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THE USE OF PCR AS AN EFFECTIVE MEANS OF DIAGNOSING MALARIA AND TOXOPLASMOSIS IN PREGNANT WOMEN IN CAMEROON

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ABSTRACT: *Toxoplasmosis, caused by the obligate intracellular protozoan Toxoplasma gondii, is an important zoonosis with medical and veterinary significance worldwide. The disease is primarily contracted by ingesting undercooked or raw meat containing viable tissue cysts, or by ingesting food or water contaminated with oocysts. Malaria, on the other hand, is caused by protozoan parasites of the genus Plasmodium, with the most serious and sometimes fatal type being caused by Plasmodium falciparum. Malaria stands as the most significant infectious disease in tropical and subtropical regions, continuing to pose a major global health challenge. A rapid and accurate diagnosis is crucial for controlling these diseases, especially among vulnerable groups such as pregnant women. However, several diagnostic methods are hindered by shortcomings such as false negatives, false positives, and accuracy issues. Hence, this study assessed the utility of Polymerase Chain Reaction (PCR) in diagnosing these parasitic diseases in pregnant women at the Garoua Regional Hospital during antenatal care. A cross-sectional analytical study was conducted, recruiting 105 participants who responded to a questionnaire and were diagnosed with malaria and toxoplasmosis using rapid diagnostic tests and microscopy. Dried blood spots were prepared from finger-prick blood samples of each participant for molecular analysis. Of the 105 participants, 70.87% originated from the North region and 21.36% from the Far North region. The women had an average age of 26 years, with the majority being married (98.06%) and housewives (79.05%), while 24.76% had never attended school. Among the 105 participants, 17/105 (16.19%) and 30/105 (28.57%) were diagnosed positive for malaria and toxoplasmosis using rapid diagnostic tests and microscopy, respectively. Meanwhile, 18/105 (17.14%) and 75/105 (71.5%) tested positive for malaria and toxoplasmosis, respectively, using PCR.*

KEYWORDS: Malaria, Toxoplasmosis, Diagnosis, Pregnant women.

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INTRODUCTION

Pregnancy is a physiological state in which a fetus develops inside a woman's womb or uterus from conception to childbirth. It is a period characterized by multiple organic and functional changes to ensure the optimal growth of the fetus (1). However, it is also a delicate period, increasing susceptibility to communicable diseases that expose both the pregnant woman and her unborn child (2). Despite reduction in the main causes of maternal mortality, such as postpartum hemorrhage, the epidemiological burden of diseases in pregnancy remains a public health concern, particularly in sub-Saharan Africa, where women still suffer and die from preventable infectious diseases (3). In a systematic review on the causes of maternal deaths conducted by Musarandega et al., it was observed that nearly 11,427 maternal deaths in sub-Saharan Africa were linked to communicable diseases (22.1%), and non-communicable conditions during pregnancy (11.5%) such as diabetes (2). Perinatal mortality is also a public health concern in sub-Saharan Africa, with an estimated 34.7 deaths per 1000 live births (3). These figures highlight the need for an adequate response to reduce this burden, which negatively impacts socio-economic development in the region (4). The reduction of maternal-fetal morbidity and mortality relies on primary and secondary prevention. Primary prevention involves addressing risk factors before disease onset, while secondary prevention involves screening for the disease before the onset of symptoms (5). Among the communicable diseases responsible for health-related complications in pregnant women are malaria and toxoplasmosis. Infections with malaria and toxoplasmosis during pregnancy can cause significant maternal/fetal morbidity and mortality (19, 20). Due to their detrimental effects on the mother and her unborn baby, early and accurate diagnosis of these infections is crucial for mitigation and treatment. Several laboratory tests are available for the diagnosis of T. gondii, including rapid diagnostic tests (RDT), serological tests (such as enzyme-linked immunosorbent assay (ELISA)), PCR, and histological and cytological examination of tissues and body fluids. Similarly, various laboratory tests exist for the diagnosis of malaria, including microscopy, RDT, and molecular-based tests like Polymerase Chain Reaction (PCR) (19). RDT is easy to perform, rapid, and does not require trained personnel or special equipment, making it suitable for convenient testing (19). However, the unreliability of RDT for T. gondii diagnosis, as well as for Plasmodium falciparum, has been reported (22, 23). Therefore, other more sensitive and specific laboratory tests like PCR may be of great importance in the diagnosis of these infections.

