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Parasitological and Molecular Studies on Co-Infection of *Plasmodium falciparum* and *Schistosoma haematobium* Amongst Women in Igedeland, Benue State, Nigeria

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ABSTRACT

This study investigated the co-infection of *Plasmodium falciparum* and *Schistosoma haematobium* among women in Igedeland of Benue State, Nigeria using parasitological and molecular approaches. Urine and blood samples were collected from women and processed parasitologically for *S. haematobium* and *P. falciparum* respectively. Well-structured questionnaires were used to obtain socio-demographic data from the women. Representative samples were afterwards processed molecularly using PCR technology. Data obtained were entered into SPSS and analyzed using chi-square test to the test the relationship between variables and co-infection rates. Results obtained revealed an overall co-infection rate of 1.25% with *P. falciparum* and *S. haematobium* recording individual rates of 17.45% and 6.07% respectively. A higher co-infection rate was recorded for women within the age range of 20-30years (1.92%) as well as those who were farmers (2.80%). Those with secondary education recorded (2.29%) while those separated had (13.04%). There was a significant difference in the co-infection rates of women in Igedeland with respect to age, education, and location ($P < 0.05$) while occupation and marital status were not significant ($P > 0.05$). Also, molecular characterization of the parasites revealed the presence of plasmodium (*Pf18S*) and *S. haematobium* (*Sh73*) DNA markers in women of Igedeland. It was concluded that the *co-infection rate is relatively low when compared to reports in time past*. Nevertheless, occurrence of single parasite infection is high with *P. falciparum* being the most dominant parasite.

INTRODUCTION

Parasitic infections are a major cause of morbidity and mortality in developing nations, and co-infection with parasite infections in humans are common. A good example of a notable co-infection is the infections by schistosomiasis and malaria, which are two of the parasitic diseases with the heaviest economic and social burdens (Lemaitre *et al.*, 2014). The overlap of malaria parasite and helminth infections is

influenced by high frequencies of the parasites in the same population, similar geographical distribution of parasites, shared risk factors, common transmission methods, and genetic and immunological predisposition (Achang-Kimbi *et al.*, 2017).

Plasmodium falciparum inflicts the greatest burden and about 90% of the populations infected with malaria live in sub-Saharan Africa where Nigeria, Benue State and Igedeland is located. Pregnant women are particularly vulnerable to *P. falciparum* especially in first pregnancy and protective interventions against malaria (WHO, 2004). Besides malaria, schistosomiasis is the second important parasitic disease in terms of socioeconomic and public health importance. More than 90% of the roughly 200 million cases of schistosomiasis occur in Africa of which approximately two-thirds are caused by *Schistosoma haematobium*, the etiologic agent of urogenital schistosomiasis (Hotez and Kamath, 2009; Achang-Kimbi *et al.*, 2017).

S. haematobium infection is caused by digenetic trematodes of the genus *Schistosoma*. 90% of worldwide cases occur in sub-Saharan Africa (WHO, 2017). More than 207 million people, 85% of who live in Africa, are infected with schistosomiasis and an estimated 700 million people are at risk of infection in 76 countries where the disease is considered endemic, as their agricultural work, domestic chores and recreational activities expose them to infested water (WHO, 2017). Transmission of the parasite takes place in permanent water bodies as well as in seasonal ponds or streams (Omonijo *et al.* 2013). Although *S. haematobium* infection does not always result in clinical diseases as many infections are asymptomatic, schistosomiasis in children can cause anaemia, stunted growth, and a reduced ability to learn (WHO, 2012).

