

Article

Helminth and Malaria Co-Infection Among Pregnant Women in Battor and Adidome Towns of the Volta Region of Ghana

Sarah Alhakimi ^{1,†}, Navneet Kaur ^{2,†}, Javeriya Choudry ^{1,†}, Naa Adjeley Frempong ³ , Charity Ahiabor ⁴ , William K. Anyan ³ , Abraham K. Anang ³ and Nilanjan Lodh ^{1,*} 

¹ Department of Medical Laboratory Science, Marquette University, Milwaukee, WI 53233, USA; s.alhakimi@berkeley.edu (S.A.); javeriya.choudry@gmail.com (J.C.)

² Department of Biomedical Sciences, Marquette University, Milwaukee, WI 53233, USA; navneet.kaur@marquette.edu

³ Parasitology Department, Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Accra P.O. Box LG 25, Ghana; ntawiah@noguchi.ug.edu.gh (N.A.F.); wanyan@noguchi.ug.edu.gh (W.K.A.); aanang@noguchi.ug.edu.gh (A.K.A.)

⁴ Science Laboratory Technology, Accra Technical University, Accra P.O. Box GP 561, Ghana; cahiabor@atu.edu.gh

* Correspondence: nilanjan.lodh@marquette.edu

† These authors contributed equally to this work.

Abstract

Aim: In sub-Saharan Africa, approximately 40 million pregnant women are exposed to parasitic diseases such as malaria caused by *Plasmodium falciparum*, Schistosome parasites, and soil-transmitted helminths (STHs). When parasitic diseases share the same habitat and overlap in distribution, then high co-infection rates occur. The co-infection can lead to consequences for the child, such as intrauterine growth retardation, low birth weight, pre-term delivery, and neonatal mortality. **Methods:** The objective of the study was to determine the nature and extent of coinfection from 100 samples collected from the Battor (50) and Adidome (50) towns of Ghana in collaboration with the Noguchi Memorial Institute for Medical Research, University of Ghana. **Results:** Out of 50 for the Adidome towns determined for *P. falciparum* by Rapid Diagnostic Test (RDT), Malaria Pan-specific Antigen (PAN), and Malaria Pf kit, 39 were true positive (TP), 8 were true negative (TN), and 30 were false negative (FN). For Battor, 19 were TP, 12 TN, and 20 FN. For *S. mansoni* in Adidome via polymerase chain reaction (PCR) and loop-mediated isothermal amplification (LAMP), 21 tested positive, and 29 were negative, with 52.5% sensitivity and 100% specificity. For *S. haematobium*, 28 were positive and 22 negative using PCR with 70% sensitivity and 100% specificity. In LAMP, 28 were positive, and 22 negatives, with 70% sensitivity and 100% specificity. In Battor PCR for *S. mansoni*, 28 positives and 22 negatives with 68.3% sensitivity and 100% specificity. In LAMP, 32 were positive, and 18 were negative, with 80% sensitivity and 100% specificity. For *S. haematobium*, PCR showed 30 positive and 20 negative, with 73.2% sensitivity and 100% specificity. With LAMP, 21 were positive, and 29 negatives, with 51% sensitivity and 100% specificity. In both towns, 20–30 years had the highest infection prevalence for *P. falciparum*, *S. mansoni*, *S. haematobium*, and *Strongyloides stercoralis*. **Conclusion:** The results will be utilized as a part of the continuous surveillance for future research aiming at gathering nationally representative data in Ghana on the prevalence of coinfection and proposing interventions based on that for the vulnerable pregnant women population.

Keywords: *Plasmodium* spp.; *Schistosoma mansoni*; *S. haematobium*; *Strongyloides stercoralis*; pregnant women; mixed infection



Academic Editor: Marcia Aparecida Speranca

Received: 21 July 2025

Revised: 11 August 2025

Accepted: 15 August 2025

Published: 22 August 2025

Citation: Alhakimi, S.; Kaur, N.; Choudry, J.; Frempong, N.A.; Ahiabor, C.; Anyan, W.K.; Anang, A.K.; Lodh, N. Helminth and Malaria Co-Infection Among Pregnant Women in Battor and Adidome Towns of the Volta Region of Ghana. *Parasitologia* **2025**, *5*, 44.

<https://doi.org/10.3390/parasitologia5030044>

Copyright: © 2025 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license

(<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

In sub-Saharan Africa, a significant proportion of the population is exposed to malaria, schistosomiasis, and soil-transmitted helminths (STHs) [1–3]. These parasitic infections remain major public health problems in the region, especially among vulnerable groups such as pregnant women. It is estimated that approximately 40 million pregnant women in sub-Saharan Africa are infected with STHs and *Schistosoma* spp., often concurrently with malaria [4]. Where parasitic diseases overlap geographically, co-infections are common, yet there is a shortage of data on the prevalence of co-infections among pregnant women in Ghana [5].

Previous studies have shown that individuals in endemic areas often remain unaware of the extent and impact of parasitic infections [6,7]. Pregnancy increases susceptibility to these infections, and co-infections can lead to severe maternal and neonatal outcomes. Particularly among primigravid (first-time pregnant) women, parasitic co-infections are associated with intrauterine growth restriction, low birth weight, preterm delivery, and neonatal mortality [8].

