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Ecological parameters and intermediate host snails of human schistosomes and other trematodes of biomedical importance from freshwater, western Côte D'Ivoire

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Abstract

Schistosomiasis, caused by *Schistosoma* trematodes transmitted by intermediate host snails, remains a waterborne disease of concern in Côte d'Ivoire. Despite mass drug administration, a high prevalence of intestinal schistosomiasis persists in the western part of the country.

This study was carried out to better understand the transmission ecology of schistosomes and other trematodes in this area by identifying the open freshwater sources most at risk.

Cross-sectional malacological studies were carried out from August to October 2022 in Biankouma, Ouaninou and Touba districts. Environmental and water parameters were recorded. Snails were collected and intermediate hosts *Biomphalaria pfeifferi* and *Bulinus globosus* spp. were tested for cercarial emission. Principal component analysis was used to assess the relationship between environmental parameters of sampling sites and snail species.

1,014 snails from seven species were collected from 23 water sites classified into four categories: rivers, streams, ponds and rice fields. The rivers and streams had more snail species than rice fields and ponds. Among the snails collected, 71.5% were *Bi. pfeifferi* and 28.5% were *Bu. globosus*. The cercariae found were echinostomes, strigeids, *Xiphidiocercariea* and schistosomes. The presence of *Bi. pfeifferi*'s was positively associated with pH and negatively with high temperature and conductivity. Bu. globosus was negatively associated with the total values of total dissolved solids.

The distribution of snail species and hence trematodes of medical importance is highly heterogeneous, associated with the type of water bodies, and with specific chemical and physical parameters. This study should be extended to assess schistosome infestation rates and, in perspective, inform preventive interventions against schistosomiasis transmission.

Keywords: Snail, transmission, trematode, freshwater, West Côte d'Ivoire

Introduction

Schistosomiasis, a waterborne parasitic disease caused by trematodes of the genus *Schistosoma*, remains prevalent in people living in tropical and subtropical regions [1, 2, 3]. Four of the six pathogenic schistosome species of humans are endemic in Africa [4]. The disease persists in areas where access to drinking water and adequate sanitation are restricted [5, 6, 7, 8, 9]. Infections or reinfection of human populations by free-living cercariae of schistosomes or other trematodes are related to their daily activities around water points [10] (dams, rivers, streams and ponds). The genera *Bulinus*, *Biomphalaria*, *Oncomelania*, and *Lymnaea (Radix)* are the most important snail hosts of trematodes in terms of causing harm to humans and animals by transmitting fascioliasis and schistosomiasis [11, 12]. Schistosomiasis is focal and its distribution depends on the distribution of the intermediate host. Due to the persistence of outbreaks, WHO recommends that drug treatment be combined with control of intermediate host snails as part of control and prevention measures to reduce transmission. However, this complementary approach requires good knowledge of the snail distribution [13, 14].

In Côte d'Ivoire, as in several sub-Saharan African countries, parasitological diagnosis and preventive chemotherapy with praziquantel administered through school and community platforms have reduced the transmission of schistosomiasis in certain highly endemic areas in recent decades [15]. However, persistent transmission hotspots with high prevalences of schistosomiasis infection ranging from 50 to 100% have been reported in the western region of the country despite the use of mass drug administration (MDA) [7, 9, 16]. This study was carried out as part of a project supported by the "Belmont Collaborative Forum for Climate, Environment and Health", which aims to map risks and target snail control in order to support schistosomiasis elimination under future climate conditions [17]. In this paper, our objective is to better understand the bioecology of snails involved in the transmission of schistosomiasis and to identify open freshwater sources that are potentially at risk for the population of the western part of Côte d'Ivoire.

Materials and Methods

Study Sites

The study was carried out in 15 villages across three health districts namely Touba (8°40'0" N; 7°30'0" W), Ouaninou (8°14'00" N; 7°52'0" W) and Biankouma (7°45'0" N; 7°40'0" W) (Figure 1). It is a mountainous region, located between the forest and the savannah. The climate is tropical (humid-dry), with a rainy season from March to October and a dry season from November to February. The mean temperature is 24.9°C in Ouaninou and 25.1°C in Touba with a precipitation of 1,627 mm. In Biankouma, the mean temperature is 23.6°C with precipitation of 2,048 mm

(https://fr.climate-data.org/afrique/cote-d-ivoire). The population in this area is engaged in coffee and cocoa cultivation, subsistence farming (rice, yam and cassava cultivation), and cattle rearing. The water drains through ponds, streams, rivers, and tributaries of the river. The vegetation consists of forest relics. When selecting the sampling sites, accessibility to water points and the prevalence of schistosomiasis were considered.