Malaria and Schistosomiasis are endemic in rural areas where there is a lack of safe water supply, poverty, ignorance, and poor hygienic practices

(Nour, 2010). Forty million women of child bearing age are infected with younger and pregnant women being at greater risk of infection. Domestic activities such as washing clothes and fetching water in infected water expose women and children to infection (Friedman *et al.* 2007). Coinfection may have considerable health consequences leading to more severe clinical symptoms and pathology than infection with single parasite species (Broker *et al.*, 2007). For example, coinfections of *P. falciparum* with hookworms and schistosomes tend to exacerbate hepatosplenic, anaemia, and malnutrition morbidities (Broker *et al.*, 2007). Considering the great effect of *P. falciparum* and *S. haematobium* infection as well as their coinfection, an investigation into their infection rates as well as their molecular characterization becomes necessary.

MATERIALS AND METHODS

Study Area

The study was carried out in Oju and Obi local government areas in the southern part of Benue State. Oju is located between latitude 6°6'N and 6°9'E and Longitude 8°10'E and 8°25'E in the south eastern part of Benue State. Oju local government area covers an area of 1,283 km². It is bounded in the south by Cross River State, in the east by Konshisha local government area, in the north by Obi and Gwer East local government areas, in the west by Ado local government area, and in the south-west by Ebonyi State. The 2006 national population and housing census put the population of Obi local government area of Benue State at 98,707, with 49,143 males and 49,564 females. Obi local government area covers an area of 423 km². It is bounded in the north-east by Gwer East local government area, in the south by Oju local government area, in the west by Ado local government area, and in the north by Otukpo local government area.

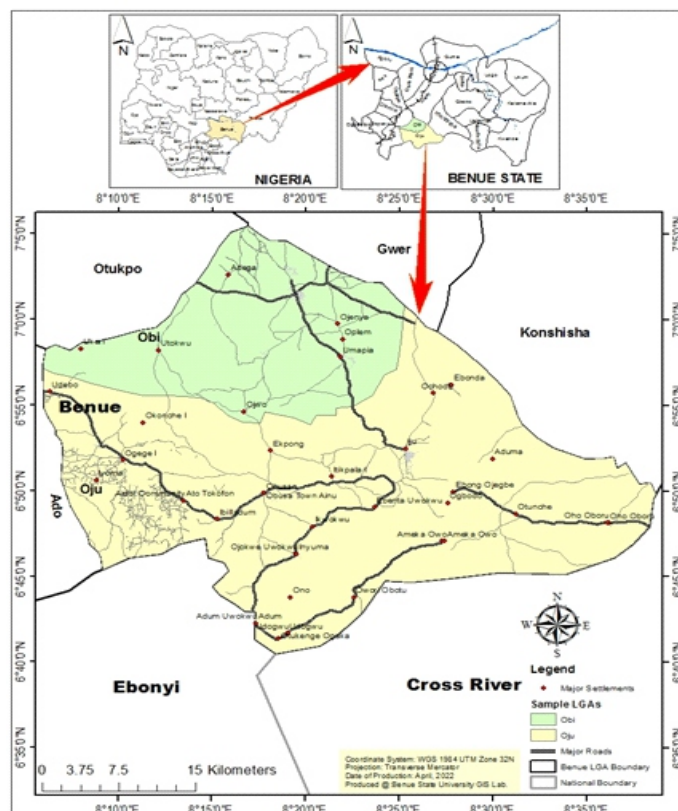


Figure 1: Map of Benue State showing Location of Oju and Obi Local Government Area
 Source: Department of Geography, Benue State University, Makurdi

Study Population

A Cross sectional study design was adopted for the study. All women in the areas visited were given equal opportunity to participate in the study. Women were briefed on the health implication of being infected with parasites, the dangers of parasitism and the relevance of the study to each participant. Participation was made voluntary. Volunteers were made to fill a written consent form indicating their willingness to participate in the study.

