a total of 2,766 investigated cases. The study period ended in 2019 because this preceded both the statewide change in disease notification procedures implemented in 2020 and the systemic effects of the COVID-19 pandemic on surveillance and diagnostic testing. The municipality was adopted as the unit of analysis for all variables included in the study to avoid problems arising from multiple associations within the dataset. Interpretation of the results considered both statistical significance and the biological plausibility of schistosomiasis transmission, given that the presence of *Biomphalaria* snails is a necessary condition for maintaining the *S. mansoni* life cycle in the environment. Likewise, the presence of the etiological agent within a municipality is required for local disease transmission.

The epidemiological variables analyzed were the number of individuals examined, the number of schistosomiasis-positive cases, the presence or absence of a historical record of *S. mansoni* in each municipality (binary variable), the presence or absence of historical records of *Biomphalaria* snails (binary variable), and the schistosomiasis positivity rate. Positivity was calculated as the number of positive examinations divided by the total number of individuals examined and multiplied by 100, and was treated as a continuous quantitative variable. The occurrence of *S. mansoni* was defined as the presence of positive cases at any time in a given

municipality, based on the map of schistosomiasis distribution in Espírito Santo (RAMOS, 2018).

Malacological variables included the presence or absence of species of the genus *Biomphalaria* in the municipalities evaluated. Municipal occurrence of each *Biomphalaria* species was determined based on the specialized reference “Moluscos Hospedeiros Intermediários de *Schistosoma mansoni* do Brasil”, by Omar dos Santos Carvalho, published in 2020. This classification allowed municipalities to be compared according to their malacological profiles, considering the three species reported for Espírito Santo: *B. glabrata*, *B. straminea*, and *B. tenagophila*.

Descriptive analyses were initially performed to characterize the epidemiological and malacological profiles of the municipalities included in the study. Quantitative variables were summarized using measures of central tendency and dispersion, including the mean, standard deviation, median, minimum, and maximum values, according to the distribution of the data. Categorical variables were expressed as absolute and relative frequencies. The total number of individuals examined, number of positive cases, mean positivity rate per municipality, occurrence of schistosomiasis, and presence or absence of *Biomphalaria* species were described. We also characterized the temporal trend in the total number of schistosomiasis cases, disease incidence according to self-reported race/color, and the types of water bodies most frequently reported as potential sites of infection.

Because the original dataset could contain multiple records for the same municipality, data were aggregated at the municipal level before inferential analyses. This procedure was adopted to avoid artificial replication of population data or municipal characteristics across multiple records, which could violate the assumption of independence required for inferential statistical tests. Accordingly, municipality-level measures were

calculated for each municipality, including the mean number of cases and mean positivity rate. After aggregation, each municipality represented an independent analytical unit, thereby reducing the risk of overestimating statistical significance due to duplicated observations.

Analysis of variance (ANOVA) was used to compare mean schistosomiasis positivity rates among groups defined by the presence of one or more of the three *Biomphalaria* species reported in Espírito Santo. In this analysis, schistosomiasis positivity rate was treated as the quantitative dependent (response) variable, whereas the presence of one, two, or three *Biomphalaria* species was treated as the categorical independent (explanatory) variable. ANOVA followed by Tukey's post hoc test was used to assess whether mean schistosomiasis positivity differed significantly among municipalities grouped according to their malacological profiles. Results were expressed as mean differences, standard errors, and p-values derived from Tukey's test. Model assumptions were evaluated, including independence of observations, approximate normality of residuals, and homogeneity of variances among groups.

To assess the association between schistosomiasis occurrence and the presence of *B. glabrata*, *B. straminea*, and *B. tenagophila*, three contingency tables were constructed using occurrence data for each species and the historical occurrence of schistosomiasis. Municipalities in Espírito Santo were classified as positive if they had a historical record of the disease and as negative if no such record was available, and were subsequently compared according to the presence or absence of each *Biomphalaria* species. Associations between categorical variables were assessed using Pearson's chi-square test to determine whether schistosomiasis occurrence differed significantly according to the presence of each potential intermediate host species.

