

## Article

# Transmission of Soil Transmitted Helminthiasis in the Mifi Health District (West Region, Cameroon): Low Endemicity but Still Prevailing Risk

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**Abstract:** The control of soil-transmitted helminthiasis (STH) in Cameroon is focused on large-scale deworming through annual mass drug administration (MDA) of albendazole or mebendazole to at-risk groups, principally pre-school and school-age children. After a decade of intervention, prevalence and intensity of infection have been significantly lowered, encouraging the paradigm shift from control to elimination. However, STH eggs are extremely resistant to environmental stressors and may survive for years in soils. It therefore appeared important to assess whether the risk of transmission was still prevailing, especially in a context where transmission of soil-transmitted helminths in the human population had almost been interrupted. A retrospective and a prospective cross-sectional surveys were conducted in five Health Areas of the Mifi Health District (West Region, Cameroon) to: (i) assess the trends in infestation rates over three-years (2018–2020) using health facility registers, and (ii) investigate, in 2020, the contamination rates of the environment by dissemination stages of soil-transmitted helminths using the sucrose centrifugal flotation method. The overall soil-transmitted helminth infestation rate was 4.9% (95% CI: 4.3–5.6), significantly lower than the overall soil contamination rate (12.0%; 95% CI: 8.2–17.2). These results are supportive of the low endemicity level of STHs in the Mifi Health District, but environmental pollution by dissemination stages of the parasites outlines that the risk of transmission is still persistent. It therefore appears compulsory to account for the environment when considering policy/recommendations for transmission interruption and stopping MDA, as it is in the case with vector-borne diseases.

**Keywords:** soil transmitted helminthiasis; transmission; persistence; Mifi Health District; Cameroon



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## 1. Introduction

Soil-transmitted helminthiasis (STH), caused by four major parasitic nematode species: *Ascaris lumbricoides*, *Trichuris trichiura*, *Necator americanus*, and *Ancylostoma duodenale*, is the most widely distributed Neglected Tropical Disease (NTD) worldwide [1,2]. It is highly endemic in the developing world and remains a major public health concern in the poorest and most deprived communities, especially where sanitation is poor. It affects one quarter of the world population and is responsible for the loss of more than three million disability-adjusted life years (DALYs) [3,4]. The harmful effects on children are diverse and alarming and including detrimental effects on the survival, appetite, growth and physical fitness, school attendance, and cognitive development of school-age children. In Cameroon, STHs are widely distributed all over the country; it is estimated that more than 10 million people are infected with the four major soil-transmitted helminths species, school-age children being the most infected [5–7].

The control of STH in Cameroon, as recommended by the World Health Organization (WHO) [8,9], is principally based on large-scale deworming through annual mass drug

administration (MDA) of mebendazole to at-risk populations, especially pre-school and school-age children [7]. This strategy has significantly reduced the prevalence and intensity of infection, indicative of transmission interruption in certain foci [10–12]. However, this intervention approach, targeting only a subset of the population, even though at high risk for infestation, does not prevent re-infestation as prevalence may return to baseline levels 6–8 months after treatment [13], and the disease was found to be persisting in some foci [14,15].

Soil-transmitted helminths are so called because of the role played by the soil in their transmission, by providing favorable conditions for the development of their infective stages. Indeed, soil-transmitted helminth eggs exhibit an extreme resistance to environmental stressors, and may survive for years in soils [16]. This suggests that even though the transmission of the disease can be considered interrupted because of the absence of parasites in the human population, the risk of transmission might still exist, especially in a context where no intervention targeting environment, such as improved sanitation that can reduce soil contamination with infective eggs, is implemented. This study aimed to assess the soil contamination rate by soil-transmitted helminth eggs, with regards to the infestation rate among human populations.