Sample Size

The sample size for the study was determined using

the formula by Beena *et al.*, (2015).

$$n = \frac{z^2 p(1-p)}{d^2}$$

Where

n = sample size

z = statistic for a level of confidence, in this case the level of confidence will be 95% (1.96)

p = expected prevalence

d = precision at 5% (0.05)

Ethical Consideration

Ethical approval was obtained from the Benue State Ministry of Health (Ref Number: MOH/STA/204/VOL1/182) prior to the

commencement of the study. This was in accordance with the requirements for conducting research on human subjects. Also, informed consent forms were distributed to the women and were verbally translated to the women in the Igede language. Only women who consented to the study by signing the consent forms were recruited for the study.

Sample Collection

Specimen bottles were distributed to the women involved in the study who were expected to return the containers with urine in them. Blood was however collected intravenously by trained laboratory technicians from women using appropriate aseptic techniques. Blood samples were examined immediately by the laboratory technicians while urine samples were taken to the laboratory for microscopic examination. Samples collected included.

Demographic data, clinical manifestations of parasites as well as information pertaining to parasitism were collected from the study subjects using well-structured questionnaires, to enable adequate correlation of results obtained with demographic variables.

Examination of Urine Samples

Urine samples were examined microscopically. About 10 ml of well mixed urine was aseptically transferred to a labeled conical tube and centrifuged at 500–1000rpm for 5 minutes. The sediment was afterwards remixed by tapping the bottom of the tube after which one drop of the *well-mixed* sediment was transferred to a slide and covered with a cover slip. The preparation was examined microscopically using the 10x and 40x objective with the condenser iris closed sufficiently to give good contrast (Cheesbrough, 2005).

Examination of Blood Samples

Blood samples were examined for malaria parasite using a Rapid Test Diagnostic Kit. The area to be

pricked was cleansed with an alcohol swab after which the end of the fingertip was squeezed gently and pierced with a sterile lancet provided in the test kit. 5µl of whole blood was collected using the pipette provided in the test kit and added into the sample well of the test device, then two drops of the assay buffer was added into the buffer well. The result was read within 20 minutes and recorded accordingly.

The result was said to be positive if two colour bands appear, one at the control line 'C' and the other at the test line 'T'. Negative result occurred if only one colour band appear, at control line 'C'. In a case where no colour band appears, or colour band appeared on the test line, the result was said to be invalid and repeated as such (Cheesbrough, 2005).

Molecular Analysis of Samples

Representative samples were sent to FOWM Biotechnology Limited, Yaba, Lagos and Molecular Laboratory, Covenant University Centre for Research, Innovation and Development (CUCRID) for DNA extraction and amplification (Polymerase Chain Reaction).

Data Analysis

Data obtained from parasitological analysis and questionnaire administration were entered into IBM Statistical Package for Social Sciences (SPSS) version 21.0. The percentage prevalence (%) was calculated in each case. Descriptive statistics such as proportion and percentages were computed for sociodemographic data while associations and relationship between risk factors and parasitic infections were tested using chi square (χ^2) at 95% confidence level. A p-value less than 0.05 ($P < 0.05$) was considered statistically significant.

RESULTS

An overall co-infection rate of 1.25% was obtained for *S. haematobium* and *P. falciparum*.

Individual infection rate of 17.45% and 6.07% was however obtained for *P. falciparum* and *S. haematobium* respectively. In terms of the prevalence of parasitic co-infection of *P. falciparum* and *S. haematobium* amongst women of Igedeland, the highest prevalence was recorded for women within the age range of 25-30years (1.92%), while age group 15-20 years recorded the least (0.88%). There was a significant association between age and co-infection with *P. falciparum* and *S. haematobium* (P<0.05) (Table 1).

The rate of co-infection was higher among farmers (2.80%) when compared to traders (2.17%), businesswomen (1.29%), civil servants (0.49%) and those from other non-specific occupations (0.32%). There was also no significant association between occupation and co-infection with *P. falciparum* and *S. haematobium* (P>0.05) (Table 2). A higher co-infection rate was observed for Oju Local government area with Idelle community recording highest prevalence (28.57%). Adiko community in Obi local government area also recorded highest prevalence of infection (40.0%) and the difference was significant (P<0.05) (Table 3).

