

Pooling poo for Strongyloides assessment in communities

Grace Opoku Gyamfi

gogyamfi@noguchi.ug.edu.gh

Noguchi Memorial Institute for Medical Research

Yvonne Ashong

Noguchi Memorial Institute for Medical Research

Millicent Opoku

La Trobe University

Millicent Selassie Afatodzie

Noguchi Memorial Institute for Medical Research

Emmanuel Frimpong Gyekye

Noguchi Memorial Institute for Medical Research

Seth Offei Addo

Noguchi Memorial Institute for Medical Research

Daniel Oduro

University of Ghana

Dziedzom K. de Souza

Noguchi Memorial Institute for Medical Research

Research Article

Keywords: Strongyloides spp., pool screening, prevalence, Ghana

Posted Date: June 29th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-10005455/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at BMC Infectious Diseases on August 21st, 2026. See the published version at <https://doi.org/10.1186/s12879-026-14237-y>.

Abstract

Background

Strongyloidiasis, caused by *Strongyloides spp.*, is a neglected tropical disease with significant but underreported public health impact. In Ghana, particularly in the Volta and Oti regions, data on prevalence is limited due to inadequate surveillance and low sensitivity diagnostics.

Methods

This cross-sectional study analyzed 1,392 archived human stool samples from seven communities across three districts (Adaklu, Hohoe, Nkwanta North). Samples were pooled (up to 10 per pool) for DNA extraction and PCR targeting the 18S rRNA gene. Positive pools were unpooled for individual testing.

Results

Overall pooled prevalence was 7.2% (95% CI: 5.5–9.3), while individual prevalence was 18.5% (95% CI: 16.5–20.6), with notable variation across communities. Lancha (27.9%) and Dawanu (27.4%) showed the highest rates. Logistic regression found no significant associations with sex, age, or district.

Conclusion

The pooling strategy proved sensitive and resource efficient, supporting its use in *Strongyloides* surveillance. This study highlights the need for sensitive diagnostics and integrated surveillance to guide targeted public health interventions.

Introduction

Strongyloides species are soil-transmitted helminths capable of persisting for decades if left untreated (1). Infection with *Strongyloides* results in strongyloidiasis, one of the overlooked neglected tropical diseases (NTDs). As of 2025, the World Health Organization (WHO) estimates over 600 million infections globally, though the true prevalence is likely higher due to diagnostic limitations despite its health implications (2–4). It is widespread throughout Asia and sub-Saharan Africa, South and central America, and the Pacific. This neglected parasitic disease is most prevalent in tropical and subtropical areas with poor sanitation and hygiene, particularly in rural settings and among socioeconomically disadvantaged populations (5, 6). Across Africa, strongyloidiasis is among the most neglected NTDs, with significant but underreported infection burdens (7, 8). The parasite exhibits unique auto-infective capabilities, facilitating chronic and severe disease, especially among immunocompromised individuals. The intricate life cycle of the worm allows it to persist, and remain undetected for extended periods, sometimes lasting decades. While many infections are asymptomatic, hyperinfection or disseminated disease can

be fatal, underscoring the importance of early, accurate detection, especially in endemic zones lacking routine screening(9, 10). Case reports have documented misdiagnosis of strongyloidiasis, including cases initially treated as meningitis, Guillain-Barre syndrome, or gastrointestinal disease, often resulting in delayed diagnosis and fatal outcomes (11, 12). Transmission occurs via skin penetration of infective filariform larvae upon contact with contaminated soil. The parasite alternates between free living and parasitic phases, complicating detection and control (9, 10). Common diagnostic methods (Kato-Katz, flotation, and sedimentation) are affordable but lack sensitivity for low intensity infections due to intermittent larval shedding, thus, cases are underestimated (13, 14). Techniques like agar plate culture and Baermann are more sensitive but require fresh samples, limiting large scale application (13–15). More advanced assays such as PCR, ELISA and sequencing are increasingly used in research settings and offer much higher sensitivity and specificity than conventional stool methods, making them suitable for accurate detection (15–17). Ivermectin remains the consensus first line treatment for *Strongyloides* infections. Traditionally, mass drug administration (MDA) programs for soil-transmitted helminths have used albendazole or mebendazole, which have limited efficacy against *Strongyloides*. Although ivermectin have been widely used to control Onchocerciasis, its incorporation into integrated MDA programs specifically to target *Strongyloides* has only occurred more recently. WHO now recommends community wide ivermectin MDA in endemic areas, supported by showing prevalence reductions near 84% (4, 18). Annual single dose ivermectin (200 µg/kg) is endorsed for school-aged and adult populations in regions with $\geq 5\%$ prevalence (3, 4). The use of pool screening assessment methods has been implemented for many diseases and shown to be effective. Pooling multiple stool samples for DNA extraction and further PCR for stool samples has shown to reduce reagent costs, time, and labour (13, 19). This method has proven effective in many parasitic and viral infections for detecting low prevalence infections (10, 13, 20). This study aimed to evaluate the pool screening assessment method for the diagnosis of *Strongyloides* infection in communities with the goal to generate baseline data and advancing surveillance capabilities for this infection.

Methods

Study Sites

The study was a cross-sectional survey conducted between September and October 2023 from seven communities in the Volta and Oti regions: Hilhave, Afeame, Dawanu (Adaklu District, Volta Region), Abledzie (Hohoe Municipality, Volta Region), Abunyanya, Lancha, and Gborsike (Nkwanta-North District, Oti Region) [Figure 1].

The environmental conditions within these districts or regions are favorable for parasite's proliferation (21, 22). Both the Volta and Oti Regions are located in the eastern part of Ghana, stretching along the country's eastern international boundary and bounded to the north by the Northern region, to the south by the Gulf of Guinea, to the west by the Volta Lake, and to the east by the Republic of Togo (23). The Volta region is one of the sixteen administrative regions of Ghana consisting of about 8 districts. The Adaklu district, spanning an area of 800.8km² is characterized by a diverse topography including

mountains, hills, lowlands, and undulating landscapes (24). The Adaklu District is characterized by a predominantly rural population engaged primarily in agricultural occupations and encompass the Volta region. Its favorable temperature regime supports robust plant growth, enabling the successful cultivation of staple food crops such as maize, sweet potato, tomato, yam, cassava, and groundnut (24, 25). The predominant vegetation is savannah woodland, however, localized semi-deciduous forest patches exist, particularly around Adaklu-Aboadi. The district is entirely rural, lacking urban centers. Its population structure is notably youthful, with 36.4% of residents below 15 years of age and only 7.6% aged 60 years or older (24–26). The Hohoe municipal, one of the 25 administrative districts in Ghana's Volta region, covers a total land area of 1,72km². This constitutes 5.6% of the Volta Region's total land area and 0.05% of Ghana's national land area. According to the 2021 population and Housing Census, the municipality had a population of 167,016, accounting for 7.9% of the Volta Region's total population (27). The local economy is sustained by diverse activities, including agriculture, petty trading, construction, and formal sector employment. The Oti region is also one of Ghana's sixteen administrative region and forms part of the six newly established regions in 2019. Nkwanta-north district of the Oti region is in the north-eastern part of Ghana. Geographically, the district lies between latitudes 7°30'N and 8°45'N, and longitudes 0°10'W and 0°45'E (28). Its administrative capital, Kpassa, is situated about 270km south of Ho, the regional capital. According to the 2021 Population and Housing Census, the district has a population 64,553 accounting for approximately 3.0% of the region's total population (25, 27).