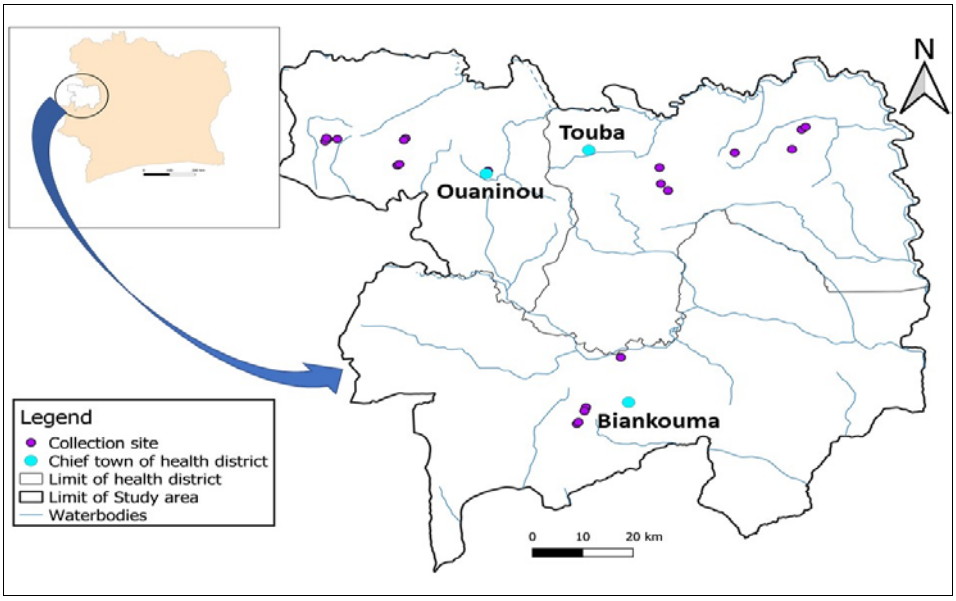

Fig 1: Map of sampling sites in the districts of Touba, Ouaninou and Biankouma in western Côte d'Ivoire

Malacological surveys

Malacological surveys were carried out during the rainy season from August to October 2022 at all human-water contact. The physico-chemical parameters, namely water temperature (°C), total dissolved solids (TDS, mg.L^{-1}), hydrogen potential (pH), and conductivity (µS. cm^{-1}), were measured using a portable multiparameter (Hanna HI98130 model). The physical parameters assessed for microhabitat characterization included size, type of waterhole, depth and vegetation cover (%). Two individuals collected snails for 15 minutes each using scoops and forceps. The snails were identified based on the morphological characteristics using Brown's key [18]. They were placed between two layers of moistened cotton wool in perforated petri dishes and then transported to the laboratory in a cool box maintained at around 20°C. In the laboratory, snails were subjected to the cercarial shedding test after exposure to artificial light for 2-3 hours. The cercariae, observed under a magnifying glass, were collected using a Pasteur pipette, and placed on a slide. After fixation and staining with 10% of Lugol's, they were identified (x40, x100) using Frandsen's key [19]. Tests were performed to determine parasite diversity and the natural infestation rate (NIR) for human intermediate host snails.

Statistical Analysis

Data were entered and validated using Microsoft Excel software and analyzed with R statistical software version 4.1.0. *P*-values < 0.05 were considered statistically significant. The Kruskal–Wallis chi-squared test (KW) was used to compare differences in physico-chemical parameters and snail abundance between districts. Associations between snail abundance and environmental/physico-chemical variables were determined using a principal component analysis (PCA).

Results

Types and characteristics of water points

A total of 23 water points, including nine rivers (39.1%), seven rivulets (30.4%), six ponds (26.1%) and one rice field (4.3%), were visited in 15 villages. Of all the water points, 39.1% (9) were open, 34.8% (8) were semi-covered and 26.1% (6) were covered by emergent vegetation and other larger rooted trees. Figure 2 shows the distribution of the type of freshwater source in the three districts. In Ouaninou, the primary water bodies were rivers and streams followed by ponds and rice paddies. In Touba, the main water sources were mostly ponds followed by rivers and streams. Whereas the district of Biankouma was dominated by rivers. The rivers in the three districts maintained permanent flow and were partially covered (50%) by the foliage of trees and shrubs along their banks.