## 2. Results

### 2.1. Retrospective Survey in Health Facilities

A total of 4856 patients, from whom a stool sample examination was requested, were retrieved from the registers of the five health facilities visited. The sex ratio was female-biased (2.1). The patients were aged 1–98 years old (Median: 22; Interquartile range, IQR: 8–36). Data collected in Djeleng Sub-Divisional Medical Centre represented 65.5% of all the entries, whereas Famchouet Sub-Divisional Medical Centre was the least represented, with 3.6% of the overall patients.

Among the 4856 patients who visited health facilities and for whom a stool examination was performed, 239 (4.9%; 95% CI: 4.3–5.6) were infected with at least one soil-transmitted helminth species. *A. lumbricoides* was almost the only soil-transmitted helminth species found (238/239; prevalence: 4.9%; 95% CI: 4.3–5.5); only one case of *T. trichiura* (0.02%; 95% CI: 0.00–0.10) and no case of hookworm (either *Necator americanus* or *Ancylostoma duodenale*) was reported. Table 1 summarizes the infestation rates for soil-transmitted helminth species, according to gender, age, community/health facility, and year of health facility attendance. Soil-transmitted helminth infections were similar between males and females (Chi-square: 0.29; df: 1;  $p = 0.592$ ) and over the three years of follow-up (Chi-square = 4.07; df: 2;  $p = 0.131$ ). Regarding age groups, school-aged children and adolescents were significantly more infected with soil-transmitted helminths than their older counterparts ( $p = 0.0001$ ). Additionally, patients attending the Bapi health facility were significantly more infected with soil-transmitted helminths compared to the other health facilities ( $p = 0.0001$ ).

The infestation rate for soil-transmitted helminths varied from one month to another during the same year (Figure 1); a significant difference was found between months of the year in 2019 ( $p = 0.0001$ ), March exhibiting the highest infestation rate.

### 2.2. Prospective Survey in the Environment

A total of 200 soil samples were collected in all five targeted communities, in different biotopes including roads, dustbins, behind houses, classrooms, and market stores. Among these 200 soil samples collected, 24 were presented with soil-transmitted helminth eggs, for an overall contamination rate of 12.0% (95% CI: 8.2–17.2) (Table 2). The distribution of soil-transmitted helminths in the environment was similar between the sampling biotopes ( $p > 0.05$ ). Eggs from the genus *Ascaris* were the most prevalent (10.0%; 95% CI: 6.6–14.9) in soil samples, those of *Trichuris* being found in 2% (95% CI: 0.8–5.0) of the samples collected. As it was observed in the retrospective survey, no case of hookworm (either *Necator americanus* or *Ancylostoma duodenale*) was found in soil samples collected.

**Table 1.** Soil-transmitted helminth infestation rates over three years according to gender, age groups, health facilities, and years of health facility attendance.

Characteristics	No. Patients Examined	No. Patients Infected	Infestation Rate (95% CI)	Chi-Square (p-Value)
<b>Gender</b>				
Female	3307	159	4.8 (4.1–5.6)	0.290 (0.592)
Male	1549	80	5.2 (4.2–6.4)	
<b>Age groups (years)</b>				
≤10	1444	105	7.3 (6.0–8.7)	65.056 (0.0001)
(11–20)	863	56	6.5 (5.0–8.3)	
(21–30)	975	13	1.3 (0.8–2.3)	
(31–50)	878	20	2.3 (1.5–3.5)	
≥51	696	45	6.5 (4.9–8.5)	
<b>Health facilities</b>				
Badiembou	324	52	16 (12.5–20.4)	1205.622 (0.0001)
Bapi	538	179	33.3 (29.4–37.4)	
Djeleng	3179	1	0 (0.0–0.2)	
Famchouet	176	7	4 (1.9–8.0)	
Kongso	639	0	0 (0.0–0.6)	
<b>Years</b>				
2018	1634	72	4.4 (3.5–5.5)	4.066 (0.131)
2019	2076	98	4.7 (3.9–5.7)	
2020	1146	69	6 (4.8–7.6)	
<b>Total</b>	<b>4856</b>	<b>239</b>	<b>4.9</b>	