In terms of marital status, highest co-infection rate was reported among separated women (13.04%) while least prevalence was recorded by married

women (0.74%) but the difference was not significant (Table 4). Also, women with secondary school level of education recorded higher co-infection rate (2.29%) while least prevalence was recorded among women with tertiary education (0.24%) and the difference was significant (Table 5).

In terms of molecular characterization of the parasites examine, the presence of plasmodium (*Pf 18S*) in blood samples of women in Obi and Oju was determined (Gel plate 1). The bands as shown in wells 1 and 14 indicated that samples OBI 58 and OJU 200 had plasmodium. Gel plate II showed the presence of *S. haematobium* (*Sh73*) in sample from OBI 31. The band is as shown in well 10.

Table 1: Prevalence of Co-infection of *Plasmodium falciparum* and *Schistosoma haematobium* amongst Women of Igedeland in Relation to Age

Age	Number examined	Number Positive (%)	χ^2	P-value
15-20years	113	1 (0.88)	14.65	0.006
20-25years	160	0 (0.00)		
25-30years	260	5 (1.92)		
30-35 years	224	3 (1.33)		
35-40years	178	2 (1.12)		
Above 40years	102	1 (0.98)		
Total	1037	13 (1.25)		

Table 2: Co-infection of *Plasmodium falciparum* and *Schistosoma haematobium* amongst Women of Igede land in Relation to Occupation

Occupation	Number examined	Number Positive	χ^2	P-value
Civil Servants	210	1 (0.47)	8.443	0.06
Trader	184	4 (2.17)		
Farmer	178	5 (2.80)		
Business Women	155	2 (1.29)		
Others	310	1 (0.32)		
Total	1037	13 (1.25)		

Table 3: Distribution of *Plasmodium falciparum* and *Schistosoma haematobium* amongst Women of Igede land.

Location	Communities	Number examined	Number Positive	Parasites	
				<i>Plasmodium spp.</i>	<i>S. haematobium</i>
Oju	Uje	140	33 (23.57)	20 (14.28)	13 (9.28)
	Uwokwu	30	6 (4.28)	3 (10.00)	3 (10.00)
	Ega	50	15 (10.71)	9 (18.00)	6 (12.00)
	Oju center	34	17 (12.14)	10 (29.41)	7 (20.58)
	Idelle	178	40 (28.57)	30 (16.85)	10 (5.61)
Obi	Adiko	220	56 (40.00)	44 (20.00)	12 (5.45)
	Obarike	150	23 (16.42)	18 (12.00)	5 (3.33)
	Itogo	150	38 (27.14)	36 (24.00)	2 (1.33)
	Adum	85	16 (11.42)	11 (12.94)	5 (5.88)
Total		1037	244 (23.52)	181 (17.45)	63 (6.07)

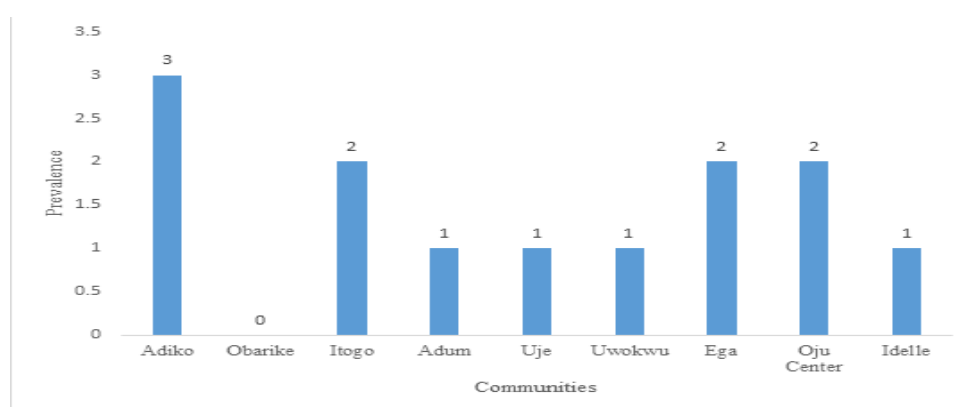
 $\chi^2 = 16.19$; $P = 0.004$ **Figure 2:** Co-infection with *Plasmodium falciparum* and *Schistosoma haematobium* in the selected Communities Surveyed