Parasitic infections have significant pathophysiological consequences, particularly in vulnerable populations such as pregnant women. *Plasmodium* spp. infections lead to hemolysis, reducing red blood cell count and oxygen delivery, which contributes to anemia. This process is often accompanied by oxidative stress, characterized by an overproduction of free radicals and disruption of redox homeostasis. The resulting lipid peroxidation generates toxic by-products such as 4-hydroxynonenal (4-HNE), which damage cellular proteins. These effects, along with systemic inflammation, can be monitored through biomarkers like Advanced Oxidation Protein Products (AOPPs) in plasma. Similarly, helminth infections can cause blood loss, gastrointestinal injury, and immune suppression. Chronic helminthiasis may impair nutrient absorption, hinder growth, and negatively affect cognitive development. Despite these clinical significances, data on the concurrent prevalence of malaria, schistosomiasis, and *Strongyloides stercoralis* among pregnant women in Ghana remain limited. Traditional diagnostic methods such as microscopy (e.g., Kato-Katz), urine filtration, and RDTs often lack the sensitivity needed to detect light or asymptomatic infections, especially for *Strongyloides* and low-intensity *Schistosoma* infections [9,10]. Furthermore, diagnostic performance can vary significantly depending on regional parasite burden and the biological sample used.

This study aims to address these gaps by determining the prevalence of single and co-infections with *Plasmodium* spp., *P. falciparum*, *Schistosoma mansoni*, *S. haematobium*, and *S. stercoralis* among pregnant women in two town of Ghana, Adidome, and Battor. Using filtered urine samples, we employed a range of diagnostic tools, including conventional RDTs, urine-based antigen detection kits (Malaria PAN and Malaria Pf), loop-mediated isothermal amplification (LAMP), and polymerase chain reaction (PCR) assay to compare their sensitivity and specificity.

A total of 100 samples were analyzed. The results revealed a high burden of parasitic infections, with notable regional differences in infection prevalence and frequent occurrence of mixed infections. Malaria PAN and Pf kits based on LAMP assays demonstrated high diagnostic accuracy compared to conventional RDTs and PCR. These findings suggest that integrating non-invasive, molecular, and antigen-based tests into antenatal care programs may enhance early detection and management of parasitic infections during pregnancy and reduce associated maternal and fetal risks [11–13].

The study objective was to determine the prevalence of co-infection among Pregnant women in Ghana by amplifying species-specific DNA from a single filtered urine sample (100 samples in total).

2. Methods

2.1. Study Site, Sample Population, and Study Design

Study locations were the Adidome and Battor towns of Ghana. 50 samples from each district of pregnant women were incorporated in this study. The age ranged between 14–45 years, with a mean and median age of 25 and 25.5 for Adidome, and 27 for both Battor (Table 1). Whatman#3 filter papers (commercially available) were used to filter urine samples. Dried filter papers were shipped to Marquette University, WI, USA, in individual zip-lock bags with a desiccant. Urine sediment on filter paper was used to extract the parasitic DNA. We have detected single, dual, or multiple infections with *P. falciparum*, *S. mansoni*, *S. haematobium*, and/or *Strongyloides* via amplification of parasite species-specific cell-free repeat DNA fragment from a single urine specimen collected from these two towns' using PCR to avoid using methods based on stool or blood.

Table 1. Demographic information of the pregnant women from Ghana's Adidome and Battor towns.

Demography	Values	
	Adidome Town	Battor Town
Total samples	50	50
Age information available	44	29
Age range	15–40 years	17–45 years
Mean age	25	27
Median age	25.5	27

2.2. Ethical Clearance

The study was approved by the institutional review board (IRB) of Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Accra, Ghana (IRB#00001276, September 2017). A Material Transfer Agreement (MTA) was established between the University of Ghana and Marquette University to receive the urine samples in the USA. The study protocol has been approved and registered by the Marquette University Institutional Biosafety Committee (IBC: BR#166). Since we could not ascertain the subjects' identities, nor were Marquette University researchers involved in the collection or interaction with subjects, the Marquette University IRB was not needed.

2.3. Parasitological Tests for *P. falciparum* (RDT)

A rapid diagnostic test (RDT) was done on pregnant women once in both towns during their antenatal visit. For the Adidome town, it was done on 45 pregnant women out of 50. For the Battor town, it was done on 32 pregnant women out of 50.

2.4. DNA Extraction and Quantification from Filtered Urine Samples

All the filter paper containing urine samples collected from the field was placed and stored in individual Ziplock bags and sent to the USA for molecular detection. DNA was extracted from the innermost region of the folded labeled filter paper cones. Using a standard paper punch, 12–15 punches were made (~1 mm in diameter) and were collected in a 1.5 mL Eppendorf tube containing nuclease-free water. To avoid contamination, the paper puncher was cleaned with 10% bleach solution and nuclease-free water and dried after every use. The Eppendorf tubes were placed on a rotator at room temperature overnight after being incubated at 95 °C for 10 min. The next day, a Qiagen QIAmp 2 mL column tube was used to hold DNA-containing water. Following the manufacturer's guidelines, DNA was purified using the QIAmp DNA Blood Mini Kit (Qiagen, Germantown, MD, USA). A

NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) was used to quantify DNA concentration and then stored at -20°C freezer for future use.