In Touba and Biankouma, water was present throughout the year in streams (permanent regime). However, in Ouaninou, it was a temporary regime. The vegetation coverage of the water access point was 100% in Touba and 50% in Ouaninou and Biankouma. The ponds and rice field surveyed were open and temporary. The measured physico-chemical parameters showed that the mean pH varied according to the site with 6.78±1.5 in the rivers 6.6±1.3 in the streams, 5.99±1.0 in the ponds and 4.4 in the rice field (KW test = 8.48, p = 0.014). The mean water temperature, among the three water bodies (rivers: 26.1°C±2.4, streams: 26.1±2.17 ponds: 26.4±1.6) were not statistically different (KW test = 0.59, p = 0.74). Total dissolved solids were higher in the ponds (96.7 mg/L±7.0) than in rivers (88.2 mg/L±45.9) and streams and the rice field (80 mg/L±49.6) (Kw test = 12.493, p = 0.0019). No significant differences were found in the conductivity values of the three water points (KW test = 1.20, p = 0.54). The mean conductivity was 57.8±25.5 µS/cm in the streams, while in the rivers and ponds, it was 56.6±24.9 µS/cm and 56.3±29.7 µS/cm, respectively. The rice pad that was surveyed had a conductivity of 115 mg/l and a total dissolved solids of 122 µS/cm.

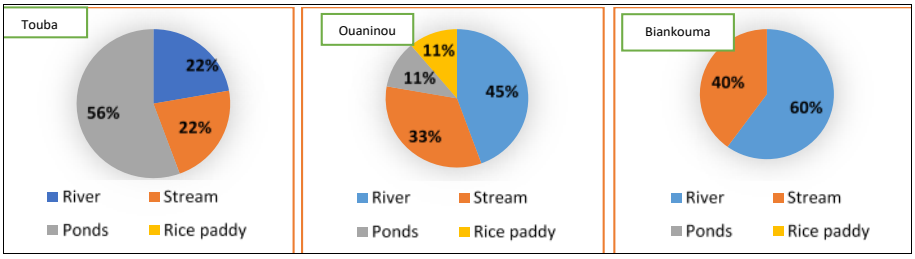

Fig 2: Relative proportion of water points surveyed in the studies sites in Touba, Ouaninou and Biankouma from August to October 2022

Results of snail survey

A total of 1,014 freshwater snails were collected. Morphological identification of snails revealed six (6) genera divided into seven (7) species: *Biomphalaria pfeifferi* (Krauss, 1848), *Bulinus globosus* (Morelet, 1866), *Bu. forskalii* (Ehrenberg, 1831), *Lymnaea (Radix) natalensis* (Krauss, 1848), *Physa acuta* (Draparnaud, 1805), *Segmentorbis kanisaensis* (Preston, 1914) and *Melanoides tuberculata* (Müller, 1774). These species were found in rivers, streams, ponds, and rice paddies (Table 1). *Bulinus forskalii*, *P. acuta*, and *S. kanisaensis* were collected in ponds, while *P. acuta* and *L. natalensis* were found in rice paddies. *Bulinus forskalii* (92.9%; 95% CI, 89.3-95.6%) and *Bi. pfeifferi* (70.8%; 95% CI, 65.4-75.9%) were collected in rivers, while the streams were mainly inhabited by *M. tuberculata* (88.9%; 95% CI, 70.8-97.6%), *Bu. globosus* (82.5%; 95% CI, 74.5-88.8%) and *L. natalensis (Radix)* (56.6%; 95% CI, 45.3-67.5%). *Segmentorbis kanisaensis* was mainly identified in ponds with a prevalence of 55.6% (95% CI, 46.8-64.2%), while *P. acuta* was found in rice paddies with a prevalence of 51.6% (95% CI, 38.6-64.5%). In Touba, *Bu. forskalii* was the most abundant species (44.6%; 95% CI 40.6-48.6%) collected in rivers and ponds. In Ouaninou, *Bi. pfeifferi* dominated the fauna (50.6%; 95% CI, 45-56.2%), while in Biankouma, *L. natalensis* was the most abundant species (45.2%; 95% CI, 34.3-56.5%). A total of 425 intermediate host snails of schistosomes were sampled from rivers and streams of which the majority were *Bi. pfeifferi* (n=305) and *Bu. globosus* (n = 120). *Biomphalaria pfeifferi*, the principal intermediate host snail for *S. mansoni*, was significantly more abundant than *Bu. globosus* in Ouaninou and Biankouma ($\chi 2$ = 48.8, $P <$ 0.001), while in Touba the two species were found at comparable frequencies (Figure 3).