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MATERIAL AND METHODS

Study Design

A cross-sectional study was conducted, recruiting 105 participants from the Gynecology Department of the Garoua Regional Hospital (GRH). Patients included in this study were pregnant women who provided consent after being administered an informed consent form. These women were at least 18 years old and were either positive for malaria, toxoplasmosis, or both.

Ethical Considerations

This study was approved by the University of Yaounde 1 Joint Institutional Review Board for Animal & Human Bioethics (JIRB) No: BTC-JIRB2022-045. The risks and benefits involved in the study were explained to the participants. Only participants who provided their approval by signing a written informed consent form were recruited. Confidentiality was maintained by assigning codes to all participants, ensuring that their names were not used.

Questionnaire Administration

A validated questionnaire, developed through a review of previous studies with similar objectives, was utilized to gather information on sociodemographic factors including age, marital status, level of education, income, clinical information, and biological assessment (malaria, toxoplasmosis). Molecular diagnosis was performed using Histidine Rich Protein 2 (HRP2) gene and B1 assays.

Sample Collection and DNA Extraction

Two milliliters (2ml) of venous blood were drawn from each participant. A portion of the blood was utilized for diagnosing malaria and toxoplasmosis using a rapid diagnostic test (RDT). The remaining blood was then used to create a dried blood spot using Whatman number 3 filter paper for molecular analysis and a microscopic slide for microscopy. DNA extraction from the dried blood spots was performed using the Chelex-100 resin boiling method, as previously described by Plowe et al. (9). The extracted DNA was subsequently stored at -20°C for subsequent analysis.

Molecular Diagnosis of Toxoplasmosis and Malaria

PCR was performed on all samples. The B1 gene of Toxoplasma gondii was used for the diagnosis of toxoplasmosis. For the amplification reaction, a 50 µL reaction mixture was prepared, each containing 5 µL of 10x PCR buffer (70 mM Tris-HCl pH 8.8, 200 mM (NH4)2SO4, 0.1% Tween 20), 2 mM MgCl2, 250 µM of each of the four deoxynucleotide triphosphates, 1.25 U Taq DNA polymerase (Fermentas), 50 pmol of each primer (Tox43': 5'CGCTGCAGGGAGGA AGACGAAAGTTG-3' and Tox55': 5'-CGCTGCAGACACAGTGCATCTGGATT-3') (10), and 5 µL of extracted template DNA. Amplification of parasite DNA was performed using a T3 thermal cycler (Biometra, UK). The PCR cycling conditions were as follows (33 cycles): initial denaturation at 94°C for 7 minutes, followed by denaturation at 94°C for 1 minute, annealing at 55°C for 1 min, extension at 72°C for 1 minute, and a final extension at 72°C for 10 minutes. PCR products were analyzed using a 1.5% agarose gel. The histidine-rich protein 2 (HRP2) gene of Plasmodium falciparum was used for the diagnosis of malaria. For the amplification reaction, a 50 µL total reaction volume

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was prepared, including 5 µL of 10x PCR buffer (70 mM Tris-HCl pH 8.8, 200 mM (NH4)2SO4, 0.1% Tween 20), 2 mM MgCl2, 250 µM of each of the four deoxynucleotide triphosphates, 1.25 U Taq DNA polymerase (Fermentas), 50 pmol of each primer (pfhrp2-F: 5'-CAAAAGGACTTAATTTAAATAAGAG-3' and pfhrp2-R: 5'-AATAAATTTAATG GCGTAGGCA-3') (11), and 5 µL of extracted template DNA. Amplification of parasite DNA was performed using a T3 thermal cycler (Biometra, UK). The PCR cycling conditions were as follows (35 cycles): initial denaturation at 95°C for 15 minutes, followed by denaturation at 95°C for 30 seconds, annealing at 50°C for 45 seconds, extension at 72°C for 1 minute, and a final extension at 72°C for 10 min. PCR products were analyzed using a 2% agarose gel.