MDA programmes in the Volta and Oti regions of Ghana were initiated in the late 1990s through to 2000s (29–31) and remains ongoing in some areas, particularly in the Oti region (32). These programmes have primarily targeted the use of ivermectin for the control and elimination of onchocerciasis (33), and later combined with albendazole for the treatment of lymphatic filariasis (34, 35). Nkwanta north district has undergone 22 rounds of ivermectin MDA since 2014 with the recent round recorded in 2024. Hohoe municipal has also conducted 16 rounds of ivermectin MDA from 2014 through to its most recent round in 2019. In contrast, Adaklu district classified as very low endemic areas have also received only a single MDA round carried out in 2019 (36). Although ivermectin is administered in these settings, there is currently no structured MDA programme specifically targeting strongyloidiasis in Ghana, and any potential impact on *Strongyloides stercoralis* is incidental rather than programmatically driven (4).

Target Population and Sample Size

This study was conducted as part of efforts aiming to assess the effectiveness of new onchocerciasis diagnostic tests. Alongside blood samples, stool samples were collected from all consenting individuals to assess the presence of other helminth infections. Demographic data were recorded via semi-structured questionnaires. The study targeted individuals ≥ 5 years, recruited prospectively in each study community. The sample size for the original study was calculated to assess non-inferiority of sensitivity of the new onchocerciasis RDTs compared to the current standard of care. Thus, a sample size of 1,700 was estimated using the Sealed Envelope online calculator for binary non-inferiority trials (Sealed

Laboratory Analysis

Sample pooling

For each individual, 100mg of stool sample was weighed and combined with stool samples from other individuals to form a pool. Samples from each community were pooled together (up to 10 samples per pool) samples to enable prevalence estimates at the community level.

Genomic DNA Extraction

Genomic DNA (gDNA) extractions were performed using both Zymo Miniprep Plus DNA extraction kit and Qiagen Invitrogen DNeasy Kit with minor yield-enhancing modifications. The Zymo kit was initially used and once it was exhausted, the Qiagen kit was used for the few remaining samples. For DNA extraction using the Zymo kit, approximately 200mg of homogenized stool was mixed with 95µl nuclease-free water, 95µl lysis buffer, and 10µL proteinase K, vortexed, and incubated overnight at 55°C. Samples were then bead beaten and horizontally vortexed, followed by addition of 400 µL Genomic Binding Buffer, vortexed briefly and centrifuged at 12,000 x g for a minute. 400 µL of the supernatant was transferred to the Zymo-Spin ICR columns and centrifuged at 12,000 x g for 1 minute. The columns were washed sequentially with 400 µL DNA Pre-Wash and (700 µL and 200 µL) DNA Wash buffers, and the DNA was eluted in 50 µL Elution Buffer after incubating for 5 minutes and centrifuging at 12,000 x g for 2 minutes. The DNA concentration and purity were assessed using a Nanodrop. The extracted DNA were stored at – 20°C until used. Following PCR, DNA was extracted individually for the samples in the positive pools and retested.

Qiagen kit was used specifically for the DNA extraction of a few individual samples. Approximately 200 mg of stool was suspended in 200 µL phosphate buffered saline (PBS) containing 2% polyvinylpolypyrrolidone (PVPP) and frozen overnight at – 20°C. After thawing, the suspension was heated at 100°C for 10 minutes and then mixed with 180 µL Tissue Lysis Buffer (ATL) and 20 µL Proteinase K, followed by overnight incubation at 55°C. Subsequently, 400 µL Buffer AL was added to 400 µL of lysate, vortexed, and incubated at 70°C for 10 minutes. Samples were homogenized with glass beads by horizontal vortexing for 15 minutes, briefly centrifuged, and the supernatant was combined with 400 µL ethanol (96–100%) and mixed thoroughly. The mixture was loaded onto a DNeasy spin column in 600 µL aliquots and centrifuged, with the remaining mixture applied to the same column. The columns were washed sequentially with 500 µL Buffer AW1 and 500 µL Buffer AW2, followed by a final spin to dry the membrane. DNA was eluted in 50 µL Elution buffer after a 1 min incubation and centrifugation at 8,000 rpm.

Conventional PCR Amplification: Genus-specific [forward: 5'-AAAGATTAAGCCATGCATG-3' and reverse: 5'-GCCTGCTGCCTTCCTTGGA-3'](37) and Species-specific primers [forward: 5'-GAATTCCAAGTAAACGTAAGTCATTAGC3', reverse: 5'-TGCCTCTGGATATTGCTCAGTTA-3' (37, 38)] targeting the 18S rRNA gene of the parasite were used. PCR conditions included initial denaturation at 95°C for 2 minutes; 35 cycles of 95°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; and a final extension at 72°C for 5 minutes. The PCR assay was carried out using the GoTaq Master Mix kit on a thermal cycler (Takara Bio Inc.). Each 10 µL reaction contained 5.0 µL of 2× GoTaq Master Mix, 2.6 µL nuclease free water, 0.2 µL each of forward and reverse primers, and 2.0 µL of DNA template. PCR amplicons were resolved on 2% agarose gels prepared in 1× TAE buffer and stained with SYBR View DNA gel stain. Approximately 5 µL of each product, alongside a 100 bp DNA ladder, was loaded and electrophoresed at 120 V (140 mA) for 40 minutes. Gels were visualized under a UV transilluminator, and samples were considered positive when bands of the expected sizes (392 bp and 101 bp) of genus and species-specific primers respectively were observed in a valid assay. Positive controls (confirmed *Strongyloides* DNA) and non-template controls (NTC) were included in all PCR reactions.

Quality Control

DNA extractions included a negative extraction control (NEC). Each PCR run included a positive, NEC and a non-template control (NTC). Valid tests required negative NEC, NTC and correct positive control bands.

Data Analysis

Data was recorded in Microsoft Excel, with charts generated in Excel and R. Pooled data was analyzed using Pooled Screen software(39) to estimate infection prevalence. Associations between individual *Strongyloides* infection and demographic factors (age, sex, community, and district) were assessed using bivariate and multivariate regression models in Stata [$p < 0.05$] and odds ratio (Ors) were estimated. All prevalence data were computed alongside their 95% confidence intervals. Prevalence data was also visualized in ArcGIS.