Table 4: Co-infection of *Plasmodium falciparum* and *Schistosoma haematobium* amongst Women of Igede land in Relation to Marital status

Marital Status	Number examined	Number Positive	χ^2	P-value
Single	277	4 (1.44)	3.484	0.43
Married	669	5 (0.74)		
Divorced	11	0 (0.00)		
Separated	23	3 (13.04)		
Widowed	57	1 (1.75)		
Total	1037	13 (1.25)		

Table 5: Co-infection of *Plasmodium falciparum* and *Schistosoma haematobium* amongst Women of Igedeland in Relation to level of education

Level of Education	Number examined	Number Positive	χ^2	P-value
No formal Education	109	1 (0.91)	9.43	0.04
Primary	217	4 (1.84)		
Secondary	305	7 (2.29)		
Tertiary	406	1 (0.24)		
Total	1037	13 (1.25)		

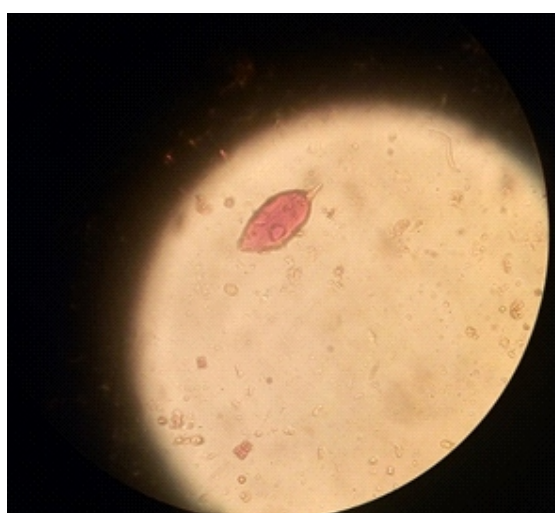
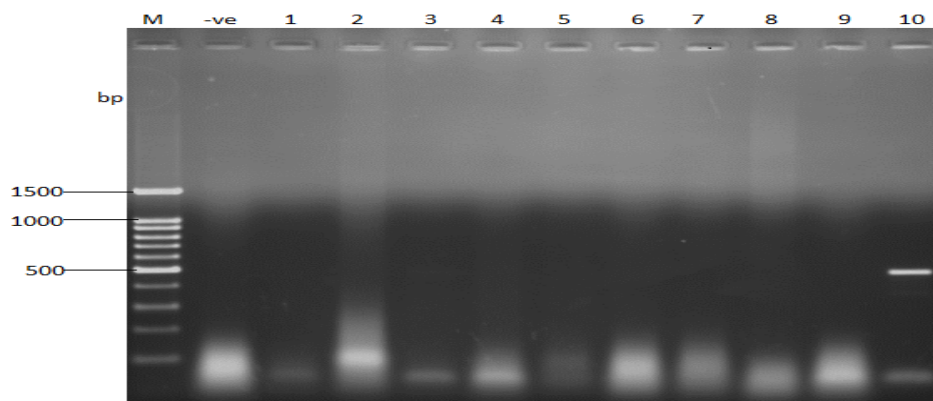