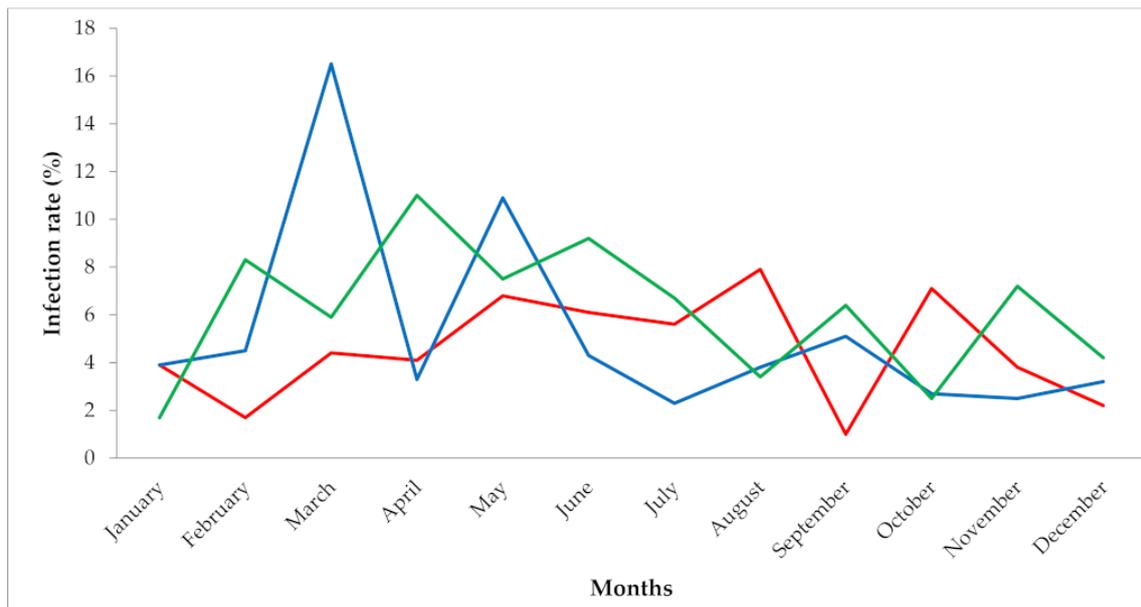
No: number of; CI: confidence interval.

**Table 2.** Soil-transmitted helminth contamination rates according to sampling biotopes.

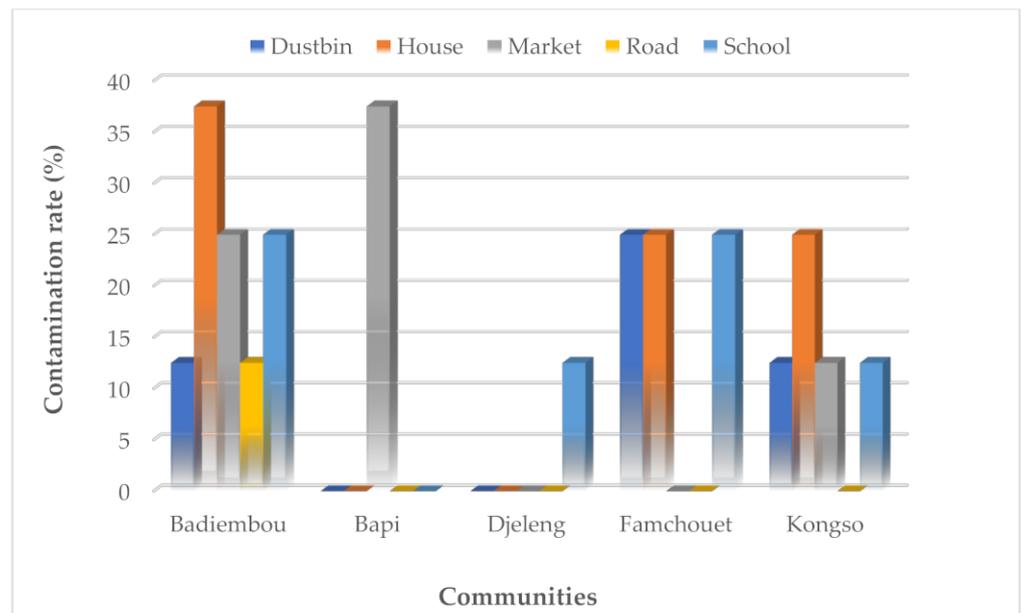
Sampling Biotopes	No. Samples Examined	N with <i>Ascaris</i> (%)	N with <i>Trichuris</i> (%)	N with STH (%)
Dustbins	40	4(10)	0	4(10)
Markets	40	6(15)	0	6(15)
Roads	40	1(2.5)	0	1(2.5)
Schools	40	5(12.5)	1(2.5)	6(15)
Houses	40	4(10)	3(7.5)	7(17.5)
<b>Overall</b>	<b>200</b>	<b>20(10)</b>	<b>4(2)</b>	<b>24(12)</b>