Results

Demographic Characteristics of Study Participants

1,392 participants provided stool samples used in this *Strongyloides* assessment study. 133 pooled stool samples were generated, with a maximum of 10 samples per pool. The pool range was 6 to 10. Females constituted 58.76%, while males accounted for 41.24%. Participants aged 10–19 years formed the largest age group (28.66%), followed by those younger than 10 years (14.58%). The smallest proportion of participants was observed in the 50–59-year age group (8.33%) [Figure 2]. In terms of geographic distribution, Nkwanta District contributed the highest proportion of samples (52.66%), followed by Adaklu District (36.35%) and Hohoe (10.99%). At the community level, Abunyanya had the highest representation (39.58%), followed by Hilhave (14.51%). The remaining communities, including Afeyeame, Abledzie, Lancha, and Gborsike, contributed smaller proportions of the total sample. About 50.8% of

respondents reported that they received ivermectin, while 48.9% indicated they did not receive it. A very small portion (0.4%) could not remember. Overall, the responses show that nearly equal proportions of the population received and did not receive ivermectin during the distribution.

Pooled and Individual Prevalence Estimations

Seventy pools were positive out of 133, resulting in an overall pooled prevalence of 7.2% (95% CI: 5.5–9.3) [Table 1]. Abledzie, in the Hohoe District, had the highest maximum-likelihood estimate at 17.3% (95% CI: 8.4–31.4), while Afeyeame recorded the lowest at 3.2% (95% CI: 0.62–9.1). District-level prevalence was highest in Hohoe at 16.7% (95% CI: 7.92–30.95), compared to the lowest in Adaklu at 5.34% (95% CI: 3.06–8.49). At the regional level, Oti Region recorded the highest pooled prevalence estimate of 7.28% (95% CI: 4.99–10.15).

Testing of individual samples from positive pools showed the highest prevalence in Lancha at 27.94% (95% CI: 20.99–36.14) and the lowest in Afeyeame at 12.86% (95% CI: 8.22–19.55) [Table 2], [Figure 3]. Hohoe district had the highest prevalence (19.88%, 95% CI: 16.29–29.56) and Nkwanta-North district the lowest (18.07%, 95% CI: 14.24–19.67). Regionally, prevalence was 18.71% in Volta and 18.07% in Oti. *Strongyloides spp.* was detected in all surveyed communities, with an overall prevalence of 18.5% [95% CI: 16.5–20.6].

Table 1
Maximum-Likelihood Prevalence of Pooled Samples

Communities	Total Number of Pools	Number of Pools Positive	Maximum Likelihood Estimate [%]	95% Confidence Interval
Hilhave	17	5	3.5	[1.1, 8.2]
Afeyeame	11	3	3.2	[6.2, 9.1]
Dawanu	16	11	11.0	[5.1, 20.0]
Abunyanya	55	23	5.3	[3.2, 8.1]
Lancha	14	11	15.2	[7.0, 28.6]
Gborsike	5	4	15.5	[3.9, 40.1]
Abledzie	15	13	17.3	[8.4, 31.4]
Overall	133	70	7.2	[5.5, 9.3]
District				
Adaklu	45	19	5.3	[3.1, 8.5]
Nkwanta-North	74	39	7.3	[4.9, 10.0]
Hohoe	14	12	16.7	[7.9, 30.9]
Region				
Volta	59	31	7.1	[4.7, 10.3]
Oti	74	39	7.3	[4.9, 10.0]

Table 2
Prevalence in Individual Samples across Community,
District, and Region

Community	Prevalence %	[95% CI Interval]
Abledzie	22.22	[16.29% – 29.56%]
Abunyanya	13.61	[11.99% – 16.74%]
Afeyeame	12.86	[8.22% – 19.55%]
Dawanu	27.44	[21.11% – 34.82%]
Gborsike	21.74	[11.91% – 36.33%]
Hilhave	18.32	[13.54% – 24.39%]
Lancha	27.94	[20.99% – 36.14%]
District		
Adaklu	18.41	[16.51% – 23.47%]
Hohoe	19.88	[16.29% – 29.56%]
Nkwanta-North	18.07	[14.24% – 19.67%]
Region		
Volta	18.71	[15.94–21.82]
Oti	18.07	[14.61–22.08]

Socio-demographic characteristics and *Strongyloides* infections of study participants

The crude (unadjusted) analysis (cOR) showed no statistically significant association between *Strongyloides* infection and sex, age group, or district [Table 3]. Although males had slightly higher odds of infection than females [cOR = 1.10, 95% CI: 0.84–1.45, $p = 0.48$], this difference was not significant. By contrast, infection risk varied significantly at the community level. In both crude and adjusted models, residence in Lancha and Dawanu were associated with higher odds of infection, with Dawanu showing a significant association (aOR = 1.72, 95% CI: 1.05–2.82, $p = 0.003$). Individuals aged 60 years and above recorded the highest prevalence (20.67%) and increased odds of infection [cOR = 1.208, 95% CI: 0.708–2.063], whereas those aged 50–59 years had the lowest prevalence (14.66%) and reduced odds [cOR = 0.796, 95% CI: 0.425–1.492]. However, these associations were not statistically significant.

Table 3
Association between Sociodemographic Characteristics and Strongyloides Infection

Characteristic	Status of <i>Strongyloides</i>			cOR [95% CI Interval]	p- value	aOR [95% CI - Interval]	p- value
	Negative [n (%)]	Positive [n (%)]	95% CIs				
Sex							
Female	672 (82.15%)	146 (17.85%)	15.23– 20.47	Ref	-	Ref	-
Male	463 (80.65%)	111 (19.35%)	16.11– 22.59	1.103 [0.839– 1.451]	0.481	1.086 [0.819– 1.437]	0.567
Age Categories							
< 10 years	167 (82.27%)	36(17.73%)	12.47– 22.99	Ref	-	Ref	-
10–19 years	321 (80.45%)	78 (19.55%)	15.66– 23.44	1.127 [0.728– 1.744]	0.591	1.137 [0.730– 01.770]	0.570
20–29 years	153 (81.38%)	35 (18.62%)	13.05– 24.18	1.061 [0.634– 1.775]	0.821	1.211 [0.714– 2.054]	0.477
30–39 years	161 (83.85%)	31(16.15)	10.95– 21.34	0.893 [0.527– 1.51]	0.674	0.968 [0.567– 1.654]	0.908
40–49 years	115 (79.86)	29 (20.14)	13.59– 26.69	1.169 [0.679– 2.014]	0.572	1.220 [0.702– 2.119]	0.480
50–59 years	99 (85.34)	17 (14.66)	8.22– 21.09	0.796 [0.425– 1.492]	0.478	0.827 [0.438– 1.562]	0.559
60 years and above	119 (79.33%)	31 (20.67%)	14.19– 27.15	1.208 [0.708– 2.063]	0.488	1.234 [0.716– 2.124]	0.449
District							
Adaklu District	567 (81.59%)	128 (18.41%)	15.54– 21.30	Ref	-	Ref	-