Plate 1: Ova of *Schistosoma haematobium* seen in a urine sample in Adiko Oju



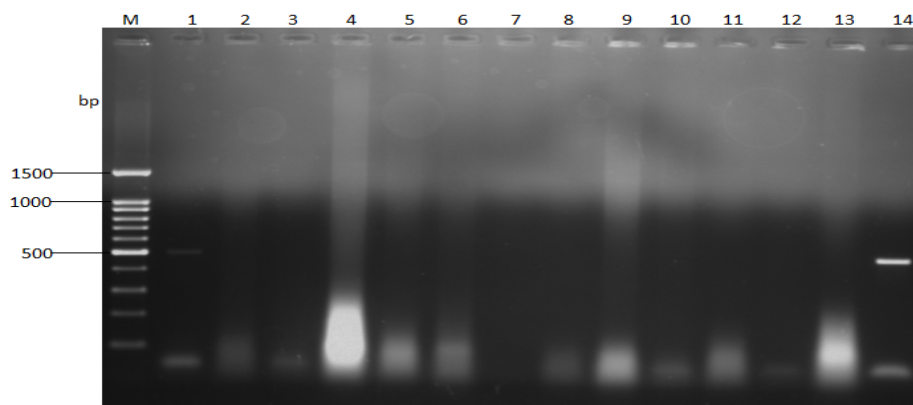
Gel pic. II: Check gel for *S. haematobium*

Sample 10 (OBI 31)

KEY:

- M= DNA ladder
- 1-10= Samples
- -ve= Negative

Figure 3: Gel Documentation of Detected *S. haematobium*: DNA ladder lane 10



Gel pic. I: Check gel for *Plasmodium falciparum*

Sample 1 and 14: OBI 58 and OJU 200

KEY:

- M= DNA ladder
- 1-14= Samples

Figure 4: Figure 3: Gel Documentation of Detected *Plasmodium falciparum*: DNA ladder lane 14

DISCUSSION

The co-infection of *P. falciparum* and *S. haematobium* among women in Igede land was investigated in this study. Findings from the study revealed a coinfection rate of 1.25% among the women in the study population while single parasite infection rate was 23.52%. Among the women in Igede land, 17.45% of them were positive for *Plasmodium* spp. only while 6.07% of them were positive for *S. haematobium* only. A higher prevalence of *Plasmodium* spp. reported in this study is in agreement with Ocheri *et al.* (2012) who reported a high ranking of the malaria parasite in Benue State, Nigeria. According to Shulman and Dorman (2003), plasmodium infection inflicts the greatest burden and about 90% of the populations infected reside in Sub-Saharan African, Igedeland inclusive. The findings of this study justifies that claim. This study however disagrees with the report of Achang-Kimbi *et al.* (2017) who reported a slightly higher prevalence of *Schistosoma haematobium* among women than *Plasmodium* spp. A higher prevalence of *Plasmodium* spp. as characteristic of this study may be attributed in part to the availability of good breeding grounds for the development of the plasmodium parasite and completion of its life cycle (CDC, 2018).

In terms of *S. haematobium*, an individual prevalence rate of 6.07% observed in this study is lower than that of Uweh *et al.* (2014) who reported a prevalence rate of 19.3% in Igedeland of Benue State. It is also lower than a 20.0% prevalence reported by Chikwendu *et al.* (2019) in Oju Local Government Area of Benue State. A variation in the rate of urinary schistosomiasis infection in Igedeland overtime might be as a result of increase in enlightenment on parasitic diseases which might have led to adoption of better prevention techniques by the people of Igedeland and hence an improvement in the prevalence of urinary schistosomiasis as days go by. A prevalence of 6.07% for *S. haematobium* as observed in this study however differs from the report of Obisike *et al.*

(2021) who reported a lower prevalence of 4.3% in Otukpo Local Government area of Benue State. This rate of co-infection with the malaria parasite and *S. haematobium* is lower than a 9.0% rate of co-infection with *P. falciparum* and *S. haematobium* reported by Dejon-Agobe in rural areas of Gabon. It is also lower than a 15.2% co-infection rate reported by Achang-Kimbi *et al.* (2017) in Munyenge, Cameroun. Variations in the rate of co-infection observed between this study and those of previous authors might be attributed in part to environmental and geographical variations which provides predisposing factors that might promote the proliferation of *Plasmodium* species and *S. haematobium*.

