

ASSESSMENT OF PLASMODIUM AND SCHISTOSOMA INFECTION AMONG PRIMARY AND SECONDARY SCHOOL CHILDREN (6-21 YEARS) IN MAKURDI, BENUE STATE, NIGERIA

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ABSTRACT

This research was conducted to determine the intensity of *Plasmodium* and *Schistosoma* infection among school-aged children in Makurdi, Benue State, Nigeria. Blood, urine and stool samples were collected from 340 school-aged in Makurdi Benue State, Nigeria. The samples were examined for *Plasmodium* and *Schistosoma* species. The primary school with high intensity of *P. falciparum* is C.A.C 21.1, while Al-Ihsan had the lowest intensity of 11.75. The school with high intensity of *S. haematobium* is Al-Ihsan with 19.00, while Holy Ghost has the lowest intensity of 9.71. The school with high intensity of *S. mansoni* is L.G.E.A 26.00, while Holy Ghost had the lowest intensity of 12.60. The secondary school with high intensity of *P. falciparum* is Al-Irshad with 22.36, while Salam Academy had the lowest intensity with 8.17. The school with high intensity of *S. mansoni* is Salim Progress with 22.00 while Al-Irshad Makurdi had the lowest intensity with 9.00. Based on the findings from the study, it is imperative that: the infected participants be treated with anti-malarial drugs for malaria and praziquantel for Schistosomiasis and monitored continuously via followed up treatment by health authorities to prevent re-infection. Public health awareness and routine investigation should be done often. It is therefore recommended that public health awareness and routine investigation should be intensified within the study population.

Keywords: *Plasmodium*, *Schistosoma*, Intensity, School Children, Benue State.

INTRODUCTION

Background of the study

Malaria and schistosomiasis are among the most important diseases of enormous public health burdens in tropical and subtropical countries of the globe (Dumbo *et al.*, 2014). Malaria is a complex and life-threatening parasitic disease caused by the protozoan parasite of the genus *Plasmodium* (Getie *et al.*, 2015). Malaria is associated with anaemia, which causes severe morbidity and mortality in vulnerable groups infected with *Plasmodium falciparum* (Ajakaye and Ibukunoluwa, 2020). Other Species that affect humans include: *P. vivax*, *P. ovale*, and *P. malariae*. Schistosomiasis is a chronic and debilitating disease caused by flukes (digenetic Trematode flatworms) known as *Schistosomes* (Okpala *et al.*, 2004).

Schistosomiasis also known as (biharziasis or snail fever) ranking second to only malaria in terms of its socio-economic and public health importance in tropical and subtropical areas (Ogbe, 2002).

It is also the most prevalent of the waterborne diseases and one of the greatest risks to health in rural areas of developing countries (Ofoezie *et al.*, 1998; Ogbe, 2002). An analysis, based on African studies, showed that there is a risk ratio of 2.4 and 2.6 for urinary schistosomiasis (caused by *S. haematobium*) and intestinal schistosomiasis (caused by *S. mansoni*), respectively, among persons living adjacent to reservoirs. The analyses also showed that persons living near land that had been irrigated for agricultural use had an estimated risk ratio of 1.1 for urinary schistosomiasis and an estimated risk ratio of 4.7 for intestinal schistosomiasis (Steinmann *et al.*, 2006).

Infection occurs through contact with water infested with the free swimming larval stages of parasitic worms (cercariae) that penetrate the skin and develop in the human body to maturity. Parasite eggs leave the human body with urine or excreta.

They hatch in freshwater and infect the appropriate aquatic snail intermediate hosts. *Bulinus* snails are intermediate host for *S. haematobium* (Okoli and Iwuala, 2004). Within the snails they develop into cercariae, which are, in turn, released into the water to infect new human hosts. Transmission can take place in almost any type of habitat from large lakes or rivers to small seasonal ponds or streams (WHO, 2001; Akue *et al.*, 2011). In urinary Schistosomiasis, it is the eggs and not the worm which cause damage to the intestines, the bladder and other organs (Brooker *et al.*, 2007).