Additionally, multiple linear regression analysis was performed to evaluate the relationship between the municipal schistosomiasis positivity rate and social, malacological, and epidemiological variables of interest. Explanatory variables included total municipal population, percentage of the population covered by sewage services, percentage of the population living in urban areas, historical occurrence of the etiological agent *S. mansoni* (binary variable classified as positive when the municipality had a documented history of confirmed infection), and historical occurrence of *B. glabrata*, *B. straminea*, and *B. tenagophila* (binary variables classified as positive when the species had been documented for the municipality in the 2020 reference by Omar dos Santos Carvalho). Multiple linear regression was used to estimate the magnitude and direction of the associations between the municipal malacological profile and schistosomiasis positivity after adjustment for the available socioeconomic variables. This approach allowed assessment of whether the presence or identity of intermediate host species was associated with higher or lower schistosomiasis positivity rates among municipalities. Results were reported as regression coefficients (estimates), confidence intervals, and p-values for each explanatory variable.

All statistical analyses were performed using jamovi software. The forest plot summarizing the multiple linear regression results was generated with the assistance of an artificial intelligence tool using the original data from the present study.

Results

The highest number of reported schistosomiasis cases in Espírito Santo was recorded in 2011 (n = 529), followed by a progressive decline until 2017 (n = 195), with a subsequent increase in 2018 (n = 307) and 2019 (n = 385). The fitted trend line indicated an overall decreasing temporal



trend, with a slope coefficient of -14.57 and accounting for 15.29% of the observed variability (**Figure 1**).

Regarding self-reported race/color, most cases occurred among White individuals ($n = 1,440$; 52.1%), followed by Brown (*pardo*) ($n = 916$; 33.1%), Black ($n = 213$; 7.7%), Asian ($n = 44$; 1.6%), and Indigenous individuals ($n = 13$; 0.5%). Race/color information was missing for an additional 98 cases (**Figure 2**).

The municipalities with the highest cumulative number of reported cases relative to population size were Marechal Floriano, Dolores do Rio Preto, Santa Maria de Jetibá, Domingos Martins, Irupi, and Muniz Freire, with values of 1.1%, 1.0%, 0.6%, 0.6%, 0.6%, and 0.4%, respectively. These values provide an estimate of disease occurrence adjusted for population size but should not be interpreted as the percentage of the population infected, because the number of cases for each municipality represents the cumulative total recorded over the entire 2011–2019 study period (**Figure 3**).

Figure 1. Temporal variation in schistosomiasis cases in the state of Espírito Santo from 2011 to 2019, showing the fitted trend line (dashed line) and its regression equation.