Women within the age group of 25-30years recorded the highest prevalence of infection. This finding differ from the report of Obisike *et al.* (2021) who reported a higher prevalence among the age group of 11-20years. It also disagrees with Achang-Kimbi *et al.* (2017) who reported higher co-infection of *S. haematobium* and *Plasmodium* species among women of age 21-25. A possible reason for the high co-infection rate observed in this age group could be as a result of the active nature of this age group who are mostly married women in the prime of their youth who are exposed to water bodies and other risk factors of infection in the course of carrying out their feminine duties. There was also a significant association between age of women and rate of co-infection with *Plasmodium* spp. and *S. haematobium* in the study location in agreement with Achang-Kimbi *et al.* (2017) and Dejon-Agobe *et al.*, (2018) who reported a significant relationship between age and co-infection rate.

The findings of this study reveal a higher co-infection rate among women who are farmers compared to other professions. A higher infection rate among this group of women might be attributed to their exposure to *Schistosoma* which

is also a soil helminth and can be transmitted when the women's foot are exposed to the ova of the parasite. The high prevalence rate among farmers however differs from that of Achang-Kimbi *et al.* (2017) who reported highest infection rate among students. A possible reason for this variation might be the dual nature of students who also act as farmers though schooling in their communities. This justifies the high prevalence obtained for farmers as reported by Achang-Kimbi *et al.* (2017). In terms of educational attainment, women who have tertiary education recorded a lower prevalence than women with other educational backgrounds. A lower prevalence among these women may be attributed to their level of enlightenment on parasitic diseases which enables them to understand the dangers associated with parasitic infections and the need to prevent them; hence the lowered prevalence. Women who were separated also presented higher prevalence rate than other women. A possible reason for this might be the ability of women who are separated to focus on their careers chief of which is farming in the study location, thus increasing their chances of becoming infected with parasites.

Amplification of genes through polymerase chain reaction indicated the presence of *Pf18S*, *Sh 73*, *Sh 77*, *Eh 1*, *Tv18S* and *Cox 1*. These genes are known to code for parasites in urine and blood and are utilized in their identification (Faten *et al.*, 2017). Their presence in the PCR analysis of blood and urine of women in Igede land confirmed the presence of Plasmodium species. and *S. haematobium* in women of Igede land. This is consistent with the findings of Cnops *et al.*, (2013); Pechangou *et al.*, (2015); Faten *et al.*, (2017); Lucrecia *et al.*, (2017); Lloyd *et al.*, (2018) and Grabias *et al.*, (2019) who reported that PCR analysis reveals the presence of parasitic DNA markers and consequently, the parasites present. Molecular analysis of parasites by PCR was seen to be more specific in identifying parasites as well as their DNA markers which not found by microscopic

techniques. This is a pointer to the high specificity of PCR techniques in the detection of parasites and for studies pertaining to co-infections with parasites.

CONCLUSION

The study revealed a co-infection rate of 1.25% among women in Igede land, Benue State. This co-infection rate is relatively low when compared to reports in time past. Nevertheless, occurrence of single parasite infection is high with *P. falciparum* being the most dominant parasite. Also, the genes *Pf18S*, *Sh 73*, *Sh 77*, *Eh 1*, *Tv18S* and *Cox 1* that codes for *Plasmodium falciparum* and *S. haematobium*, were present in the women examined thus confirming the presence of parasitic co-infection in women of Igedeland. Despite a lowered prevalence of co-infection with *Plasmodium* species and *S. haematobium*, continuous efforts is necessary to ensure a complete eradication of the malaria parasite and urinary schistosomiasis from Igedeland Benue State and Nigeria at large.

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