Schistosomiasis appears to be a neglected tropical disease. However, due to irrigation programs and hydroelectric power development, the incidence of infections is increasing in endemic areas of Africa and the near east, and the risk of infection is highest amongst those who lived near lakes or rivers (Kabatercine *et al.*, 2004; Brooker *et al.*, 2007). More than 207 million people are infected worldwide, with 75% of them living in Africa alone (WHO, 2011). Recent estimates from sub-Saharan Africa have indicated that approximately 280,000 deaths each year can be attributed to schistosomiasis (Van der Werf *et al.*, 2003). In Nigeria *S. haematobium* infection is widespread, constituting a public health problem particularly in children (Sulyman *et al.*, 2009; Griffiths *et al.*, 2011; Dumbo *et al.*, 2014). Although there is no current estimate of the disease in the country, past estimates have put the infection at about 25 million people, and 101 million at risk of infection (Chitsulo *et al.*, 2000). The distribution of the disease is focal, aggregated and usually related to water resources and development schemes such as irrigation projects, rice/fish farming and dams. It occurs in all the states of the federation, with a high infection rate among school children (Okpala *et al.*, 2004; Mafe *et al.*, 2005; Akue *et al.*, 2011).

In Nigeria, Schistosomiasis due to *S. haematobium* is widespread, constituting a public health problem particularly in children (Okpala *et al.*, 2004; Sulyman *et al.*, 2009 and Griffiths *et al.*, 2011). The distribution of the disease is focal, aggregated and usually related to water resources and development schemes such as irrigation projects, rice/fish farming and dams. It occurs in all the states of the federation, with a high infection rate among school children (Mafe *et al.*, 2000; Okpala *et al.*, 2004). There are reports of Bilharziasis in Benue State (Amali, 1989; Atu and Galadima, 2003; Houmsou, *et al.*, 2012). Four species of *Schistosomes* are responsible for human schistosomiasis: *Schistosoma mansoni*, *S. haematobium*, *S. japonicum* and *S. intercalatum* (Swai *et al.*, 2006; Dawaki *et al.*, 2015; Dawaki *et al.*, 2016).

Infection with multiple Species of parasites is often the norm in developing countries (Griffiths *et al.*, 2011). Malaria and schistosomiasis are highly endemic in tropical and sub-tropical areas and their epidemiologic co-existence is frequently observed (Adegnika and Kremsner, 2012; Anchang-Kimbi *et al.*, 2017). The prevalence of malaria-schistosomiasis co-infection reported to be 15 % and caused high prevalence of anemia, as compared to those infected only with malaria (Degarege *et al.*, 2012). Schistosomiasis plays an antagonistic role against malaria, but the egg intensity of *Schistosoma* species and the age of infected individuals could determine the type of interaction (Briand *et al.*, 2005; Sangweme *et al.*, 2010). Although most studies were conducted, reported that *Schistosoma* co-infection contributes to severe malaria presentation. *Schistosoma* co-infection resulted in high *Plasmodium parasitemia* and increased susceptibility of infection to mortality (Yoshida *et al.*, 2000; Legesse *et al.*, 2004; Laranjeiras *et al.*, 2008; Sangweme *et al.*, 2009). In contrast, others illustrated that *Schistosoma* co-infection contributed to low *Plasmodium parasitemia* and inhibited cerebral malaria (Lwin *et al.*, 1982; Wankine-Grinberg *et al.*, 2010; Bucher *et al.*, 2011).

Both Malaria and schistosomiasis are endemic in Nigeria (Terer *et al.*, 2013). Malaria environmental risk factors include: low utilization of nets, low utilization of indoor residual spray, and availability of multiple mosquitoes breeding site or stagnant water near the home and staying outdoor overnight. Schistosomiasis risk factors are: lack of access to safe water, contact/exposure to fresh water bodies, outdoor activities, low Socio-economic status, and poor educational access for schistosomiasis (FMOH, 2007; Getie *et al.*, 2015).

MATERIALS AND METHOD

Study area

Makurdi, Benue State's capital, is situated at latitude 7.732152° 41° N and longitude 8.539144° 28° E (Fig. 1). The Benue River runs through Makurdi, and the city's primary settlements are around 671 meters long (Udo, 1981). The rainy season lasts seven months (April to October), with an annual rainfall of 120-200cm on average (Akaahan *et al.*, 2010). Peak flows occur from August to early October, while low levels occur from December to April, depending on the rainy season. Intensive agricultural activity, bathing, swimming, and washing in rivers and streams are all common during this time (Houmsou *et al.*, 2012). Throughout the year, the area has high temperatures ranging from 28-33 °C, with the months of March and April being the hottest. During the months of December and January, Harmattan winds are accompanied by cooling effects (Nyagba, 1995)