2.5. LAMP PAN and Pf for *Plasmodium* spp. and *P. falciparum*

The purpose of the LoopAmp Malaria PAN kit was to detect the *Plasmodium* genus, which includes *P. falciparum*, *P. ovale*, *P. malariae*, and *P. vivax*, and then to detect *Plasmodium falciparum* from Malaria PAN positives by using the LoopAmp Malaria Pf kit (Tokyo, Japan). The LoopAmp Malaria PAN kit was used to test 100 samples. Malaria PAN detection reagent (dMAL PAN) was available in each test tube in a dried state, composed of *Bst* DNA polymerase, deoxynucleotide triphosphate, magnesium sulfate, calcein, manganese chloride, and genus-specific primers. A pipette was used to add 30 μL of extracted DNA solution into each tube along with negative (NC Mal) and positive controls (PC Mal; Human Diagnostic). According to the manufacturer, positive control comprises a product derived from in vitro amplification of an artificial gene derived from *Plasmodium* genus mitochondrial DNA. Both controls were available in the test tubes labeled NC Mal and PC Mal, and they were prepared by spinning down the tubes to collect the content at the bottom before use. All the other tubes were flipped downwards to gather the solution at the bottom of each tube and then were flipped upside down for 2 min to reconstitute the dried reagents. The tubes were then inverted five times to mix the contents. After that, the tubes were placed in a thermocycler, and the amplification happened at 65°C for 40 min. Then the tubes were photographed using a gel imager (Azur Biosystem C200, Dublin, CA, USA), which allowed us to see the color change in the test tubes. The test tubes showing a green, fluorescent light consistent with the positive control were all considered positive samples, and the remaining tubes with no light were considered negative. After saving the pictures to a pen drive, the results were entered into a computer for further analysis.

After detecting 78 positives of the *Plasmodium* genus, the LoopAmp Malaria Pf Kit was used to detect *Plasmodium falciparum*. In this case, the Malaria Pf detection reagent was available in a dried state in the test tube, composed of *Bst* DNA polymerase, deoxynucleotide triphosphate, magnesium sulfate, calcein, manganese chloride, and *P. falciparum*-specific primers. Next, using a pipette, 30 μL of the extracted DNA solution of malaria PAN-positive samples was added to each tube along with positive and negative controls for quality control. In this case, both negative and positive controls were different from those used in the Malaria PAN kit. According to Human Diagnostic, the positive control contained a product made via in vitro amplification of an artificial gene from Pf's mitochondrial DNA. Both controls were available in the test tubes and were prepared by spinning to collect all the contents. All the remaining tubes were then flipped downwards, then upside down for 2 min, and at last were inverted 5 times to mix the content. For the amplification, all the test tubes were placed in the thermocycler at 65°C . After 40 min, all the test tubes were placed in the gel imager (Azur Biosystem C200) and images were captured. The test tubes showing a green-fluorescent light matched with a positive control were considered positive, and the remaining were negative samples. All the pictures were stored in the pen drive and then on a computer for further analysis.

2.6. PCR Amplification for *S. mansoni* and *S. haematobium*

The PCR test detected the presence of *S. haematobium* and *S. mansoni* among 104 samples. The samples were obtained from both sexes and three age groups in the range of 14–19 years, 20–30 years, and 31–40 years old (Table 1). The PCR test included forward and reverse primers to amplify non-coding, short tandem repeats of 121 bp species-specific DNA fragments of both *S. haematobium* and *S. mansoni* [3]. The amplification of *S. mansoni* was done by using forward primer SmPF (5'-GATCTGAATCCGACCAACCG-3')

and reverse primer SmPR (5'-ATATTAACGCCCACGCTCTC-3') [3]. This allowed for the amplification of a 110 bp fragment from a highly repetitive 121 bp region of the species DNA [3]. For *S. haematobium*, two species-specific primers were used. One was ShDra1F (5'-GATCTCACCTATCAGACGAAAC-3') and the other was ShDra1R (reverse: 5'-TCACAACGATACGACCAAC-3'). These two primers amplified 121 bp of Dra 1 repeats [3]. The cell-free repeat DNA fragments of both species of Schistosomiasis were detected from urine samples using species-specific primers [3]. The repetitive fragment consists of 12–16% of the genome for both species (approximately 600,000 copies per cell), to allow for high sensitivity and specificity [4]. All the DNA samples contained a 1–3 ng/μL concentration of DNA, and 1 μL of DNA was used as the PCR template. The PCR reaction consisted of 10 μL total reaction volume, of which 5 μL was Master Mix, 10X (Promega, Madison, WI, USA), 0.5 μL (10 mM) of forward and reverse primers, 0.5 μL of 25 mM MgCl₂, and 2 μL of nuclease-free water for filling the reaction to its final volume of 10 μL. For quality control, first, the genomic DNA of *S. haematobium* served as the negative control for *S. mansoni*, while the genomic DNA of *S. mansoni* was used as the negative control for *S. haematobium* [2]. Both genomic DNAs were used as the positive controls for the same species. Second, the nuclease-free water was also used as the negative control. To ensure that there was no cross-contamination, each sample was amplified twice. If cross-contamination was observed after PCR amplification, the sample was thrown away, and that trial was run again. The steps of PCR amplification were as 5 min of initial denaturation at 95 °C, 30 s of 40 cycles at 95 °C, annealing for 45 s at 63 °C, elongation for 30 s at 72 °C, and finally, an extension step for 5 min at 72 °C. For visualization, 4 μL of each sample was used to run agarose gel electrophoresis. The gel electrophoresis contained 2% agarose gel, with each sample stained with SYBR green, and a 50 bp ladder as a reference for band sizes. After running the agarose gel, all the amplified bands were visualized in the Azure C200 gel documentation system.

2.7. LAMP Amplification for *S. mansoni* and *S. haematobium*

The LAMP technique was used to detect the presence of *S. haematobium* and *S. mansoni* as single and co-infections among 100 samples of pregnant women (Table 2). The LAMP technique consisted of two sets of primers to amplify four regions of the target DNA. The 10 μL mixture for amplification was made using 2X LAMP ready-to-use buffer mix (from 10X LAMP buffer), 5 M betaine, and 10 mM dNTPs [2]. Within the 10 μL mixture, there was 4 μL ready mix buffer, 0.5 μL each of the F3, B3, FIP, and BIP primers, 1 μL of *Bst* DNA polymerase, 2–3 μL of the extracted DNA, and 1 μL of nuclease-free water as the negative control [2]. For negative controls, *S. haematobium* genomic DNA served as the negative control for *S. mansoni*, while *S. mansoni* was the negative control for *S. haematobium*. The positive control was the corresponding genomic DNA of each species (i.e., genomic DNA of *S. mansoni* and *S. haematobium*). All samples were run twice via LAMP. If at any point cross-contamination was observed, then the entire sample run was discarded, and samples were repeated (for quality control). The 2-h amplification was carried out at 65 °C with 5 min of inactivation at 80 °C at the end [2]. The final amplified products were visualized first through gel imaging, and then by adding 1 μL of SYBR green (1:20 dilution) to each of the samples to note for a color change. After adding SYBR green to all the samples and waiting to observe the color change, a cell phone picture was taken [2].