Table 1: Proportion of snail species found in freshwater sources in the district of Biankouma, Ouaninou and Touba, western Côte d'Ivoire, from August to September 2022

Species	Rivers		Streams		Ponds		Rice Paddy		
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	Total
Bulinus globosus	21 (17.5)	11.2-25.5	99 (82.5)	74.5-88.8	0 (0.0)	0.0-0.3	0 (0.0)	0.0-0.3	120
Biomphalaria pfeifferi	216 (70.8)	65.4-75.9	89 (29.2)	24.1-34.6	0 (0.0)	0.0-1.2	0 (0.0)	0.0-1.2	305
Bulinus forskalii	264 (92.9)	89.3-95.6	7 (2.5)	0.66-4,27	13 (4.6)	2.4-7.7	0 (0.0)	0.0-1.3	284
Segmentorbis kanisaensis	17 (12.8)	7.6-19.7	42 (31.6)	23.8-40.2	74 (55.6)	46.8-64.2	0 (0.0)	0.0-2.7	133
Physa acuta	6 (9.7)	3.6-19.9	0 (0.0)	0.0-5.8	24 (38.7)	26.6-51.9	32 (51.6)	38.6-64.5	62
Melanoides turberculata	3 (11.1)	2.3-29.1	24 (88.9)	70.8-97.6	0 (0.0)	0.0-12.8	0 (0.0)	0.0-12.8	27
Lymnae natalensis (Radix)	23 (27.7)	18.4-38.6	47 (56.6)	45.3-67.5	0 (0.0)	0.0-4.3	13 (15.7)	8.6-25.3	83

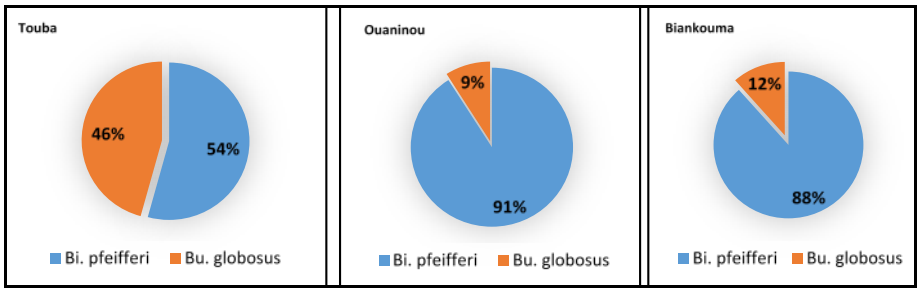

Fig 3: Relative proportions of schistosome intermediate host snails in the study area from August toOctober 2022.

In Touba, *Bi. pfeifferi* was found in the same proportions in rivers (30,7%; 95% CI, 24.6-36.8%) and streams (23.5%; 95% CI, 17.9-29.1%), while *Bu. globosus* occurred mainly in streams (44.8%; 95 % CI, 38.2- 51.3 %) compared to rivers (0.9 %; 95 % CI, 0.1-2.1 %). In Ouaninou, *Bi. pfeifferi* was significantly more abundant in rivers (82.6%; 95% CI, 76.2-87.8%) than in streams (8.4%; 95% CI, 4.3-12.50%). A few *Bu globosus* (8.99%; 95 % CI, 4.79-13.19%) were collected in rivers but none in the streams. In Biankouma, *Bi. pfeifferi* was collected only in rivulets (84.6%; 95% CI, 70.7-98.5%). No intermediate hosts for schistosomes were identified in ponds or rice paddies.

Infection in intermediate host snails

Among the 425 *Biomphalaria* and *Bulinus* snails that are a competent hosts for schistosome parasites, 422 were tested for natural infection; 302 *Bi. pfeifferi* (71.5%) and 120 *Bu. globosus* (28.5%). Forty-eight snails were infected with trematodes, giving an overall infection rate of 11.4% (95% CI, 8.3-14.4%). Specifically, we found 44 infected *Bi. pfeifferi* (pooled prevalence of infection: 14.6%) and 4