Data Analysis

Statistical analysis was conducted using the statistical software packages SPSS version 20 and EPI Info (version 7.1.3). Graphs were generated using Excel 2013 software. The significance threshold for our study was set at 5% for the analysis conducted.

RESULTS

Characteristics of the Population

The average age of the study participants was 26 years old. A higher proportion of participants (70.87%) came from the North region compared to the Far North region. The majority of participants were married women (98.06%), with most being housewives (79.05%). Additionally, 24.76% had no formal education, 35.24% had completed primary education, and 5% received a salary as income (see Table 1). In terms of ethnicity, the majority of participants were Sudano-Sahelian women (98.06%). None of the participants identified as semi-Bantu.

Table 1: Socioeconomic Characteristics of the Study Population

Variables	Effective (n=105)	Total (N=105)
Marital status		
Unmarried	2 (1,94%)	2 (1,94%)
Married	103 (98,06%)	103 (98,06%)
Divorced	0 (0,00%)	0 (0,00%)
Widow	0 (0,00%)	0 (0,00%)
Level of Education		
Primary	37 (35,24%)	4 (35,24%)
Secondary	29 (27,62%)	34 (27,62%)
Higher	13 (12,38%)	22 (12,38%)
None	26 (24,76%)	0 (24,76%)
Profession		
Private sector employee	2 (1,90%)	15 (1,90%)
Public sector employee	5 (4,79%)	21 (4,79%)
Merchant	15 (14,26%)	22 (5,71%)
Housewife	83 (79,05%)	1 (79,05%)
Ethnicity		
Bantu	2 (1,94%)	37 (61,66%)
Semi-Bantu	00 (0,00%)	00 (0,00%)

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Sudano-Sahelian	103 (98,02%)	1 (1,66%)

Result of Biological diagnosis of pathologies in the study population

Among the 105 women interviewed, the pregnancy-related pathologies are presented in Table 2. The most common was toxoplasmosis (28.57%), followed by malaria with a proportion of (16.19%).

Table 2: Biological Diagnosis of Pathologies in the Study Population

Pathology	Effective (n=105)
Malaria	17 (16,19%)
Toxoplasmosis	30 (28,57%)

Result of PCR diagnosis of pathologies in the study population

The same results were obtained when the participants were assessed for malaria and toxoplasmosis using the rapid diagnostic test (RDT) and microscopy.

Following the amplification of DNA extracts from the 105 participants, 18 out of 105 (28.33%) tested positive for the Plasmodium falciparum Histidine Rich Protein 2 (HRP2), and 75 out of 105 (71.42%) tested positive for the B1 gene of Toxoplasma gondii. The electropherograms (see Figures 1 and 2 below) illustrate the different forms of gene alleles.