Table 2. Distribution of *Plasmodium* spp. and *P. falciparum* infection among pregnant women was detected by rapid diagnostic test (RDT), Malaria PAN, and Malaria Pf kit from filtered urine samples collected from Battor and Adidome towns of Ghana. Total samples = 100.

Variables	Adidome Town			Battor Town		
	RDT	Malaria PAN	Malaria Pf	RDT	Malaria PAN	Malaria Pf
Test conducted	45 samples	50 samples	47 Malaria PAN + samples	32 samples	50 samples	31 Malaria PAN + samples
Positive *	7 (15.5%)	47 (94%)	39 (83%)	0	31 (62%)	19 (61.3%)
Negative *	38 (84.5%)	3 (6%)	8 (17%)	32 (100%)	19 (38%)	12 (38.7%)
Sensitivity *	14%	100%	100%	0%	100%	100%
Specificity *	50%	100%	100%	100%	100%	100%
Infection prevalence [@] (95% CI)	15.6% (6.5–29.5%)	94% (83.5–98.8%)	83% (69.2–92.4%)	0% (0–10.9%)	62% (47.2–75.4%)	61.3% (42.2–78.2%)

* Percentage = calculated based on total samples evaluated. @ Infection prevalence = proportion of positive infections by each test out of a total number of samples evaluated.

2.8. PCR Amplification for *Strongyloides*

For DNA standardization and amplification, specific primers were designed to amplify 125 bp fragments from an *S. stercoralis* dispersed repetitive sequence. The forward primer (SSC-F) 5'CTCAGCTCCAGTAAAGCAACAG3' and reverse primer (SSC-R) 5'AGCTGAATCTGGAGAGTGAAGA3' were designed by using the PrimerQuest Tool (IDT, Coralville, IA, USA). 15 µL volume contained Taq 2X Master mix (New England Biolabs, Ipswich, MA, USA), 0.75 µL of 10 µL of each primer, 1–2 µL of DNA, and PCR-grade water for PCR amplification. Initial denaturation occurred at 95 °C for 10 min, followed by 35 cycles at 95 °C for 1 min, 63 °C for 1.5 min, 72 °C for 1 min, and final extension at 72 °C for 10 min. The PCR products were then evaluated via 2% agarose gel stained with SYBR green, and a 50 bp ladder as a reference for band sizes. All the amplified bands captured via gel electrophoresis were visualized in the Azure C200 gel documentation system. For quality control, the genomic DNA of *S. stercoralis* was used as a positive control. *S. mansoni* and *S. haematobium* DNA were used as negative DNA controls. In addition, nuclease-free water was used as a negative control.

2.9. Statistical Analysis

Data was collected for positive and negative amplification of Malaria PAN, Malaria Pf, *S. mansoni*, *S. haematobium*, and *S. stercoralis*. The statistical analyses were done by converting the results of all amplifications to numerical values, such as 1 = positive and 0 = negative. The total positive and negative infections for each targeted species (via PCR and LAMP) were calculated by JMP 12 (JMP® v12, SAS Institute Inc., Cary, NC, USA). Data was recorded for three different age groups for both districts and all targeted species separately. The sensitivity, specificity, and infection prevalence of all amplifications were calculated using MedCalc 12.4.0 (MedCalc Software, Ostend, Belgium). These were compared against PCR amplification.

3. Results

3.1. Distribution of *Plasmodium* spp. and *P. falciparum* Infection by RDT, Malaria PAN, and Malaria Pf

3.1.1. Infection Prevalence in the Adidome Town

For the Adidome town, 50 urine samples were evaluated for *P. falciparum* by RDT, Malaria PAN, and Malaria Pf kit. Of which 39 were true positives (TP), 8 were true negatives (TN), and 30 were false negatives (FN) (Table 2). In RDT testing, 7 were tested positive, and 38 were negative, with 14% sensitivity and 50% specificity (Table 2). In comparison, the *Plasmodium* malaria PAN kit detected 47 true positives and only 3 true negatives out of

50 urine samples. Next, the Malaria Pf kit was used to detect *P. falciparum* from 47 Malaria PAN positives, where 39 were TP, and 8 were TN (Table 2). There were no false positives with 100% sensitivity and specificity (Table 2). Infection prevalence in the case of RDT was 15.6% (CI: 6.5–29.5%), Malaria PAN was 94% (CI: 83.5–98.8%), and Malaria Pf was 83% (CI: 69.2–92.4%) (Table 1).

3.1.2. Infection Prevalence in the Battor Town

For the Battor town, 50 urine samples were evaluated, of which 19 were TP, 12 were TN, and 20 were FN (Table 2). For RDT testing, 0 were positive, and 32 were negative out of 32 samples, with 0% sensitivity and 100% specificity (Table 2). In comparison to Malaria PAN, out of 50 samples, 31 were positive and 19 were negative, with 100% sensitivity and specificity (Table 2). Next, with the Malaria Pf test, 10 were positive and 12 negatives out of 31 Malaria PAN positives, with 100% sensitivity and specificity. Infection prevalence in the case of RDT was 0% (CI: 0–10.9%), Malaria PAN was 62% (CI: 47.2–75.4%), Malaria Pf was 61.3% (CI: 42.2–78.2%) (Table 2). Overall, *P. falciparum* was predominant in the district of Adidome as compared to Battor.