Bu.globosus (3.3% infection prevalence). Four species of trematodes were identified: *Schistosoma mansoni* (schistosome), *Echinostoma* sp. (*Echinostoma*), *Plagiorchis* sp. (Xiphidiocercaria), and *Cotylurus* sp. (Strigeids). Trematode infections were found mainly in *Bi. pfeifferi* with a natural infection rate (NIR) of 14.6% (95% CI, 10.5-18.5). The cercariae found in *Bi. pfeifferi* were mainly from echinostomes (10.7%), strigeids (6%) and schistosomes (2.8%). The *Bi. pfeifferi* species from rivers were significantly more infected (19.5%; 95% CI, 14.5-25.5%) than those from streams 2.3% (95% CI, 0.3-8.1%). Of the *Bu. globosus* collected from stream, 2.5%, (95% CI, 0.0-5.3%) were infected with schistosomes and 0.8% (95% CI, 0.0-5.3%) with strigeid cercariae.

Snails and environmental parameters

To relate the type of collection site to the presence of snail species, principal component analysis (PCA) was used (Fig 4). The F1 axis contrasts individuals from the ponds and rice fields group with those from the streams and rivers group, and the F2 axis contrasts the individuals of the ponds and rivers group with those of the streams group. The species *Bi. pfeifferi* found in the rivers was positively associated with low pH and negatively associated with high temperatures. *Bu. globosus* and *L. natalensis* were correlated with streams and associated with shallow depths and low total dissolved solids values. The species *P. acuta* was correlated with rice fields as well as high temperatures and conductivity values.

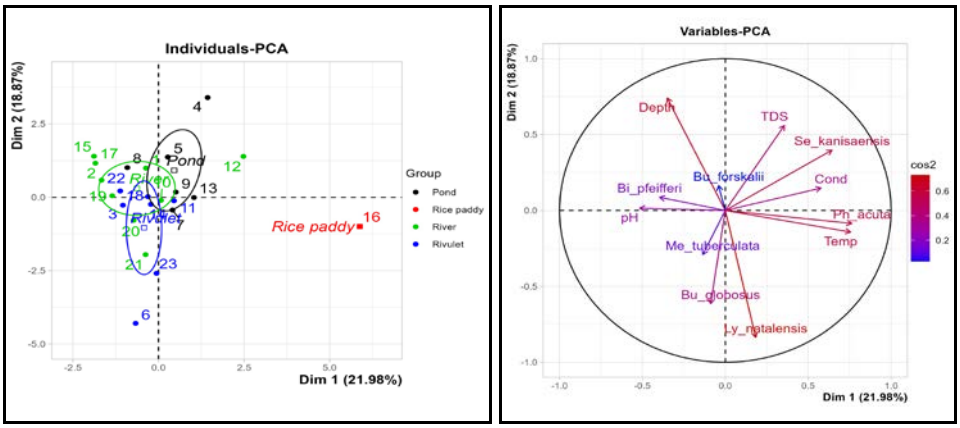

Fig 4: Relationship between physical-chemical parameters of water sources and abundance of snails in Touba, Ouaninou and Biankouma, western Côte d'Ivoire, from August to October 2022. Temp: temperature; TDS: total dissolved solids Bi_pfeifferi = Biomphalaria pfeifferi; Bu_globosus = Bulinus globosus; Ly_natalensis = Lymnaea natalensis; Me_tuberculate = Melanoides tuberculata; Ph_Acuta = Physa acuta; Bu_Forskalii = Bulinus forskalii; Se_kanisensis = Segmentorbis kanisaensis.

Discussion

The malacological investigation carried out in the Touba, Ouaninou and Biankouma district revealed that the freshwater environments consist of rivers, streams, ponds and rice paddies. These water-points had different physical and chemical characteristics due to the topography of the soils, the flow rates, the accumulation of organic matter and the structure of the water [20]. The species of snails identified in this study, are common and have already been observed in Côte d'Ivoire [21, 22, 23].