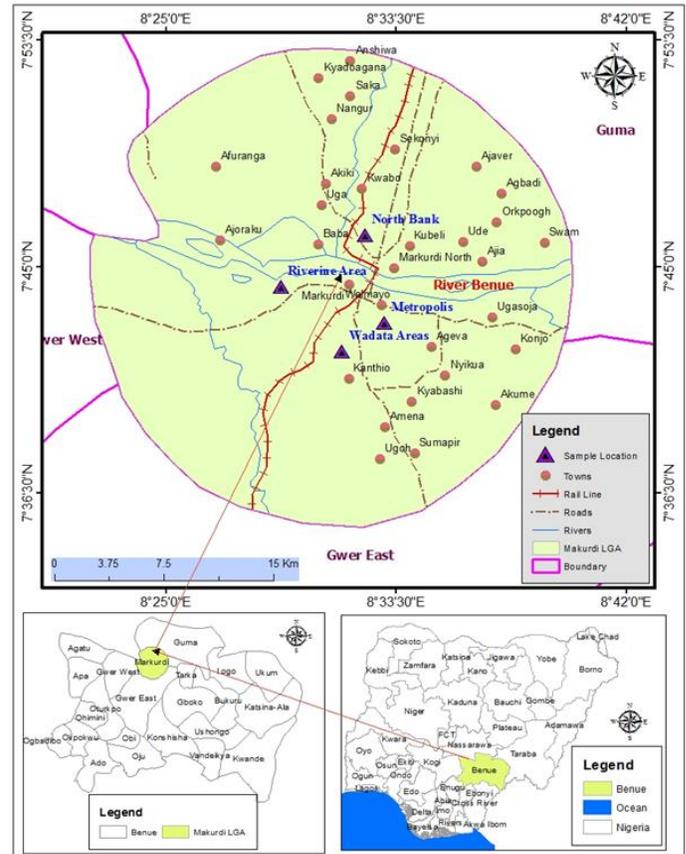


Figure 1: Map of Makurdi, Benue State.
 Source: Modified from Administrative Map of Benue State

Sample size determination

The sample size (n) was estimated using Chocran formula ($n = Z^2 (1-p)/L^2$) (Chocran, 1963)

$$N = \frac{Z^2 (1-p)}{2L^2}$$

Where:

- n = is the sample size required.
- Z = The normal distribution at 95% confidence interval.
- P = Proportion of infected individuals on a scale of 1.
- q = Proportion of infection free individuals on a scale of 1.
- L = Precision level or allowable error on a scale of 1.

Ethical approval

Before the commencement of the study, ethical approval was obtained from Benue State Ministry of Health with the reference number MOH/STA/204/VOL.1/124, Federal Medical Centre, Makurdi with the reference number FMH/FMC/MED.108/VOL.1/X and permission was sought from Makurdi Local Government Education Authority, Riverine Communities Head, and Parents of children.

Study Period

The study was conducted for a period of nine (9) months, from June, 2020 to February, 2021.

Study Design

Random sampling was used in selecting participants from each school and the riverine communities.

Study Population

Seventeen (17) Government and private schools were randomly selected based by balloting on proximity to source of water.

Inclusion Criteria

Primary 1-3 and 4-6 pupils of age range 6-9 and 10-13 years respectively, secondary school students of age range 14-17 and 18-21 years respectively were recruited. And 1-10, 11-20, 21-30 and 31-40 years age groups from the riverine communities were also recruited. The anonymity of each pupil was treated with high level of confidentiality as the purpose of this research is concerned.

Data Collection and Questionnaire Administration

The data was gathered from the respondents using a structured questionnaire. Socio-demographic data such as age, sex, parent's occupation (for school-aged children and children from riverine communities), water source and water contact activities, method of water treatment, housing distance from water body, outdoor activities, and other variables were all asked. With the help of health educator workers, community-based research assistants, and teachers, some of the questions were translated and delivered to them in their native language for better understanding. The students were grouped into their respective classes and directed to appropriately fill the form and riverine communities were also grouped at their respective working places, as adopted by Kapito-Tembo *et al.* (2009).