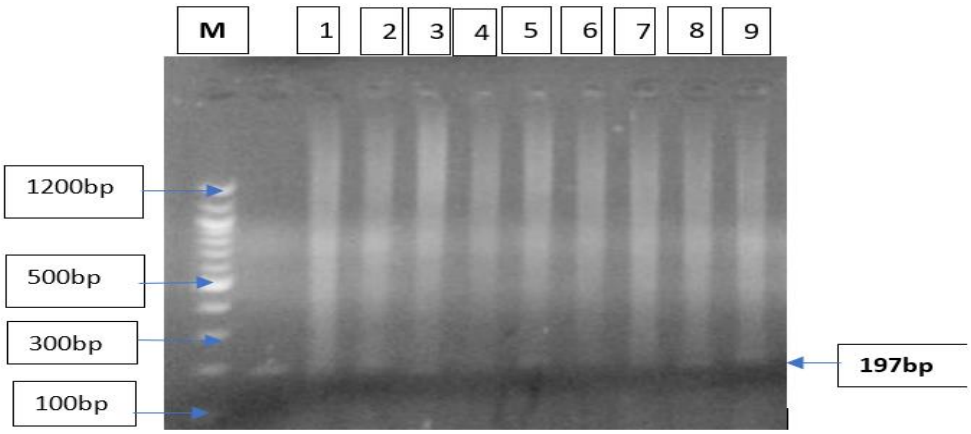

Figure 1: Representative photograph of the PCR of HRP2gene for plasmodium falciparum. M= 100 bp DNA Ladder, 1 = Positive control, 2 - 9 = Test samples.

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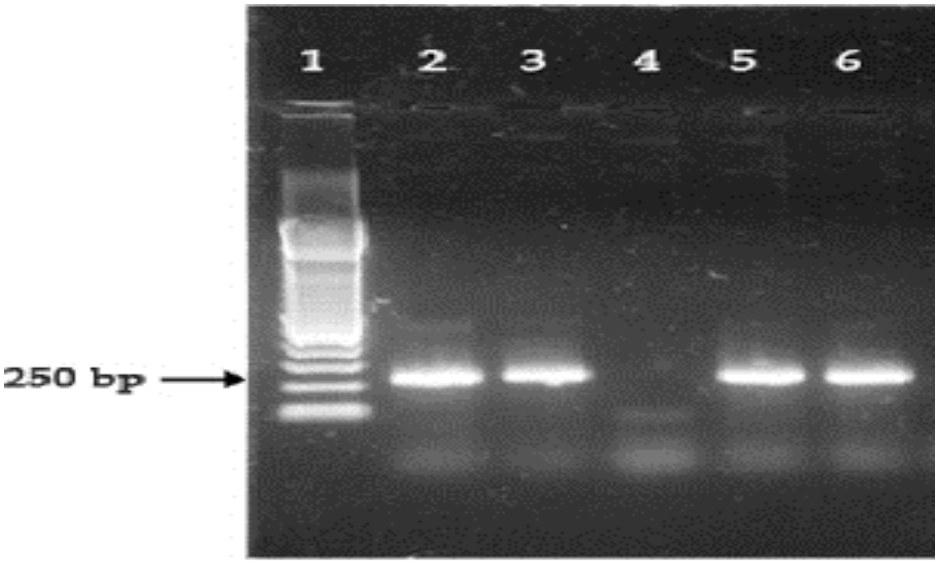

Figure 2: Representative photograph of the PCR of B1 gene for Toxoplasmosis gondii. 1= 100 bp DNA Ladder, 2 = Positive control, 3 - 6 = Test samples.produit.

Comparing the results obtained by microscopy, PCR, and RDT, we observed that PCR is significantly more specific than microscopy and RDT (see Table 3) in diagnosing malaria and toxoplasmosis in pregnant women. The proportions were 16.19% for RDT and microscopy compared to 17.14% for PCR for malaria, and 28.57% for RDT against 71.42% for toxoplasmosis

Table 3: Frequency of Molecular Diagnosis

Variables	Eff Biological Diagnostic (n=105)	Eff Molecular Diagnostic (n=105)	P-value
Malaria (HRP2)	17 (16,19%)	18 (17,14%)	
Toxoplasmosis (B1)	30 (28,57%)	75 (71,42%)	**0,00739**

Average number of births per group of patients with or without toxoplasmosis

According to the following graph, patients with toxoplasmosis give birth more than those without toxoplasmosis. On average, they have at least two children, while those without the disease have fewer than two children.