3.2. Distribution of *Schistosoma mansoni*, *S. haematobium*, and *Strongyloides Stercoralis* Infection by PCR and LAMP

3.2.1. Infection Prevalence in the Adidome Town

In the Adidome town, 50 samples were evaluated for *S. mansoni* using PCR and LAMP (Table 3(A)). In PCR, 21 samples were tested positive and 29 were tested negative, with 52.5% sensitivity and 100% specificity (Table 3). In LAMP, 32 samples tested positive, and 18 were negative, with 80% sensitivity and 100% specificity (Table 3). 50 samples were evaluated for *S. haematobium*, 28 were tested positive, and 22 samples were tested negative using PCR with 70% sensitivity and 100% specificity (Table 3). In LAMP using the same 50 samples, 28 samples were tested positive and 22 were negative, with 70% sensitivity and 100% specificity (Table 3). Moreover, 50 samples were evaluated for *S. stercoralis*, 3 were tested positive, and 47 were tested negative with 100% sensitivity and 100% specificity (Table 3).

Table 3. Distribution of *Schistosoma mansoni*, *S. haematobium*, and *Strongyloides stercoralis* infection among pregnant women detected by PCR, LAMP from filtered urine samples collected from Battor and Adidome towns of Ghana. Total samples = 100. [@] Infection prevalence = proportion of positive infections by each test out of a total number of samples evaluated.

(A) Adidome Town					
Variables	<i>S. mansoni</i>		<i>S. haematobium</i>		<i>S. stercoralis</i>
	PCR	LAMP	PCR	LAMP	PCR
Test conducted	50	50	50	50	50
Positive	21 (42%)	32 (64%)	28 (56%)	28 (56%)	3 (6%)
Negative	29 (58%)	18 (36%)	22 (44%)	22 (44%)	47 (94%)
Sensitivity	52.5%	80%	70%	70%	100%
Specificity	100%	100%	100%	100%	100%
Infection prevalence [@] (95% CI)	42% (28.2–56.8%)	64% (49.2–77.1%)	56% (41.3–70%)	56% (41.3–70%)	6% (1.3–16.6%)
(B) Battor town					
Variables	<i>S. mansoni</i>		<i>S. haematobium</i>		<i>S. stercoralis</i>
	PCR	LAMP	PCR	LAMP	PCR
Test conducted	50	50	50	50	50
Positive	28 (56%)	25 (50%)	30 (60%)	21 (42%)	12 (24%)
Negative	22 (44%)	25 (50%)	20 (40%)	29 (58%)	38 (76%)
Sensitivity	68.3%	61%	73.2%	51%	100%
Specificity	100%	100%	100%	100%	100%
Infection prevalence [@] (95% CI)	56% (41.3–70%)	50% (35.5–64.5%)	60% (45.2–73.6%)	42% (28.2–56.8%)	24% (13.1–38.2%)

3.2.2. Infection Prevalence in the Battor Town

In the Battor town, 50 samples were evaluated in PCR and LAMP for *S. mansoni* (Table 3). PCR showed 28 positives with 22 negatives, with 68.3% sensitivity and 100% specificity (Table 3). In LAMP, 32 were tested positive and 18 were tested negative, with 80% sensitivity and 100% specificity (Table 3). For *S. haematobium*, PCR showed 30 positives and 20 were tested negative with 73.2% sensitivity and 100% specificity (Table 3). With LAMP, 21 tested positive and 29 tested negative, with 51% sensitivity and 100% specificity (Table 3). Moreover, the 50 samples were tested for *S. stercoralis* with PCR; 12 were tested positive and 38 were tested negative, with 100% sensitivity and 100% specificity (Table 3).

3.3. Distribution of Infection by Age Group

3.3.1. Infection Prevalence of the Adidome Town by Age Group

In age group A (14–19 years) of the Adidome town, 9 (20.5%) were infected with *Plasmodium* spp., 6 (14.6%) were infected with *P. falciparum*, 8 (18.2%) with *S. mansoni*, 6 (13.6%) with *S. haematobium*, and none with *Strongyloides stercoralis* (Table 3). In age group B (20–30 years), 24 (54.6%) were infected with *Plasmodium* spp. (Table 4). 21 (51%) were infected with *P. falciparum*, 16 (36.4%) with *S. mansoni*, 15 (34.1%) with *S. haematobium*, and 3 (6.8%) with *S. stercoralis* (Table 4). In age group C (31–40 years), 8 (18.2%) were infected with *Plasmodium* spp., 6 (14.6%) with *P. falciparum*, 4 (9.1%) with *S. mansoni*, 5 (11.4%) with *S. haematobium*, and none with *S. stercoralis* (Table 4). Out of the three groups, group B was found to be the most susceptible to all the parasitic infections tested for in the Adidome district of Ghana (Table 4).

Table 4. Distribution of *Plasmodium* spp. (by Malaria PAN), *P. falciparum* (by Malaria Pf), *Schistosoma mansoni* (by LAMP), *S. haematobium* (by LAMP), and *Strongyloides stercoralis* (by PCR) infection among three different age groups. The participants are divided into three different age groups: teens (14–19 years), early adulthood to thirties (20–30 years), and 30+ (31–45 years). The age groups are respective of the participants' age and number from Adidome (age data available = 29) and Battor (age data available = 44) towns of Ghana.