Characteristic	Status of <i>Strongyloides</i>			cOR [95% CI Interval]	p- value	aOR [95% CI - Interval]	p- value
	Negative [n (%)]	Positive [n (%)]	95% CIs				
Sex							
Hohoe District	137 (80.12%)	34 (19.88%)	13.90- 25.86	1.099 [0.721– 1.676]	0.660	1.296[0.767– 2.189]	0.333
Nkwanta North District	431 (81.93%)	95 (18.07%)	14.77– 21.35	0.976 [0.728– 1.309]	0.873	1.299 [0.589– 2.865]	0.517
Community							
Abledzie	119 (77.78%)	34 (22.22%)	15.63– 28.81	1.029 [0.463– 2.284]	0.945	-	-
Abunyanya	476 (86.39%)	75 (13.61%)	10.75– 16.47	0.567 [0.270– 1.190]	0.134	0.548 [0.259– 1.159]	0.116
Afeyeame	122 (87.14%)	18(12.86%)	7.33– 18.38	0.531 [0.225– 1.252]	0.148	0.686 [0.372– 1.266]	0.228
Dawanu	119 (72.56%)	45 (27.44%)	20.61– 34.27	1.361 [0.624– 2.969]	0.438	1.717 [1.045– 2.823]	0.033
Gborsike	36 (78.26%)	10 (21.74%)	9.83– 33.65	Ref	-	Ref	-
Hilhave	165 (81.68%)	37(18.32%)	12.98– 23.66	0.807 [0.368– 1.771]	0.593	-	-
Lancha	98 (72.06%)	38 (27.94%)	20.40- 35.48	1.396 [0.138– 0.559]	< 0.001	1.361 [0.612– 3.028]	0.449

Preventive Measures and Treatment History in Relation to *Strongyloides* infection

No statistically significant difference was observed in recent deworming within the past 12 months between *Strongyloides* positive and negative participants (about 85–87%; $p = 0.801$). Ivermectin uptake for onchocerciasis was also similar, between groups, with roughly half of participants in each group having received treatment ($p = 0.582$). Among those treated, most reported receiving two to five doses ($p = 0.063$). Among infected participants, the frequency of ivermectin use over the previous three years

showed no meaningful variation ($p = 0.225$). Reported barriers to ivermectin uptake including travel, fear of side effects, dislike of medication, lack of availability, pregnancy or breastfeeding, and uncertainty about reason for treatment were not associated with infection status, as all p -values exceeded 0.05. Almost all participants rejected “absence of disease” as a reason for non-uptake, with no differences observed between groups [Table 4].

Table 4
Preventive Measures and Treatment History in Relation to Strongyloides infection

Variable / Category	Status of <i>Strongyloides</i>		
	Negative [n (%)]	Positive [n (%)]	p-value
Dewormed in the past 12 months			
Don't remember	17 (1.50%)	4 (1.56%)	0.801
No	968 (85.29%)	223 (86.77%)	
Yes	150 (13.22%)	30 (11.67%)	
Received ivermectin for onchocerciasis			
Don't remember	4 (0.35%)	1 (0.39%)	0.582
No	547 (48.19%)	133 (51.75%)	
Yes	584 (51.45%)	123 (47.86%)	
Number of times received ivermectin			
2 to 5 times	405 (69.35%)	85 (69.11%)	0.063
6 or more times	61 (10.45%)	18 (14.63%)	
Don't remember	6 (1.03%)	4 (3.25%)	
Once	112 (19.18%)	16 (13.01%)	
Number of times received ivermectin in the last three years			
Don't remember	18 (3.08%)	3 (2.44%)	0.225
None	70 (11.99%)	24 (19.51%)	
Once	84 (14.38%)	14 (11.38%)	
Thrice	175 (29.97%)	32 (26.02%)	
Twice	237 (40.58%)	50 (40.65%)	
Reasons for the non-uptake of ivermectin			
Travel			0.113
No	1124 (99.03%)	257 (100.00%)	
Yes	11 (0.97%)	0 (0.00%)	
Fear of adverse effect			

Variable / Category	Status of <i>Strongyloides</i>		
	Negative [n (%)]	Positive [n (%)]	p-value
Dewormed in the past 12 months			
No	1126 (99.21%)	257 (100.00%)	0.152
Yes	9 (0.79%)	0 (0.00%)	
Dislike for medications			
No	1132 (99.74%)	257 (100.00%)	0.409
Yes	3 (0.26%)	0 (0.00%)	
Absence of disease			
No	1135 (100.00%)	257 (100.00%)	
Absence/unavailable			
No	1064 (93.74%)	240 (93.39%)	0.831
Yes	71 (6.26%)	17 (6.61%)	
Pregnancy/breastfeeding			
No	1131 (99.65%)	254 (98.83%)	0.095
Yes	4 (0.35%)	3 (1.17%)	
Don't know			
No	922 (81.23%)	195 (75.88%)	0.051
Yes	213 (18.77%)	62 (24.12%)	

Discussion

This study provides evidence of *Strongyloides spp.* infection across the study communities in Ghana's Volta and Oti regions. Pooled results showed variable community level prevalence, with the highest MLE in Abledzie (17.3%) and the lowest in Afeyeame (3.2%). Testing of individual samples revealed a higher overall prevalence of 18.5% compared to 7.2% in pooled samples, indicating that pooled sampling may underestimate infection rates (13, 20, 40). Presenting the data for both the pooled and unpooled data provides an understanding of disease burden and guides the design of future surveys in similar endemic settings, as demonstrated in studies by Papaiakevou et al. and James E. Truscott (10). These findings, nonetheless, suggest evidence of transmission.

Continuous monitoring is important due to autoinfection risks and potential disease severity. The observed prevalence underscores the need for integrated public health interventions, including sanitation improvements, community education, and routine surveillance. The widespread prevalence of infection across age groups and sex suggests that *Strongyloides* is not restricted to a specific demographic, as reported by Forrer et al. (41). The higher prevalence observed in individuals aged 60 years and above may reflect the parasite's persistence due to its autoinfective life cycle, weak immune system, and occupational exposures such as farming, which involves frequent soil contact. These findings are consistent with observations from elsewhere (41, 42).