No: number of; N: Number.

Soil-transmitted helminths' contamination rates varied from one sampling biotope to another in each of the selected communities (Figure 2). This variation in contamination rates was not significantly different between sampling biotopes, except in Bapi where 37.5% of samples collected behind the market stores were positives (Chi-square = 12.97;  $p = 0.011$ ).



**Figure 1.** Monthly soil-transmitted helminths' infestation trends over three years. Lines represent infestation rates in 2018 (red), 2019 (blue), and 2020 (green).



**Figure 2.** Soil-transmitted helminth contamination rates in different sampling biotopes in selected communities.

### 2.3. Comparison between Infestation Rates in Health Facilities and Contamination Rates of the Environment

Globally, out of the 4856 patients who visited health facilities and 200 soil samples collected in different environmental biotopes, 239 (4.9%; 95% CI: 4.3–5.6) and 24 (12%; 95% CI: 8.2–17.2), respectively, harbored/were positives for at least one soil-transmitted helminth species. The overall contamination rate of the environment was significantly higher than the overall infestation rate among patients visiting health facilities of the same communities (Chi-square = 19.52,  $p < 0.0001$ ). When considering only the year 2020, especially the months (September to December) when soil samples were collected, the infestation rate at the health facility was 4.8% (95% CI: 3.0–7.7), significantly higher than the soil contamination rate (Chi-square = 9.26,  $p = 0.0023$ ). In almost all the selected com-

munities, soil-transmitted helminths were significantly more abundant in soil samples than among the human population ( $p < 0.05$ ), except in Bapi, where soil-transmitted helminth infestation recorded in health facilities was significantly higher than the contamination rate of the environment ( $p = 0.0007$ ) (Table 3).

**Table 3.** Comparison between soil-transmitted helminth contamination rates in the environment and infestation rates in the health facilities.

Community	Sites	N Examined	No Positives (%)	Chi-Square	<i>p</i> -Value
Badiembou	Health facility	121	23 (19.0)	0.23	0.6315
	Environment	40	9 (22.5)		
Bapi	Health facility	122	44 (36.1)	11.94	0.0005
	Environment	40	3 (7.5)		
Djeleng	Health facility	614	0 (0.0)	Fisher exact test	0.0612
	Environment	40	1 (2.5)		
Famtchouet	Health facility	84	2 (2.4)	Fisher exact test	0.0136
	Environment	40	6 (15.0)		
Kongso	Health facility	205	0 (0.0)	Fisher exact test	<0.0001
	Environment	40	5 (12.5)		
<b>Overall</b>	<b>Health facility</b>	<b>1146</b>	<b>69 (6.0)</b>	<b>9.46</b>	<b>0.0021</b>
	<b>Environment</b>	<b>200</b>	<b>24 (12.0)</b>		

N: Number; No: number of. Only retrospective infestation rates computed from data collected in the registers of health facilities in 2020 were compared to contamination rates in the environment as soil samples were collected in 2020.