Species	Adidome Town			Battor Town		
	Group A (14–19 Years)	Group B (20–30 Years)	Group C (31–40 Years)	Group A (15–19 Years)	Group B (20–30 Years)	Group C (31–45 Years)
<i>Plasmodium</i> spp.	9 (20.5%)	24 (54.6%) *	8 (18.2%)	1 (3.5%)	14 (48.3%) *	5 (17%)
<i>P. falciparum</i>	6 (14.6%)	21 (51%) *	6 (14.6%)	1 (5%)	10 (50%) *	3 (15%)
<i>Schistosoma mansoni</i>	8 (18.2%)	16 (36.4%) *	4 (9.1%)	2 (7%)	8 (28%) *	2 (7%)
<i>Schistosoma haematobium</i>	6 (13.6%)	15 (34.1%)	5 (11.4%)	1 (3.5%)	11 (38%) *	2 (7%)
<i>Strongyloides stercoralis</i>	0	3 (6.8%)	0	1 (3.5%)	7 (24%)	4 (14%)

* Important findings are highlighted.

3.3.2. Infection Prevalence in the Battor Town by Age Group

In age group A (14–19 years) of Battor district, 1 (3.5%) was infected with *Plasmodium* spp., 1 (5%) was infected with *P. falciparum*, 2 (7%) was infected with *S. mansoni*, 1 (3.5%) was infected with *S. haematobium* and 1 (3.5%) was infected with *Strongyloides stercoralis* (Table 4). In age group B (20–30 years), 14 (48.3%) were infected with *Plasmodium* spp., 10 (50%) with *P. falciparum*, 8 (28%) with *S. mansoni*, 11 (38%) of the people were infected with *S. haematobium*, and 7 (24%) with *S. Stercoralis* (Table 4). In group C (31–45 years), 5 (17%) were infected with *Plasmodium* spp., 3 (15%) with *P. falciparum*, 2 (7%) with *S. mansoni*, 2 (7%) with *S. haematobium*, and 4 (14%) with *S. Stercoralis* (Table 4). Out of the three groups in the Battor district, group B was found to be most susceptible to all the parasitic infections, the same as in the Adidome district (Table 4).

3.4. Comparison of Infection Prevalence Between Adidome and Battor

The parasite infection that was most prevalent among both towns was found to be different. Within the Adidome town, the parasitic infection of *Plasmodium* spp. was found to be the highest in prevalence. In addition, *P. falciparum* was found to be the highest in prevalence in Adidome (Table 4). The lowest infection rate (0%) in the Adidome town group A was detected with *S. Stercoralis*, while for the Battor town Group A, the lowest infection rate (3.5%) was detected with *Plasmodium* spp., *S. haematobium*, and *S. Stercoralis* (Table 4). The highest infection rate (20%) in the Adidome town group A was with *Plasmodium* spp. (Table 4). The highest infection rate (7%) in the Battor town group A was also found to be with *S. mansoni* (Table 4). In terms of the lowest infection rate (0%) in the Adidome town group C, this was also found to be with *S. stercoralis* (Table 4). For the Battor town Group C, the lowest infection rate (7%) was found to be with *S. mansoni* and *S. haematobium* (Table 4). The highest infection rate (18.2%) in the Adidome town group C was found to be with *Plasmodium* spp. (Table 4). For the Battor town group C, the highest infection rate (17%) was also found with *Plasmodium* spp. (Table 4).

3.5. Distribution of Mixed Parasitic Infection

Single and Mixed Parasitic Infection

The highest detection rate (12) was for *S. mansoni* as a single infection in the Battor town, as compared to the infection rate (11) in the Adidome town. The highest single infection rate (33) in the Adidome town was for *S. haematobium* (Table 5). In the Battor town, the coinfection of *S. mansoni* and *S. haematobium* was found to be higher (22) as compared to the coinfection of *S. mansoni* and *S. haematobium* in the Adidome town (18) (Table 5). The co-infection rate consisting of all three parasitic infections (*S. mansoni*, *S. haematobium*, and *S. stercoralis*) was detected in the Battor town as 4 cases, but 0 cases within the Adidome town (Table 5).

Table 5. Detailed numerical distribution of single and mixed parasitic infections among pregnant women in two towns of Ghana. * = Significant.

Infection Type	Battor District	Adidome District
Sm only	12	11
Sh only	11	33
Ss only	4	0
Sm + Sh	22 *	18
Sm + Ss	2	1
Sh + Ss	2	2
Sm + Sh + Ss	4	0

4. Discussion

This study provides a comparative assessment of parasitic infections, *Plasmodium* spp., *P. falciparum*, *S. mansoni*, *S. haematobium*, and *S. stercoralis*, among pregnant women in the Adidome and Battor towns of Ghana. Utilizing a combination of rapid diagnostic tests (RDT), urine-based Malaria PAN and Pf kits involving nucleic acid amplification methods (PCR and LAMP), we evaluated infection prevalence, diagnostic performance, and age-related distribution patterns. The potential severity of parasitic infections in pregnant women and the associated risks to fetal health underscore the need for accurate diagnostic tools. In this study, molecular methods such as PCR and LAMP were employed to enhance detection capability. The use of a non-invasive, urine-based approach further emphasizes

the clinical relevance and practical applicability of the methodology, particularly in resource-limited settings.

4.1. Malaria Detection and Diagnostic Performance

The findings demonstrate that *Plasmodium* infections, particularly *P. falciparum*, were more prevalent in the Adidome town (up to 95.6%) than in Battor (62%). While conventional RDTs showed limited sensitivity (14% in Adidome, 0% in Battor), the urine-based Malaria PAN and Pf LAMP kits demonstrated excellent sensitivity and specificity (100% each), confirming their potential for non-invasive, field-friendly malaria surveillance. This performance aligns with previous studies indicating the limitations of RDTs in low-parasitemia or asymptomatic infections [3,14] and the value of alternative urine-based diagnostics [10,15].