The exclusive presence of schistosome intermediate host snails (*Bi pfeifferi* and *Bu. globosus*) in rivers and streams is certainly due to the favorable conditions offered by these biotopes, unlike ponds and rice paddies. Indeed, the availability of food resources, the association with low pH and low temperature, and total dissolved solids (in the observed range) can explain their presence in these water bodies. Temperature and total dissolved solids are key factors that can influence key features of the life history of snails. The negative association with temperature suggests that the temperature at the study site is near or above the thermal optimum of *Bi. pfeifferi*, in agreement with recent analyses based on thermal-sensitive mechanistic models [24]. These in turn can influence distribution, abundance, and persistence in the environment. In general, natural aquatic environments are influenced by biotic and abiotic ecological factors that regulate the distribution and abundance of species. Several studies have highlighted the wide distribution of *Bi. pfeifferi* in rivers in Man [23] in western Côte d'Ivoire and elsewhere [25, 26] which is correlated with temperature, pH, and the level of total dissolved solids. Similarly, *Bu.globosus* was collected in shallow streams and negatively correlated with a high total dissolved solids. Permanent streams with woody vegetation cover and low level of total dissolved solids are suitable habitats for this species. Similar results have been reported in Man and Agboville [27], confirming that natural habitats with woody vegetation cover and low level of total dissolved solids, such as permanent streams, are suitable biotopes for this species. As reported by Mohammed *et al*. in Sudan [28], *Bi. pfeifferi* is capable of adapting to rivers and streams. The high abundance of *Bi. pfeifferi* in almost all rivers and streams in the three districts establishes the fact that it is a cosmopolitan species. This pervasiveness may be due to the permanence of water, plant cover, and the absence of predators that favour their reproduction and proliferation in the environment. Natural infestation tests revealed four species of trematodes harboured by *Bi. pfeifferi*: *S. mansoni* (schistosomes), *Echinostoma sp*. (Echinostomes), *Plagiorchis sp*. (Xiphidiocercaria) and *Cotylurus sp* (Strigeides). These species were found in Man district [29], in western Côte d'Ivoire with a natural infestation rate of 13.7%. Lower rates have been reported in Zimbabwe (1.3%) and Tanzania (1.3%) [30, 31]. The diversity of trematodes hosted by *Bi. pfeifferi* may certainly be a consequence of its high abundance in the environment and its exposure to parasitic pressures. Furthermore, the presence of different vertebrate or invertebrate species of hosts involved in their life cycle [32]. *Echinostoma* sp. was the dominant species and mixed with schistosomes, strigeids and Xiphidiocercariae

with a low natural infection rate. Our results are in line with a study in western Kenya that confirmed that some echinostomes clades use *Biomphalaria* and *Bulinus* as their first intermediate hosts [33].

This predominance of echinostomes within *Bi. pfeifferi* has been reported by several authors [34, 35] However, the results of this study differ from those of Tian Bi *et al* [29], who found only schistosomes in monoinfections in *Bi. pfeifferi.* The low natural infection of *Bi. pfeifferi* by schistosomes can be explained firstly, by antagonistic interactions with other trematodes and secondly by the rainy season. Studies have shown that when multiple species of trematodes colonize the same snail host, echinostomes have usually been shown to be dominant to other species [36, 37] Also, studies have shown that a high natural infection rate for *Bi. pfeifferi* is recorded during the dry season [38], when human-water contact is greater, leading to defecation and a high risk of miracidia transmission to people. The high proportion of *Bi. pfeifferi* in the rivers and streams and the schistosome infection rates confirm the active and persistent transmission of *S. mansoni* in the three districts. In addition, the presence of *Bulinus globosus* and the infection rates recorded in Touba make it a high-risk area for both snail species.

Even if low natural infestation rate can cause a high prevalence of schistosomiasis in a region, according to De Kock *et al* [39]. More studies should be carried out to assess schistosome infestation rates and, in perspective, inform preventive interventions against schistosomiasis transmission.

Conclusion

Six genera and seven species of snails were identified in four types of water bodies (streams, rivers, ponds, and rice paddies). Intermediate hosts of schistosomes were found only in rivers and streams. In Touba, *Bi. pfeifferi* was found in the same proportions in rivers and streams, while *Bu. globosus* occurred mainly in streams. In Ouaninou, *Bi. pfeifferi* was more abundant in rivers than streams and in Biankouma, *Bi. pfeifferi* was collected only in streams. *Biomphalari pfeifferi* found in the rivers was positively associated with pH and temperatures while, *Bu. globosus* found in streams was associated with total dissolved solids values. In addition to schistosomes, freshwater human host snails harboured three species of parasitic trematodes (echinostomes, xiphidiocercariae and strigeids). *Bi. pfeifferi* is the most widespread snail species with mixed infestations, while *Bu. globosus* was lightly infested.

These two types of water sources are potentially high-risk and indicate the need to step up prevention through health education and, above all, interventions in the field of water sanitation and hygiene (WASH). The high abundance of *Bi pfeifferi* and the infection rates could be the cause of persistent transmission in these three districts.

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Competing interests

The authors declare that they have no competing interests.

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