The absence of a significant association between ivermectin receipt and *Strongyloides stercoralis* infection in many settings may reflect several important factors. First, ivermectin MDA programs in these settings have largely been designed to reduce the burden of onchocerciasis, rather than to specifically interrupt transmission of *S. Stercoralis*. As a result, the frequency of MDA and target populations of these programs may not be optimized for Strongyloidiasis control. Although periodic ivermectin distribution for onchocerciasis has in some cases reduced *Strongyloides* prevalence, these effects vary with coverage and epidemiologic context (43). Secondly, the dosing regimens commonly used in onchocerciasis MDA programs differ from those recommended for treating *Strongyloides* infection. Clinical guidelines and studies indicate that effective treatment of uncomplicated strongyloidiasis generally involves ivermectin dosed at approximately 200 µg/kg (4), whereas MDA programs for onchocerciasis often use a dosage around 150 µg/kg with spacing focused on controlling microfilariae rather than full parasite eradication(44). These differences in both dosage and treatment strategy likely influence the effectiveness of MDA against *S. stercoralis*. Also, the unique autoinfective lifecycle of *S. stercoralis* allows the parasite to sustain infection within a host for extended periods, potentially reducing the apparent impact of intermittent community programs (45). Although strongyloidiasis prevalence may decrease in areas with high ivermectin coverage over time, autoinfection can maintain chronic infection and complicate interpretations of cross-sectional prevalence data (43, 45). Finally, reinfection and incomplete treatment coverage further limit the observable impact of MDA on strongyloidiasis. Even where ivermectin distribution is widespread, heterogeneous uptake and ongoing environmental exposure to infective larvae may diminish the protective effect in population surveys (4, 43, 45).

There were a couple of limitations to this study. Firstly, pooling was initially performed during DNA extraction to conserve resources. Although this strategy is cost-efficient and commonly used in surveillance, it can lower sensitivity for detecting very low intensity infections because of dilution. This limitation was partly addressed by unpooling PCR positive samples and repeating extraction at the individual level, which helped ensure more accurate estimates of individual prevalence. However, PCR negative pools were not subsequently unpooled and rested, meaning that low burden infections concealed by pooling may have gone unnoticed. Additionally, environmental and behavioral risk factors such as sanitation conditions, footwear practices, contact with domestic animals, and occupational exposures were not systematically evaluated in this study.

In conclusion, this study provides evidence of *Strongyloides* spp. infection across rural communities in Ghana's Volta and Oti regions. It validates pooled sampling surveillance as a feasible tool for detecting *Strongyloides* spp. in under resourced endemic communities, despite its underestimation of the prevalence estimates compared to the testing of individual samples. Integrating this methodology with national NTD programs could enhance control efforts. The findings also highlight the public health significance of Strongyloidiasis in Ghana, emphasizing the need for enhanced surveillance, targeted community interventions, integration into the national NTD program, and the need for strategies to reduce the burden of this neglected parasite.

Abbreviations

NTDs

Neglected Tropical Diseases

MDA

Mass Drug Administration

PCR

Polymerase Chain reaction

WHO

World Health Organization

DNA

Deoxyribonucleic reaction

cOR

crude odds ratio

aOR

adjusted odds ratio

MLE

Maximum Likelihood Estimate

Declarations

Ethical Statement

This study was conducted in accordance with the principles of the Declaration of Helsinki. Ethical approval for the study was obtained from the Noguchi Memorial Institute for Medical Research Institutional Review Board (NMIMR-IRB; CPN 013/23-24). Written informed consent was obtained from all adults. For participants aged 12–17 years, written assent was obtained alongside parental/guardian consent. Parental consent was obtained for children 5 to 11 years. Participants consented to long term storage and future use of their samples for further diagnostic evaluations.

Consent for publication

Not applicable

Availability of data and materials

The datasets generated/ analyzed during the study are not publicly available due to privacy and ethical considerations but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare that they have no competing interests.

Funding

Financial support for this work was provided through grant (NTDSC 270U) awarded to DKdS by the Coalition for Operational Research on Neglected Tropical Diseases (COR NTD), funded by The Task Force for Global Health by the United States Agency for International Development (USAID) through its Neglected Tropical Diseases Program, as well as the Bill & Melinda Gates Foundation (BMGF OPP1190754). The funders played no part in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Author Contributions:

The concept for this manuscript was developed by GOG, YA, MO, and DKdS. GOG, MSA, YA, acquired the data from the field. Data analysis was analyzed by EFG, SOA and GOG. GOG drafted the manuscript and YA, MO, DKdS and DO reviewed the manuscript. All authors reviewed and gave their final approval to the manuscript.

Acknowledgments: We sincerely thank the study participants for their invaluable contributions.