4.2. Helminth Infections: Prevalence and Diagnostic Insights

LAMP assays proved more sensitive than PCR for detecting *S. mansoni* in both districts (80% vs. 52.5–68.3%), confirming its value in field diagnostics where resource constraints may limit PCR application. Conversely, for *S. haematobium*, PCR demonstrated slightly higher or equal sensitivity compared to LAMP, depending on the district. The discrepancies in assay sensitivity are likely due to differences in parasite biology, assay design, and field conditions. LAMP showed higher sensitivity for *S. mansoni* (80% vs. 52.5–68.3%) due to its robustness, simplicity, and suitability for resource-limited settings. In contrast, PCR performed slightly better or equally for *S. haematobium*, possibly due to better assay optimization or more stable DNA in urine samples. These results are consistent with prior work supporting LAMP's higher field sensitivity and reduced technical complexity [12,16].

Infection with *S. stercoralis* was substantially higher in Battor, particularly among the 20–30 age group (24%), raising concerns about underdiagnosed strongyloidiasis in endemic rural populations. This is important because it is very underreported among the African population, especially among pregnant women. Additionally, the potential pathophysiological effects on this vulnerable group are not known individually for *Strongyloides* or when co-infected with other helminths. PCR, with its high specificity and sensitivity, remains the gold standard for detecting *S. stercoralis*, particularly in non-invasive urine samples [13].

4.3. Age Group Susceptibility and Mixed Infections

Participants aged 20–30 years (Group B) across both districts had the highest burden of all parasitic infections. This may reflect behavioral and occupational exposure or immunological factors unique to pregnancy in this age group. Consistent with similar studies in sub-Saharan Africa [1,5]. These findings highlight the importance of targeting this demographic for integrated parasitic disease screening during antenatal care.

Mixed infections were common. The co-occurrence of *S. mansoni* and *S. haematobium* was more frequent in Battor (22%) than Adidome (18%), and triple infections involving *S. mansoni*, *S. haematobium*, and *S. stercoralis* were exclusive to Battor. These polyparasitic cases emphasize the need for multiplex diagnostics and integrated treatment strategies to mitigate compounded disease burden [17].

4.4. Study Limitations

Several limitations must be acknowledged. First, the relatively small sample size (100) limits the generalizability of the findings. Second, the study was restricted to urine samples, which may have impacted the sensitivity of parasite detection, particularly for *S. stercoralis*, which typically requires stool or serological analysis for accurate diagnosis. Third, due to logistical constraints, not all participants could be matched with complete demographic and clinical data, such as gravidity, nutritional status, or use of preventive treatment.

Lastly, the lack of longitudinal follow-up prevents assessment of treatment outcomes or reinfection rates.

4.5. Future Directions

Future studies should expand the sample size and include longitudinal follow-ups to evaluate treatment efficacy, reinfection patterns, and maternal–fetal outcomes. Integration of urine-based testing could enhance the detection of intestinal helminths like *S. stercoralis*. Moreover, combining parasitic screening with nutritional and anemia assessments could improve maternal health strategies. The promising performance of urine-based diagnostics warrants further validation in broader populations and across different ecological zones.

5. Conclusions

This study highlights the high burden of parasitic infections among pregnant women in southern Ghana and the diagnostic efficacy of urine-based Malaria PAN/Pf LAMP assays and LAMP assays for *Schistosoma* spp. Diagnostic performance varied by town, with PCR and LAMP consistently showing high specificity. PCR demonstrated strong malaria detection, while rapid tests (RDTs) yielded frequent false negatives, highlighting limitations in field diagnostics. Schistosomiasis sensitivity varied by method and location. Co-infections were more prevalent among women aged 20–30 in both regions. These findings support surveillance efforts and may guide targeted interventions to mitigate pregnancy-related complications from parasitic infections.

Moreover, given the limitations of conventional RDTs and PCR accessibility, these non-invasive alternatives using urine offer promising tools for community-level diagnosis and surveillance. Integrated, age-targeted screening and treatment programs, especially among young adult women, could significantly reduce the health impacts of malaria and neglected tropical diseases during pregnancy.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/parasitologia5030044/s1>, Table S1: MAHEVA_Samples_Adidome and Batt.

Author Contributions: Study conducted, field study, sample collection: N.A.F., C.A., W.K.A., and A.K.A. Prepared the samples for molecular assays: S.A., N.K., and J.C. Performed molecular assays and data acquisition: S.A., N.K., and J.C. Performed data analysis: N.L. Prepared the manuscript: N.K., J.C., and N.L. Takes responsibility for the integrity of this work as a whole from inception to the published article and should be designated as the guarantor: N.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was approved by the Institutional Review Board (IRB) of Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana, Ghana (IRB#00001276). A Material Transfer Agreement (MTA) was established between the University of Ghana and Marquette University to receive the urine samples in the USA. The study protocol has been approved and registered by the Marquette University Institutional Biosafety Committee (IBC: BR#166). Since we could not ascertain the subjects' identities, nor were Marquette University researchers involved in the collection or interaction with subjects, the Marquette University IRB was not needed.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data are contained within the article and supplementary materials.

Acknowledgments: *S. mansoni* genomic DNA was provided by the Schistosomiasis Resource Center for distribution by BEI Resources, NIAID, NIH: Genomic DNA from Adult Male and Female

Schistosoma mansoni, Strain NMRI, NR-28910. *S. haematobium* genomic DNA was provided by the Schistosomiasis Resource Center for distribution by BEI Resources, NIAID, NIH: Genomic DNA from Adult Male and Female *Schistosoma haematobium*, Egyptian Strain, NR-31682.