### 3. Discussion

This study aimed to assess the trend towards elimination of STH in the Mifi Health District through a retrospective survey, and to investigate whether the risk of soil-transmitted helminth transmission was still present through a prospective survey to search for dissemination stages of the parasites in soil samples.

The three-year retrospective survey revealed that the overall prevalence of STH was quite low (4.9%), suggestive of low endemicity levels, as was previously demonstrated during a cross-sectional prospective survey, both in the human population and the environment, conducted in the Bangangte Health District (West Region, Cameroon), located about 50 km from the Mifi Health District [17]. In fact, a continuing decreasing trend has been observed since 1987 (prior to intervention in the Mifi Division, with prevalence of 68.2% and 82.9% for *A. lumbricoides* and *T. trichiura*, respectively), suggesting a good momentum towards elimination [5,6,10–12]. This could be the result of the public health interventions (Mebendazole-based deworming campaigns) that have been implemented since 2007 by the Ministry of Public Health through its National Program for Schistosomiasis and Soil-Transmitted Helminthiasis Control [18]. In addition, it is worth mentioning that these populations are used to self-deworming by self-medication, especially adult populations that are not targeted by deworming campaigns, as this was declared by health staff as part of informal discussions during the retrospective survey. The comparison of the prevalence of STHs between the age groups revealed a significant difference between adult and young patients ( $p = 0.0001$ ), children less than 10 years old (mostly pre-school and school-age children) and adolescents (less than 20 years old) being the most infected as they are the most exposed to the infestation, and primarily targeted by MDA against STHs [18]. The monthly follow-up revealed a significantly high infestation rate in March 2019, the Bapi Health Area presenting with the highest infestation rate. This peak in March 2019 is difficult to explain with available information regarding both the epidemiological pattern of the disease and the drug coverage, and further investigations are needed to shed light on this particular situation for further and appropriate decision making.

The prospective survey revealed that the overall soil contamination rate was 12.0%, indicative of an important contamination of the environment by disseminating stages

of soil-transmitted helminths. This contamination rate is relatively high compared to the soil contamination rate in the Bangangte Health District (3.3%) [17], but quite low compared to the rates (26.79%, 28.5%, and 78%) in other studies conducted in Cameroon, Nepal, and Bangladesh, respectively [19–21]. The differences in contamination rates of the environment between these studies may be due to environmental factors such as climate, topography, altitude, temperature, type of soil, rainfall, the use of human feces as fertilizer for crops [22], and the level of hygiene and sanitation [23], as well as the performance of programs (drug coverage during deworming campaigns) that can favor the dissemination of the eggs. This environmental pollution is supportive of persisting risk of transmission of STH in the Mifi Health District, as these disseminating stages can survive for many years in the environment. *Ascaris* spp. was the most prevalent soil-transmitted helminth species in all the five selected communities and sampling biotopes. This confirms the cosmopolitan character of that species as previously largely documented [24,25]. This can be explained by the fact that its eggs can survive for several months in soil, even in adverse environmental conditions due to their inner shell being of a lipoprotein nature, which makes them resistant to drastic environmental conditions [26]. In addition, it is known that *Ascaris* eggs can be over dispersed in the environment, as a single female may lay more than 200,000 eggs per day. The contamination rate by soil-transmitted helminths was similar ( $p = 0.25$ ) between sampling biotopes, suggesting that the distribution of these parasites in all the environmental biotopes is even and mostly depends on population habits. However, a significant difference in the distribution of soil-transmitted helminths between biotopes was observed in the Bapi community where only samples collected behind market stores were positive ( $p = 0.011$ ). This can be explained by the fact that Bapi is a junction community where people from neighboring communities come during market days to buy and sell food crops, thus taking advantage to do their laboratory examinations at the health center. This can further explain why the infestation rate is the highest in Bapi health facility despite the low contamination rate in all the biotopes other than the market, as people are likely to practice open defecation despite the availability of latrines because of crowds during market days. This might indicate that there is no (or low) active transmission of soil-transmitted helminths in the Bapi community, as the sanitation level in this community is of a high standard, with good sanitation and hygiene behaviors.