Author's Information

Grace Opoku Gyamfi – gogyamfi@noguchi.ug.edu.gh/amagracegy21@gmail.com

Yvonne Ashong - yashong@noguchi.ug.edu.gh

Millicent Opoku - 20883266@students.latrobe.edu.au

Millicent Selassie Afatodzie - msafatodzie@noguchi.ug.edu.gh

Emmanuel Frimpong Gyekye - Egyekye@noguchi.ug.edu.gh

Seth Offei Addo – saddo@noguchi.ug.edu.gh

Daniel Oduro – doduro@noguchi.ug.edu.gh

Dziedzom Komi de Souza - DdeSouza@noguchi.ug.edu.gh

References

1. Seeger D, Cornejo Cisneros E, Lucar J, Denyer R. *Strongyloides* and COVID-19: challenges and opportunities for future research. *Trop Med Infect disease*. 2023;8(2):127.
2. Buonfrate D, Bisanzio D, Giorli G, Odermatt P, Fürst T, Greenaway C, et al. The global prevalence of *Strongyloides stercoralis* infection. *Pathogens*. 2020;9(6):468.
3. Buonfrate D, Bradbury RS, Watts MR, Bisoffi Z. Human strongyloidiasis: complexities and pathways forward. *Clin Microbiol Rev*. 2023;36(4):e00033–23.
4. World Health Organization. WHO guideline on preventive chemotherapy for public health control of strongyloidiasis. World Health Organization; 2024.
5. Assaré RK, Ouattara M, Becker SL, Bassa FK, Diakité NR, Utzinger J, et al. *Strongyloides stercoralis* Infection in Humans in West Africa, 1975–2024: Systematic Review and Meta-Analysis. *Trop Med Infect Disease*. 2025;10(11):321.
6. Castelletto ML, Akimori D, Patel R, Schroeder NE, Hallem EA. Introduction to *Strongyloides stercoralis* anatomy. *J Nematology*. 2024;56(1):20240019.
7. Hailu T, Nibret E, Amor A, Munshea A. Strongyloidiasis in Africa: Systematic Review and Meta-Analysis on Prevalence, Diagnostic Methods, and Study Settings. *Biomed Res Int*. 2020;2020(1):2868564.
8. Tiruneh A, Zemene E, Mekonnen Z. Strongyloidiasis: the most neglected tropical disease in Ethiopia. *Infect Dis Poverty*. 2021;10(1):65.
9. Buonfrate D, Fittipaldo A, Vlieghe E, Bottieau E. Clinical and laboratory features of *Strongyloides stercoralis* infection at diagnosis and after treatment: a systematic review and meta-analysis. *Clin Microbiol Infect*. 2021;27(11):1621–8.
10. Papaïakovou M, Wright J, Pilotte N, Chooneea D, Schär F, Truscott JE, et al. Pooling as a strategy for the timely diagnosis of soil-transmitted helminths in stool: value and reproducibility. *Parasites vectors*. 2019;12(1):443.
11. Qu J, Zong Z. Strongyloidiasis in a patient diagnosed by metagenomic next-generation sequencing: a case report. *Front Med*. 2022;9:835252.
12. Rahim S, Drabu Y, Jarvis K, Melville D. Strongyloidiasis: a mistaken diagnosis and a fatal outcome in a patient with diarrhoea. *Trans R Soc Trop Med Hyg*. 2005;99(3):215–7.
13. Becker SL, Piraisoody N, Kramme S, Marti H, Silue KD, Panning M, et al. Real-time PCR for detection of *Strongyloides stercoralis* in human stool samples from Côte d'Ivoire: diagnostic accuracy, inter-laboratory comparison and patterns of hookworm co-infection. *Acta Trop*. 2015;150:210–7.
14. Buonfrate D, Formenti F, Perandin F, Bisoffi Z. Novel approaches to the diagnosis of *Strongyloides stercoralis* infection. *Clin Microbiol Infect*. 2015;21(6):543–52.
15. Hailu T, Amor A, Nibret E, Munshea A, Anegagrie M, Flores-Chavez MD, et al. Evaluation of five diagnostic methods for *Strongyloides stercoralis* infection in Amhara National Regional State, northwest Ethiopia. *BMC Infect Dis*. 2022;22(1):297.

16. Buonfrate D, Requena-Mendez A, Angheben A, Cinquini M, Cruciani M, Fittipaldo A, et al. Accuracy of molecular biology techniques for the diagnosis of *Strongyloides stercoralis* infection—a systematic review and meta-analysis. PLoS Negl Trop Dis. 2018;12(2):e0006229.
17. Mendez P, Walsh B, Hallem EA. Using newly optimized genetic tools to probe *Strongyloides* sensory behaviors. Mol Biochem Parasitol. 2022;250:111491.
18. Le B, Clarke NE, Legrand N, Nery SV. Effectiveness of ivermectin mass drug administration in the control of soil-transmitted helminth infections in endemic populations: a systematic review and meta-analysis. Infect Dis Poverty. 2024;13(01):1–15.
19. Leonardi-Cattolica A, Kayastha S, Miller M, Guag J, Tkachenko A, Lowe J, et al. Evaluation of Fecal Sample Pooling for Real-Time RT-PCR Testing SARS-CoV-2 in Animals. Viruses. 2024;16(11):1651.
20. Maurizio A, Marchiori E, Tessarin C, Cassini R. Comparing pooled and individual samples for estimation of gastrointestinal strongyles burden and treatment efficacy in small ruminants. Vet Parasitol. 2023;318:109935.
21. Mensah SSG, Larbi JA, Streit A. Molecular taxonomic characterisation of *Ancylostoma* spp. and *Strongyloides* spp. in three protected areas in Ghana. Front Parasitol. 2026;5:1798958.
22. Dumevi CY, Kretchy J-P, Kyei GB, Tetteh-Quarcoo PB, Ayi I, Ayeh-Kumi PF. Human Strongyloidiasis in Ghana: A scoping review of prevalence and geographic distribution (2003–2023). Health Sci Investigations J. 2025;7(1):1148–60.
23. Ceesay B, Taal A, Kalisa M, Odikro MA, Agbope D, Kenu E. Analysis of snakebite data in Volta and Oti Regions, Ghana, 2019. Pan Afr Med J. 2021;40:131.
24. Agbemaflle E, Kubio C, Bandoh D, Odikro M, Azagba C, Issahaku R, et al. Evaluation of the malaria surveillance system—Adaklu District, Volta Region, Ghana, 2019. Public Health Pract. 2023;6:100414.
25. Agyekum MW, Afrifa-Anane GF, Kyei-Arthur F. Prevalence and correlates of disability in older adults, Ghana: evidence from the Ghana 2021 Population and Housing Census. BMC Geriatr. 2024;24(1):52.
26. Owusu G, Yankson P. Urbanization in Ghana. The economy of Ghana Sixty years after independence. Oxford: Oxford University Press; 2017.
27. Service GS. 2010 Population & housing census report: Disability in Ghana: Ghana Statistical Service; 2014.
28. Gbogbo S, Amu H, Dowou RK, Ayanore MA. Management of onchocerciasis among adolescents in nkwanta north district of Ghana: Qualitative study of adolescents’ perception, community and health system support. PLoS Negl Trop Dis. 2023;17(8):e0011577.
29. Biritwum NK, Frempong KK, Verver S, Odoom S, Alomatu B, Asiedu O, et al. Progress towards lymphatic filariasis elimination in Ghana from 2000–2016: Analysis of microfilaria prevalence data from 430 communities. PLoS Negl Trop Dis. 2019;13(8):e0007115.
30. de Souza DK, Otchere J, Sumboh JG, Asiedu O, Opare J, Asemanyi-Mensah K, et al. Finding and eliminating the reservoirs: Engage and treat, and test and treat strategies for lymphatic filariasis programs to overcome endgame challenges. Front Trop Dis. 2022;3:953094.