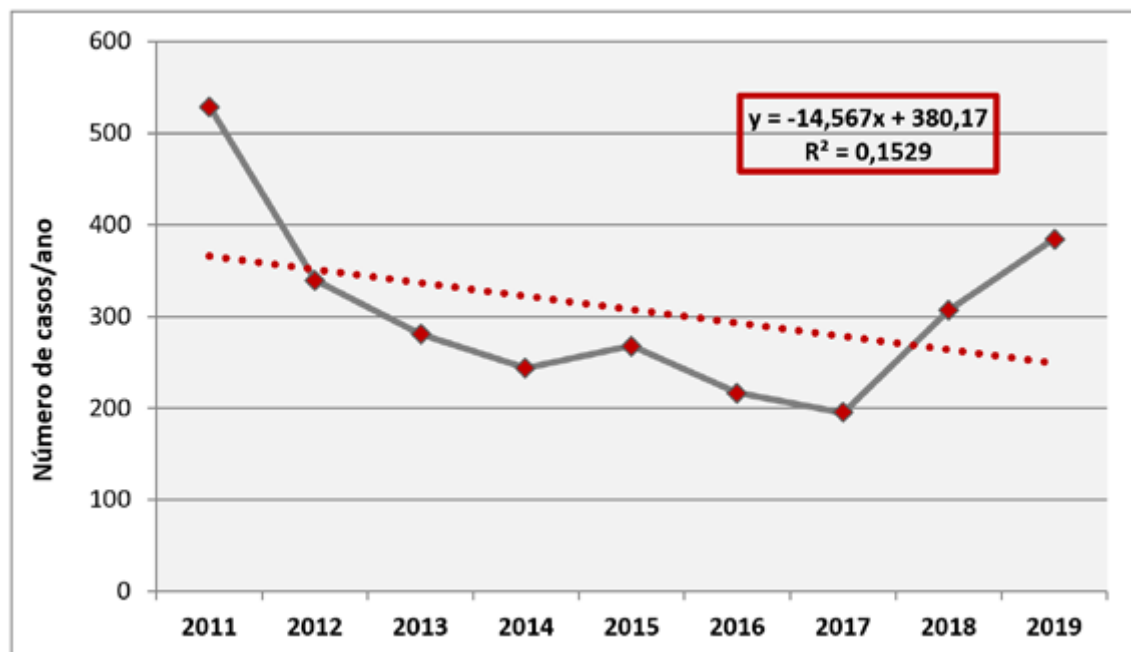


Figure 2. Total number of schistosomiasis cases in Espírito Santo from 2011 to 2019, grouped by self-reported race/color.

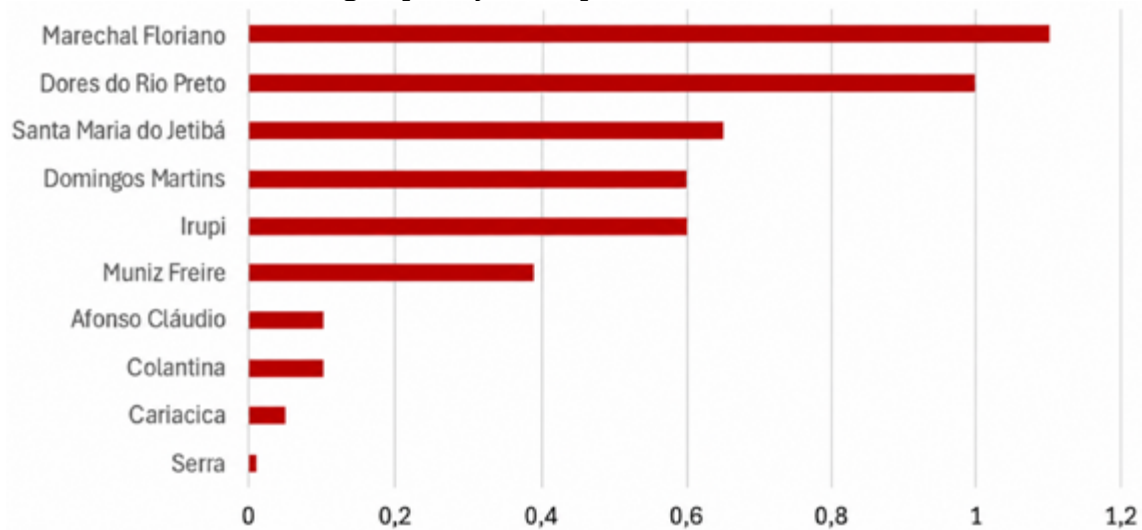
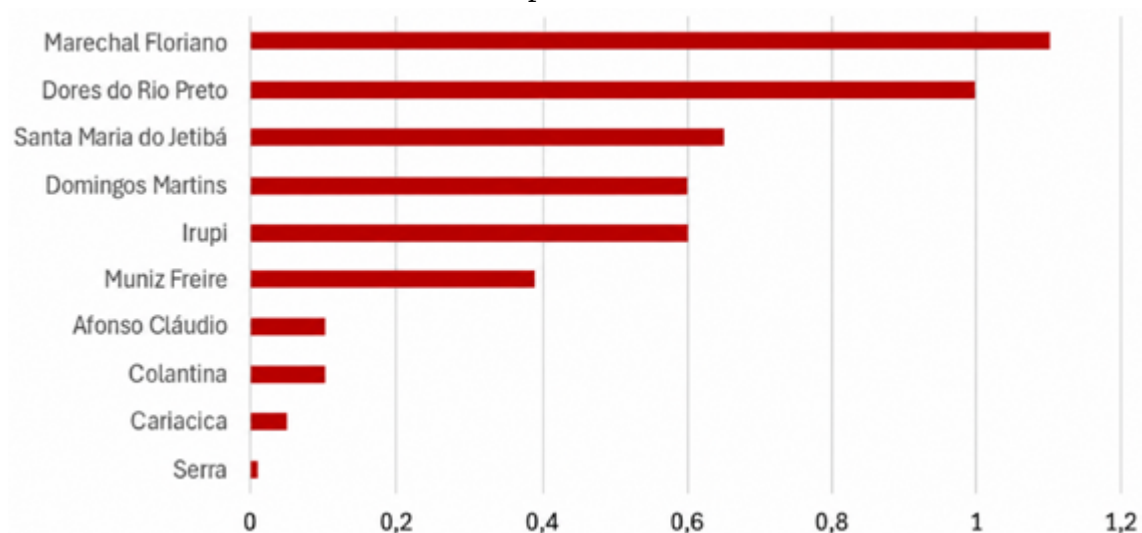