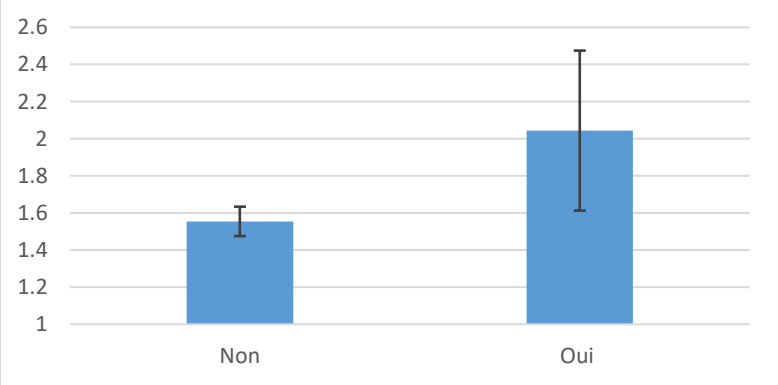

***Figure 3**: Average number of births per group of patients with or without toxoplasmosis*

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DISCUSSION

Of the 105 patients sampled, the majority of them came from the North (70.87%) and Far North (21.36%). Almost all of them were married (98.06%), with an average age of 26 years. The data obtained was slightly different from a similar study carried out in Ghana by Bhavana et al. where they compared the diagnostic performance of the rapid diagnostic test (RDT) and enzyme-linked immunosorbent assay (ELISA) for T. gondii diagnosis. This study is somehow different from another study conducted in Yaounde Cameroon by Akindeh et al.. in that they compare the performers of RDT, microscopy and PCR in the diagnosis of malaria in asymptomatic children whereas as study compared this technique in an adult population of pregnant women **(14, 23).** A low level of education was observed among the study participants this was similar to studies conducted by Bhavana et al. and Kwakye-Nuako all in Ghana. These studies demonstrated an association between low educational level and seroprevalence of T. gondii in their study populations. **(24, 25).** The study showed a 17 (16,19%) for the rapid and microscopic diagnosis of malaria and a 18 (17,14%) for molecular diagnosis of malaria. These results differ from those of a similar study carried out by Bekindaka et al. in the kumba health district where there demonstrated a great difference in results obtained from diagnosing malaria in pregnant women with a rapid diagnostic test, microscopy and PCR. Here the PCR was more sensitive and more specific than the RDT and microscopy **(26).** This difference may be justified by the fact that there was a possible deletion of the histidine rich protein 2 gene from Bekindaka et al. study population as compared to ours **(27).** A 30 (28,57%) for the rapid and microscopic diagnosis of toxoplasmosis and a 75 (71,42%) for molecular diagnosis of toxoplasmosis. This result was similar to another study conducted in Ghana, however it differs from another study conducted in Cameroon by Olivia et al. where there was no significant difference between toxoplasmosis diagnosis results using a rapid diagnostic test and those using PCR as was non-statistically significant results was obtained (pv = **0,00739)**. This difference might be due to a difference in the study population since Olivia et al. used children as their study population whereas in our study, pregnant women were the targeted population. **(24, 28).** Our results also differ from those obtained by Guemgne Todjom et al. (2019) **(15)** in the West Region of Cameroon. This difference could be explained by the difference in sample size since Todjom et al. used a larger sample size than ours**.**

CONCLUSION

This study demonstrates that Polymerase Chain Reaction (PCR) is more sensitive in the diagnosis of malaria and toxoplasmosis in pregnant women.

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DECLARATIONS

Ethical approval: This study was approved by the University of Yaounde 1 Joint Institutional Review Board for Animal & Human Bioethics (JIRB) No: BTC-JIRB2022-045

Competing interest: We declare no conflict of interest.

Authors' contributions: WFM, AMN, JPKC, IA, contributed to the design of the study. IA, JPKC, coordinated the study. IA, ASH, KA, KM, supervised the enrolment of patients and sample collection. IA, TC, BML, performed the molecular analysis. IA, JPKC, NLN, writing up the manuscript. All authors contributed to the revision of the manuscript and approved the final version of the manuscript prior to submission.

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Data Availability

The dataset used for this analysis is available and we can let you have it if needed.

Consent to participate and consent to publish

All participants consented to the study by signing a consent form before they were recruited.

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