Soil-transmitted helminths are more abundant in the environment than among the population in all communities ( $p < 0.05$ ), except for Bapi. Despite the low endemicity of STH among the population, the presence of dissemination stages of these parasites in the environment suggests that the risk of infestation and transmission of soil-transmitted helminths is still prevailing in the selected communities, thus raising the need to complement preventive chemotherapy with interventions in the environment, as well as water, sanitation, and hygiene (WASH) and health education for behavior change, to sustain transmission interruption/elimination of STH and avoid potential recrudescence of the disease.

In this study, purposive sampling was used for selection of communities where soil samples were collected, as the presence of a health facility was compulsory for comparison of soil-transmitted helminth infestation rate among human populations and the contamination rate of the environment. Purposive sampling can be prone to representativeness bias because of the subjectivity and non-probabilistic nature of unit selection, thus limiting the generalization of the findings. However, this potential bias can be mitigated as the judgement was based on clear criteria (availability of health facility), especially when considering the fact that a Health Area is usually served by only one health facility. Additionally, the selection of the first and then the subsequent sampling spot in each biotope was done by convenience. This approach can bias the repeatability of the study, which would have been ensured by random sampling among a list of biotopes.

## 4. Materials and Methods

### 4.1. Study Area and Population

This study was carried out in the Mifi Health District (West Region, Cameroon), chosen because it is ranked among foci where STH transmission has almost been interrupted, based on the decreasing trend in parasitological indicators. Indeed, STH prevalence significantly decreased over two decades (1987–2012), from 68.2%, 82.9%, and 7.2% to 3.4%, 2.9%, and 0.0% for *A. lumbricoides*, *T. trichiura*, and hookworms, respectively [5,6,10,11]. The climate prevailing in the Mifi Health District is tropical with two seasons: the dry season from November to May and the rainy season from June to October. The mean annual temperature is about 20.9 °C, with the warmest (27.6 °C) in February and the coldest (14.3 °C) in September; the mean annual rainfall is about 1871 mm [27]. The population of the study area is about 362,142 inhabitants [28]; their main occupations are farming of food crops and trading because Bafoussam (5.39999° N; 10.41666° E; 1450 m above sea level), the city capital of the West Region of Cameroon, is the third most financially important city of the country, with many trading activities.

### 4.2. Study Design

A two-stage cluster sampling was used in this study, the first sampling-stage being Health Areas, and the second one being communities. Indeed, the Health Areas constituting the Mifi Health District were purposively organized into five clusters (north, south, east, west, and center), with one Health Area being randomly selected in each cluster to ensure an optimal geographical coverage. Five Health Areas (Badiembou, Bapi, Djeleng, Famchouet, and Kongso) were therefore chosen among the 20 constituting the Mifi Health District (Supplementary Table S1). In each Health Area, one community was purposively selected where a health facility was available.

In each community, a prospective cross-sectional survey was carried out from September to December 2020, which is from the end of the dry season to the beginning of the rainy season. Soil samples, in various biotopes not exposed to direct sunlight along roads, around dustbins, behind houses, behind classrooms, and behind market stores, were collected to assess the contamination rate of the environment by soil-transmitted helminths. In addition to the prospective cross-sectional survey, a three-year (January 2018–December 2020) retrospective survey was conducted in the health facilities of each of the five communities to collect available STH historic data in registers. The retrospective survey was focused on the previous three years because of availability of data over these last three years. Information taken in consideration from the registers of each health facility laboratory were age, gender, the community of residence, the month, and the year when the patient visited the laboratory for stool examination, using the direct wet mount technique [29].