Figure 3. Municipalities in Espírito Santo with the highest cumulative schistosomiasis occurrence from 2011 to 2019, adjusted for total population and expressed as the number of cases per 100 inhabitants.



Among the reported water bodies, the most frequent probable sites of infection were rivers, lakes, streams, waterfalls, and drainage ditches, accounting for 11.68%, 5.60%, 3.76%, 2.82%, and 1.08% of cases, respectively.

vely. Of the 2,766 cases investigated, 1,905 had no information available regarding the probable site of infection (**Table 1**).

Comparison of schistosomiasis positivity rates among categories defined by the occurrence of the three *Biomphalaria* species using analysis of variance (ANOVA) showed no statistically significant difference in the overall analysis ($F = 1.47$; $p = 0.231$) or in pairwise comparisons among municipalities with no recorded *Biomphalaria* species and those with one, two, or three species (**Table 2**). In contrast, a historical record of *S. mansoni* in the municipality was significantly associated with schistosomiasis positivity, with municipalities with a documented history of the etiological agent showing a mean positivity rate 16 percentage points higher than municipalities without such a record ($F = 8.0$; $p = 0.006$).

Table 1. Distribution of schistosomiasis cases according to the suspected site of infection in Espírito Santo from 2012 to 2019.

Site	Number of cases	Percentage of cases
Rio	323	11,68
Lagoa	155	5,60
Córrego	104	3,76
Cachoeira	78	2,82
Valão	30	1,08
Lago	16	0,58
Brejo	14	0,51
Nascente	13	0,47
Barragem	11	0,40
Outros	117	4,23
Não sabe informar	1905	68,87
TOTAL	2766	100,00

Table 2. Results of the ANOVA using *Biomphalaria* species richness (S; 0, 1, 2, or 3 species) and the presence or absence (0/1) of a historical *S. mansoni* record in each municipality of Espírito Santo as categorical variables, and the municipal schistosomiasis positivity rate as the quantitative response variable. MD = mean difference; SE = standard error.