31. Tekle AH, Zouré HG, Noma M, Boussinesq M, Coffeng LE, Stolk WA, et al. Progress towards onchocerciasis elimination in the participating countries of the African Programme for Onchocerciasis Control: epidemiological evaluation results. *Infect Dis poverty*. 2016;5(1):66.
32. World Health Organization (WHO). Summary of Presentations and Discussions of GONE Webinar: Country Progress Towards NTDs 2030 Road Map Targets for Onchocerciasis in Ghana. 2024 Accessed 18 April 2024.
33. Otabil KB, Basáñez MG, Ankrah B, Opoku SA, Kyei DO, Hagan R, et al. Persistence of onchocerciasis and associated dermatologic and ophthalmic pathologies after 27 years of ivermectin mass drug administration in the middle belt of Ghana. *Tropical Med Int Health*. 2023;28(11):844–54.
34. Anto F, Asoala V, Anyorigiya T, Oduro A, Adjuik M, Akweongo P, et al. Simultaneous administration of praziquantel, ivermectin and albendazole, in a community in rural northern Ghana endemic for schistosomiasis, onchocerciasis and lymphatic filariasis. *Tropical Med Int Health*. 2011;16(9):1112–9.
35. Fimbo AM, Mnkugwe RH, Mlugu EM, Kunambi PP, Malishee A, Minzi OM, et al. Efficacy of ivermectin and albendazole combination in suppressing transmission of lymphatic filariasis following mass administration in Tanzania: a prospective cohort study. *Infect Dis Poverty*. 2024;13(1):44.
36. World Health Organization. Expanded Special Project for Elimination of Neglected Tropical Diseases (ESPEN). Ghana: onchocerciasis data 2024 [Available from: <https://espen.afro.who.int/maps-data/countries/ghana#onc--data>. Accessed 08 May 2026.
37. Sitta R, Malta F, Pinho J, Chieffi P, Gryschek R, Paula F. Conventional PCR for molecular diagnosis of human strongyloidiasis. *Parasitology*. 2014;141(5):716–21.
38. Verweij JJ, Canales M, Polman K, Ziem J, Brienens EA, Polderman AM, et al. Molecular diagnosis of *Strongyloides stercoralis* in faecal samples using real-time PCR. *Trans R Soc Trop Med Hyg*. 2009;103(4):342–6.
39. Katholi CR, Unnasch TR. Important experimental parameters for determining infection rates in arthropod vectors using pool screening approaches. *Am J Trop Med Hyg*. 2006;74(5):779–85.
40. Leta GT, French M, Dorny P, Vercruysse J, Levecke B. Comparison of individual and pooled diagnostic examination strategies during the national mapping of soil-transmitted helminths and *Schistosoma mansoni* in Ethiopia. *PLoS Negl Trop Dis*. 2018;12(9):e0006723.
41. Forrer A, Khieu V, Vounatsou P, Sithithaworn P, Ruantip S, Huy R, et al. *Strongyloides stercoralis*: Spatial distribution of a highly prevalent and ubiquitous soil-transmitted helminth in Cambodia. *PLoS Negl Trop Dis*. 2019;13(6):e0006943.
42. Wiraphongthongchai W, Sithithaworn P, Thinkhamrop K, Suwannatrai K, Kopolrat KY, Worasith C, et al. Epidemiology of *Strongyloides stercoralis* and *Opisthorchis viverrini* infections in northern and northeastern Thailand: Insights from urine-ELISA surveys. *Parasitol Res*. 2024;123(12):417.
43. Anselmi M, Buonfrate D, Guevara Espinoza A, Prandi R, Marquez M, Gobbo M, et al. Mass administration of ivermectin for the elimination of onchocerciasis significantly reduced and

maintained low the prevalence of *Strongyloides stercoralis* in Esmeraldas, Ecuador. PLoS Negl Trop Dis. 2015;9(11):e0004150.

44. Frallonardo L, Di Gennaro F, Panico GG, Novara R, Pallara E, Cotugno S, et al. Onchocerciasis: Current knowledge and future goals. Front Trop Dis. 2022;3:986884.

45. Page W, Judd JA, Bradbury RS. The unique life cycle of *Strongyloides stercoralis* and implications for public health action. Trop Med Infect disease. 2018;3(2):53.