### 4.3. Soil Samples Collection and Processing

Soil samples were collected using a hand shovel. In each Health Area, eight soil samples were collected in each of the five sampling biotope (along roads, around dustbins, behind houses, behind classrooms, and behind market stores), for a total of 40 samples per Health Area. In each biotope, sample spots were separated from one another by a distance of about 60 m. About 100 g of each soil sample, taken from a depth of about 50 cm from the surface, was collected, stored in an airtight plastic bag to avoid any extra contamination, and transferred to the laboratory [17,30]. In the laboratory, samples were dried overnight at room temperature and protected from light. The next morning, the samples were sieved in a 150 µm mesh. Then, 2 g of the powdered soil were put into four different test tubes and 13 mL of distilled water added. Test tubes were centrifuged at 100 rpm for 5 min, the supernatants were discarded, and sediments homogenized again with the same volume of distilled water. The test tubes were centrifuged again at 800 rpm for 5 min and the supernatants discarded. Sucrose solution with a specific gravity ( $d = 1.12$  g/mL) was added in the test tubes that were vigorously shaken then centrifuged at 100 rpm for 5 min. The supernatants were transferred into four new test tubes and a sucrose solution was added

for the flotation of helminth eggs, if present. Cover slips were then placed on top of each test tube for 10 min. Thereafter, the cover slips were carefully removed and placed on slides for microscopical examination (magnitude 100 X and 400 X). If present in the preparation, parasitic stages were identified based on the morphological characteristics such as the shape, the nature of eggshell, and the number of blastomeres [31,32]. Samples collected from the same spot were processed in quadruplicate. During the sample processing, when one of the four replicates was positive for any of the targeted soil-transmitted helminths, the soil sample was considered to be contaminated.

#### 4.4. Data Analysis

All relevant data were entered on a purpose-built Microsoft Excel spreadsheet. Data analysis was performed using the software PASW Statistics 18.0 (SPSS Inc., Chicago, IL, USA). Contamination (soil samples) and infestation (human population) rates were expressed as percentage with 95% confidence interval. Pearson's Chi-square test was used to compare contamination rates between sampling biotopes and communities/Health Areas, as well as infestation rates between genders, age groups, communities/Health Areas, months, and years of health facility attendance. Contamination rates of the environment were finally compared to infestation rates among human populations attending health facilities using Pearson's Chi-square and Fisher Exact probability tests. The threshold for significance was set at 5% for all statistical analyses.

## 5. Conclusions

The present study revealed that although infestation rates assessed at the level of health facilities indicate that transmission might be interrupted or is progressing towards interruption, the contamination rates of the environment are supportive of persistent risk of STH transmission. Therefore, even though MDA can be useful to interrupt STH transmission, interventions in the environment (water, sanitation, and hygiene, so-called WASH) as well as health education for behavior change appear compulsory to prevent re-infestation, reduce soil contamination with infective eggs, and avoid potential recrudescence for successful and sustainable elimination of the infection. Further studies might be worth carrying out to (i) confirm the soil-transmitted helminth species, likely using molecular assays, as parasite eggs from humans and animals are similar, and (ii) to check whether these parasite eggs that are present in the environment are viable.

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/article/10.3390/parasitologia1030011/s1>, Table S1: Number of communities and population of each Health Areas of the Mifi Health District.

**Author Contributions:** Conceptualization, L.S. and H.C.N.-D.; methodology, L.S., E.N.O.A., E.M., T.G., and H.C.N.-D.; software, L.S. and H.C.N.-D.; validation, L.S., E.N.O.A., T.G., and H.C.N.-D.; formal analysis, L.S., T.G., and H.C.N.-D.; investigation, E.M.; resources, L.S., E.M., and T.G.; data curation, L.S., E.M., T.G., and H.C.N.-D.; writing—original draft preparation, L.S. and H.C.N.-D.; writing—review and editing, L.S., E.N.O.A., E.M., T.G., and H.C.N.-D.; visualization, L.S.; supervision, L.S., E.N.O.A., and T.G.; project administration, L.S. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Patient consent was waived because data involving human participants was collected retrospectively.

**Data Availability Statement:** The data presented in this study are available on request from the corresponding author. All relevant data are within the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

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