S de <i>Biomphalaria</i>		DM	EP	ptukey
0	1	-7,012	6,83	0,734
	2	-6,329	8,37	0,874
	3	9,265	8,82	0,720
1	2	0,683	7,84	1,000
	3	16,277	8,32	0,214
2	3	15,594	9,62	0,374
Ocorrência de <i>S. mansoni</i>				
0	1	-16	5,64	0,006

In municipalities where the occurrence of *B. glabrata* was confirmed, the mean positivity rate of diagnostic tests reached 71.1%, indicating a high level of schistosomiasis transmission in areas where this species occurs, despite differences in demographic characteristics and sanitation infrastructure. Contingency-table analysis showed a significant association between municipal schistosomiasis occurrence and the presence of *B. glabrata* ($\chi^2 = 6.06$; $p = 0.014$), with an odds ratio (OR) of 3.96 (95% CI: 1.27–12.3), indicating that the odds of schistosomiasis occurrence were nearly four times higher in municipalities where this species was recorded. For *B. straminea*, no statistically significant association with schistosomiasis occurrence was observed ($\chi^2 = 0.00178$; $p = 0.966$), with an OR of 0.974 (95% CI: 0.292–3.25). Finally, the presence of *B. tenagophila* was significantly associated with municipal schistosomiasis occurrence ($\chi^2 = 6.06$; $p = 0.014$). However, the odds ratio was below 1 (OR = 0.253), indicating an

inverse association between the occurrence of this species and schistosomiasis occurrence (**Table 3**).

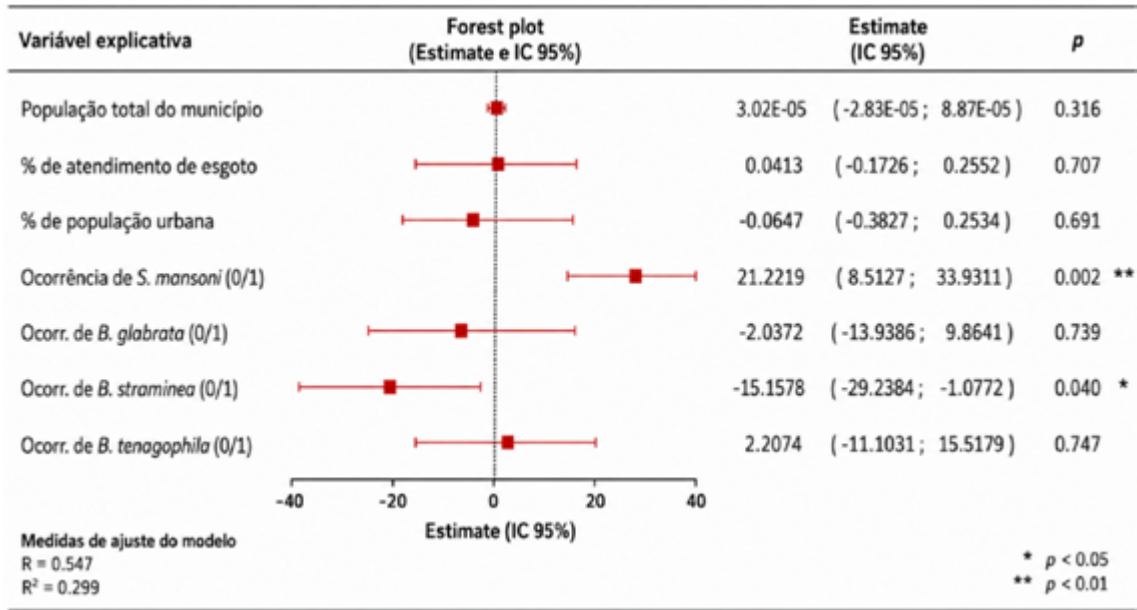
In the multiple linear regression analysis, the model yielded a correlation coefficient (R) of 0.547 and a coefficient of determination (R^2) of 0.299, indicating that approximately 29.9% of the variability in municipal schistosomiasis positivity rates was explained by the variables included in the model. Historical occurrence of *S. mansoni* was positively and significantly associated with the positivity rate (Estimate = 21.2219; SE = 6.489; $t = 3.27$; $p = 0.002$), corresponding to an average increase of approximately 21.2 percentage points in municipalities with a historical record of the etiological agent. In contrast, the occurrence of *B. straminea* showed a significant negative association (Estimate = -15.1578; SE = 7.182; $t = -2.11$; $p = 0.040$), corresponding to an average reduction of approximately 15.2 percentage points in municipalities where this species was recorded. Total municipal population (Estimate = 3.02×10^{-5} ; $p = 0.316$), sewage service coverage (Estimate = 0.0413; $p = 0.707$), percentage of the population living in urban areas (Estimate = -0.0647; $p = 0.691$), occurrence of *B. glabrata* (Estimate = -2.0372; $p = 0.739$), and occurrence of *B. tenagophila* (Estimate = 2.2074; $p = 0.747$) were not significantly associated with schistosomiasis positivity after adjustment for the effects of the other variables (**Figure 4**).