Conflicts of Interest: All authors declared that there are no conflicts of interest.

References

1. Brooker, S.; Clements, A.C.A.; Hotez, P.J.; Hay, S.I.; Tatem, A.J.; Bundy, D.A.P.; Snow, R.W. The co-distribution of *Plasmodium falciparum* and hookworm among African schoolchildren. *Malar. J.* **2006**, *5*, 99. [\[CrossRef\]](#) [\[PubMed\]](#)
2. Hotez, P.J.; Kamath, A. Neglected tropical diseases in sub-Saharan Africa: Review of their prevalence, distribution, and disease burden. *PLoS Neglected Trop. Dis.* **2009**, *3*, e412. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Venkatesan, P. The 2023 WHO World Malaria Report. *Lancet Microbe* **2024**, *5*, e214. [\[CrossRef\]](#) [\[PubMed\]](#)
4. Hotez, P.J.; Molyneux, D.H.; Fenwick, A.; Kumaresan, J.; Sachs, S.E.; Sachs, J.D.; Savioli, L. Control of neglected tropical diseases. *N. Engl. J. Med.* **2007**, *357*, 1018–1027. [\[CrossRef\]](#) [\[PubMed\]](#)
5. Degarege, A.; Velede, E.; Degarege, D.; Erko, B.; Nacher, M.; Madhivanan, P. *Plasmodium falciparum* and soil-transmitted helminth co-infections among children in sub-Saharan Africa: A systematic review and meta-analysis. *Parasites Vectors* **2016**, *9*, 344. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Chimbari, M.J.; Dhlomo, E.; Mwadiwa, E.; Mubila, L. Transmission of schistosomiasis in Kariba, Zimbabwe, and a cross-sectional comparison of schistosomiasis prevalences and intensities in the town with those in Siavonga in Zambia. *Ann. Trop. Med. Parasitol.* **2003**, *97*, 605–616. [\[CrossRef\]](#) [\[PubMed\]](#)
7. Ezeamama, A.E.; McGarvey, S.T.; Acosta, L.P.; Zierler, S.; Manalo, D.L.; Wu, H.-W.; Kurtis, J.D.; Mor, V.; Olveda, R.M.; Friedman, J.F. The Synergistic Effect of Concomitant Schistosomiasis, Hookworm, and Trichuris Infections on Children's Anemia Burden. *PLoS Neglected Trop. Dis.* **2008**, *2*, e245. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Steketee, R.W.; Nahlen, B.L.; Parise, M.E.; Menendez, C. The burden of malaria in pregnancy in malaria-endemic areas. *Am. J. Trop. Med. Hyg.* **2001**, *64*, 28–35. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Knopp, S.; Mgeni, A.F.; Khamis, I.S.; Steinmann, P.; Stothard, J.R.; Rollinson, D.; Marti, H.; Utzinger, J. Diagnosis of soil-transmitted helminths in the era of preventive chemotherapy: Effect of multiple stool sampling and use of different diagnostic techniques. *PLoS Neglected Trop. Dis.* **2008**, *2*, e331. [\[CrossRef\]](#) [\[PubMed\]](#)
10. Mharakurwa, S.; Simoloka, C.; Thuma, P.; Shiff, C.; Sullivan, D. PCR detection of *Plasmodium falciparum* in human urine and saliva samples. *Malar. J.* **2006**, *5*, 103. [\[CrossRef\]](#) [\[PubMed\]](#)
11. Katzin, A.M.; Kimura, E.S.; Alexandre, C.O.; Ramos, A.M. Detection of antigens in urine of patients with acute falciparum and vivax malaria infections. *Am. J. Trop. Med. Hyg.* **1991**, *45*, 453–462. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Mwangi, I.N.; Agola, E.L.; Mugambi, R.M.; Shiraho, E.A.; Mkoji, G.M. Development and Evaluation of a Loop-Mediated Isothermal Amplification Assay for Diagnosis of *Schistosoma mansoni* Infection in Faecal Samples. *J. Parasitol. Res.* **2018**, *2018*, 1267826. [\[CrossRef\]](#)
13. Requena-Méndez, A.; Chiodini, P.; Bisoffi, Z.; Buonfrate, D.; Gotuzzo, E.; Muñoz, J. The Laboratory Diagnosis and Follow Up of Strongyloidiasis: A Systematic Review. *PLoS Neglected Trop. Dis.* **2013**, *7*, e2002. [\[CrossRef\]](#)
14. Moody, A. Rapid diagnostic tests for malaria parasites. *Clin. Microbiol. Rev.* **2002**, *15*, 66–78. [\[CrossRef\]](#) [\[PubMed\]](#)
15. Nwakanma, D.C.; Gomez-Escobar, N.; Walther, M.; Crozier, S.; Dubovsky, F.; Malkin, E.; Locke, E.; Conway, D.J. Quantitative detection of *Plasmodium falciparum* DNA in saliva, blood, and urine. *J. Infect. Dis.* **2009**, *199*, 1567–1574. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Notomi, T.; Okayama, H.; Masubuchi, H.; Yonekawa, T.; Watanabe, K.; Amino, N.; Hase, T. Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.* **2000**, *28*, E63. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Hotez, P.J.; Brindley, P.J.; Bethony, J.M.; King, C.H.; Pearce, E.J.; Jacobson, J. Helminth infections: The great neglected tropical diseases. *J. Clin. Investig.* **2008**, *118*, 1311–1321. [\[CrossRef\]](#) [\[PubMed\]](#)

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.