Figures

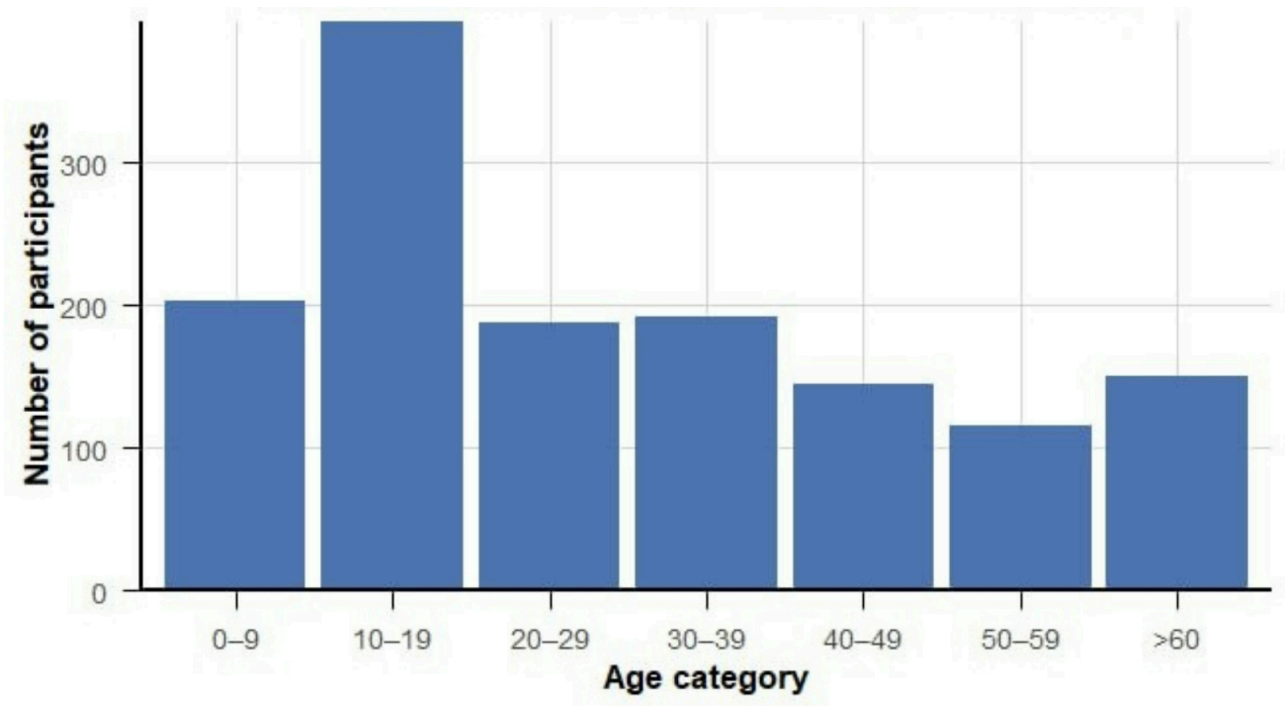


Figure 1

Age Demographics of Study Participants

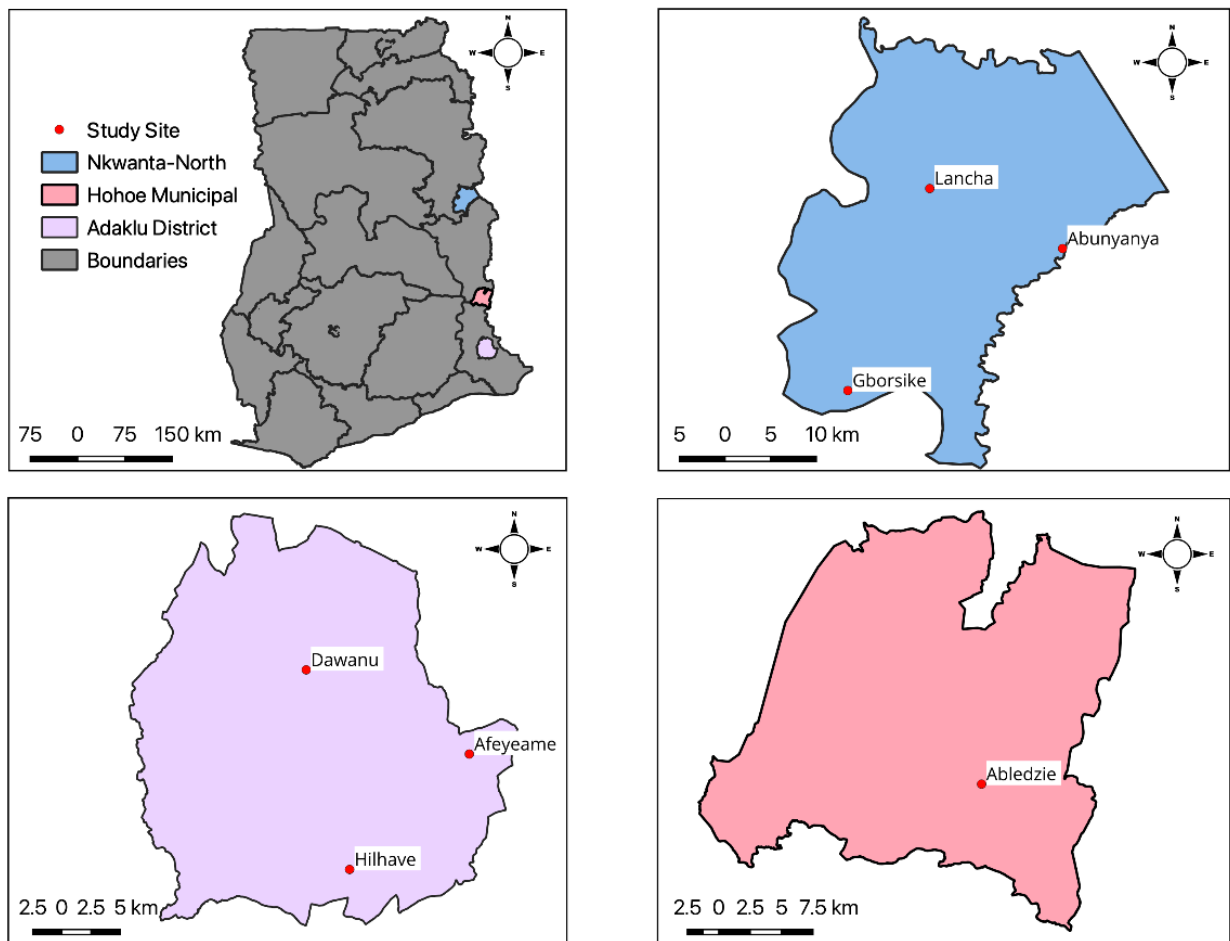


Figure 2

Map of Study Sites

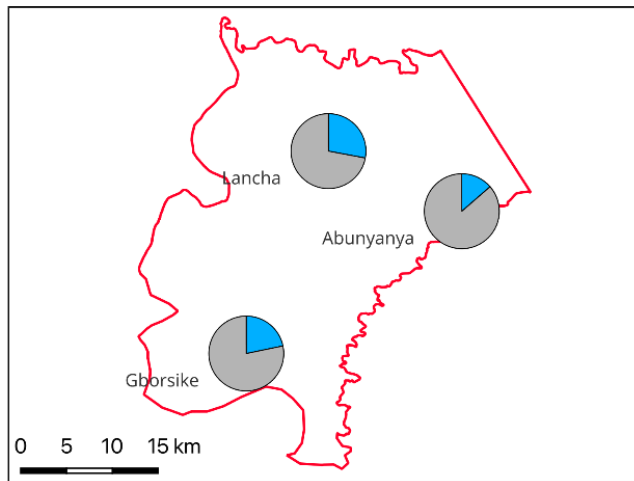
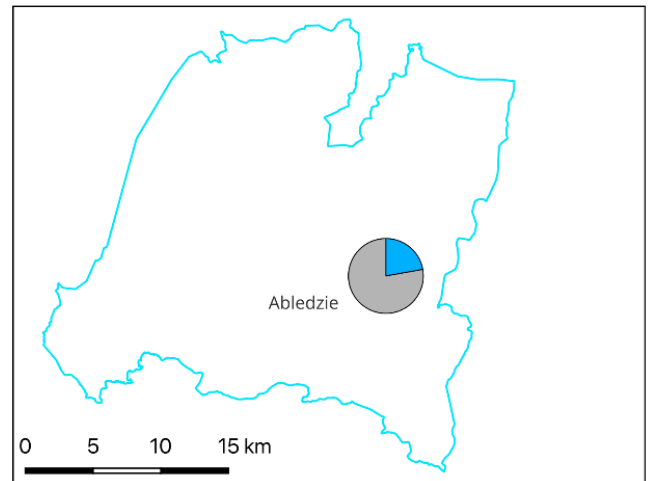
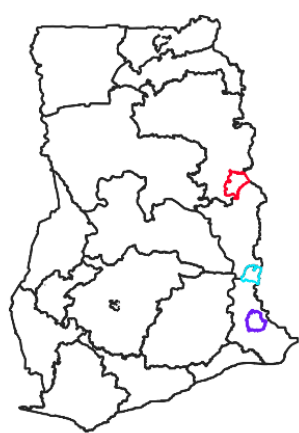
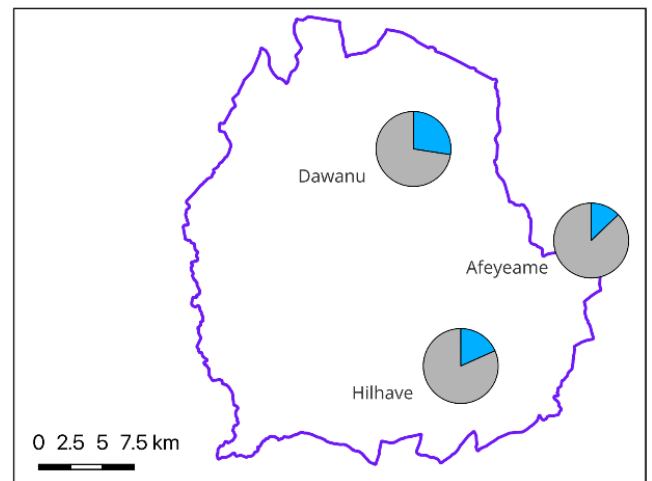


Figure 3

Spatial Representation of Strongyloides infection across study districts