Table 3. Contingency tables for the categorical variables schistosomiasis occurrence (No/Yes) and the occurrence of each *Biomphalaria* species in municipalities of Espírito Santo. OR = odds ratio; 95% CI = 95% confidence interval.

<i>B. glabrata</i> occurrence		Schistosomiasis occurrence		
		No	Yes	Total
No	Observed	19	24	43
	%	44,2%	55,8%	100,0%
Yes	Observed	5	25	30
	%	16,7%	83,3%	100,0%
Total	Observed	24	49	73
	%	32,9%	67,1%	100,0%
<i>B. glabrata</i> x <i>Esquistossomose</i>		OR	IC(95%)	
		0,014	3,96	1,27 12,3
<i>B. straminea</i> occurrence		Schistosomiasis occurrence		
		No	Yes	Total
No	Observed	19	39	58
	Line (%)	32,8%	67,2%	100,0%
Yes	Observed	5	10	15
	Line (%)	33,3%	66,7%	100,0%
Total	Observed	24	49	73
	Line (%)	32,9%	67,1%	100,0%
<i>B. straminea</i> x <i>Esquistossomose</i>		OR	IC(95%)	
		0,966	0,974	0,292 3,25
<i>B. tenagophila</i> occurrence		Schistosomiasis occurrence		
		No	Yes	Total
No	Observed	5	25	30
	Line (%)	16,7%	83,3%	100,0%
Yes	Observed	19	24	43
	Line (%)	44,2%	55,8%	100,0%
Total	Observed	24	49	73
	Line (%)	32,9%	67,1%	100,0%
<i>B. tanegophila</i> x <i>Esquistossomose</i>		OR	IC(95%)	
		0,014	0,253	0,0813 0,785



Figure 4. Forest plot showing the results of the linear regression analysis, with the percentage of positive results in each municipality as the response variable.



Discussion

The study hypothesis that historical records of *S. mansoni* and species of the genus *Biomphalaria*, its potential intermediate hosts, would be significantly associated with schistosomiasis incidence across municipalities in the state of Espírito Santo after adjustment for population size and municipal sewage treatment coverage was only partially supported. Our results are also consistent with the alternative hypothesis that some *Biomphalaria* species may be associated with a lower risk of disease, as they showed inverse associations with schistosomiasis incidence.

Despite the essential role of *Biomphalaria* species in the life cycle of *S. mansoni* (CARVALHO & AMARAL, 2008), the mere presence of intermediate hosts in a municipality was not associated with parasitological test positivity. This finding suggests that factors related to transmission intensity, local environmental conditions, sanitation, and human contact with contaminated water bodies may be more important in explaining the spatial



distribution of schistosomiasis than the occurrence of host species alone, since these snails may be present locally without effectively transmitting the parasite (SOUZA, 2018; CONCEIÇÃO et al., 2016; SANTOS et al., 2023).

Moreover, among the three *Biomphalaria* species recorded in Espírito Santo, only *B. glabrata* showed a significant positive association with schistosomiasis incidence, consistent with the literature identifying this species as the main intermediate host of *S. mansoni* in Brazil (CARVALHO; AMARAL, 2008). In contrast, *B. straminea* and *B. tenagophila* showed null and/or negative associations with the percentage of positive diagnostic results. This departure from the expected transmission pattern may be explained by taxonomic issues involving the genus *Biomphalaria*. As reported in the literature, *B. straminea* and *B. tenagophila*, both susceptible to *S. mansoni*, are morphologically indistinguishable from *B. kuhniana* (CALDEIRA et al., 2000) and *B. occidentalis* (COIMBRA JÚNIOR; ENGEL, 1982; PARAENSE, 1981; PARAENSE; CORRÊA, 1982), respectively, whereas the latter two species are refractory to infection by the parasite (CARVALHO et al., 2008; TEODORO et al., 2010). Historically, this marked phenotypic overlap has already resulted in mapping errors in other regions of Brazil, requiring subsequent epidemiological reassessment (PARAENSE, 1986; PIZA et al., 1972; TELES, 1989; VAZ et al., 1983). Therefore, our findings suggest the possibility of taxonomic misidentification bias in statewide distribution records, whereby species naturally resistant to *S. mansoni* may have been mistakenly classified as species associated with transmission risk. This represents an important point of concern and highlights the need for future studies on snail spatial distribution in Espírito Santo to incorporate molecular biology techniques alongside morphological identification (CALDEIRA et al., 2000). Such an approach would improve taxonomic accuracy and may lead to updates in species distribution

maps and, consequently, in estimates of schistosomiasis transmission risk across municipalities in Espírito Santo.

The occurrence of cases associated with natural aquatic environments, particularly rivers, lakes, streams, and waterfalls, together with variation in basic sanitation coverage, reinforces the complexity of the transmission cycle and the importance of integrated public health approaches for effective schistosomiasis control in Espírito Santo, including improvements in sanitation infrastructure and strengthened malacological surveillance (ESPÍRITO SANTO, 2010; CONCEIÇÃO *et al.*, 2016). The higher occurrence of the disease in inland municipalities was accompanied by a high proportion of cases among self-identified White individuals, as observed in the present study, in areas with a substantial population of Pomeranian descendants (SPAMER, 2016). These findings reinforce the need to expand sewage collection and treatment systems and strengthen control measures targeting intermediate hosts in regions farther from the state's main political and economic centers, thereby promoting more equitable public health coverage across the population of Espírito Santo.

Conclusions

This study has limitations inherent to the use of administrative systems and secondary databases, which may introduce biases related to surveillance coverage, data quality, and completeness of records. Potential underreporting of schistosomiasis cases and inconsistencies in records of parasite detection and potential intermediate hosts may affect the accuracy of the estimates and comparability among the municipalities analyzed. Nevertheless, the results indicate that the presence of snails of the genus *Biomphalaria* alone was not associated with schistosomiasis occurrence in Espírito Santo. In contrast, a significant association was observed between

en disease occurrence and *B. glabrata*, reinforcing the role of this species as the main intermediate host involved in schistosomiasis transmission in the state. Conversely, *B. tenagophila* and *B. straminea* did not show the same pattern and were associated with lower disease occurrence.

Molecular studies are needed to reassess the geographic distribution of these species in Espírito Santo and to investigate the possibility that some records may correspond to alternative species refractory to *S. mansoni*, as previously reported in other regions of Brazil. Such studies could contribute to a more accurate identification of areas at risk for schistosomiasis transmission in the state. Surveillance and disease-control measures should be targeted toward priority areas, while health education should be strengthened as a permanent strategy for schistosomiasis prevention.

Author contributions

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Writing — review & editing: SASM

Conflict of Interest

The authors declare no conflicts of interest related to this study. This work was conducted independently, without influence from institutions, organizations, or sponsors that could affect the results or the interpretation of the data presented.

Data Availability

The authors declare that the data supporting the results of this study are available in the article. Derived data supporting the study's conclusions that are not found in the article may be requested from the corresponding author.

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