Sample Collection and Laboratory Analysis of Samples

One thousand and sixty (1,060) blood, urine and stool samples were collected from each of the 1,060 individuals. The samples were collected in clean, wide mouth, screw capped and dry transparent containers for stool, sterile urine sampling bottles for urine, and anticoagulant blood sampling bottles (EDTA bottles) for blood samples (Cheesbrough, 2006; Mu'azu, 2008). Collection was done between the hours of 07- 10am and samples were transported to the laboratory, Federal Medical Centre Makurdi for analysis according to the method of Cheesbrough (2005).

Microscopic Assessment of *Plasmodium* species

Two drops of venous blood was placed separately on a clean microscopic glass slide as adopted by WHO (1991) and Nyarko *et al.* (2018) Thick and thin blood films were prepared and air dried. Thin blood films were fixed with absolute methanol and both films were stained with 10% Geimsa stain for 10 min. Both thin and thick blood films were examined using light-camera microscope (LEICA DM 2500 model) with a $\times 100$ objective lens for the presence of malaria parasite. The density of the parasite was counted as "Number Parasites Found" (NPF) using recommendations of WHO standard, the parasitized RBCs are counted against leukocytes (WBCs) in the thick film, the number of leukocytes (WBCs) used is 8,000 (WHO, 1991). In addition, two tally counters (one to count parasites and the other to count leukocytes, and a simple electronic calculator) was used and expressed by this formula.

Parasites per microliter

$$= \frac{\text{Number of parasites counted}}{\text{Number of leukocytes (WBCs)}} \times 8000$$

The infected individuals were treated with Artemisinin combination based therapy (ACT), anti-malarial drugs which are used for the treatment of malaria.

Analyses of Urine Samples for Schistosomiasis Detection

For haematuria examination, chemical reagent strip methods as described by (Cheesbrough, 2002; WHO, 2011) were employed. Reagent strip combi-9 and combi-10 (Medi-Test Macherey-Nagel, Germany) was dipped into each urine sample and the color was matched with the standard color by the side of the container as recommended by the manufacturer to determine the presence of blood in urine. Urine samples were examined to detect the presence of eggs using sedimentation technique. Each urine sample was thoroughly shaken and 10ml decanted into a test-tube and centrifuged at 3,000 rpm for 5 min. in haematocrit centrifuge machine and the supernatant was discarded leaving the sediments. A drop of the sediment was placed on a clean microscope slide and stained using Lugol's iodine and left for 15 sec. for the stain to penetrate the eggs and viewed under microscope at low power ($\times 10$) and then $\times 40$ objective lens (Cheesbrough, 2002; WHO, 2011). The number of *S. haematobium* eggs per 10ml of urine was counted for each positive sample and the result was calculated by multiplying the crude egg numbers per slide with the number of ml of the respective urine sample and dividing by 10 to represent the intensity. Heavy intensity of infection was defined as >50 *S. haematobium* eggs per 10ml (WHO, 1991 and WHO, 2011). Presence of terminal spine indicates *S. haematobium*. The infected individuals were treated with praziquantel, anti schistosomal drugs which are used for the treatment of schistosomiasis.

Analyses of Stool Samples for Schistosomiasis

Using the standard Kato-Katz technique adopted by WHO (1991), WHO (2011), Solomon *et al.*, (2013), Rasoamanahaja *et al.*, (2016) and Feleke *et al.*, (2017), about 1-2 mg of the stool sample was emulsified in a drop of normal saline (0.85 % NaCl) on the center of the slide using a disinfected stick. A cover-slip was placed on the sample and was examined systematically with $\times 10$ and $\times 40$ objective lenses. A disinfected stick was used to emulsify an estimated 1g (pea-size) of representative faeces in about 4 ml of 10% formol water contained in a screw-cap tube. Then further 3-4 mL of 10% w/v formol water was added and mixed well by shaking. The emulsified faeces were sieved using gauze and the suspension was transferred to a centrifuge tube. Then 3-4 mL of diethyl ether was added. The tube was capped and mixed for 1 min, and immediately centrifuged at 3,000 rpm for 1 min. After centrifugation, the fecal debris was separated in a layer between the diethyl ether and the 10% formal-saline layers. A faecal debris layer was loosened by disinfected wooden stick and the tube was rapidly inverted to discard the ether, faecal debris, and formol water. The bottom of the tube was tapped to re-suspend and mix with the sediment. Finally, the sediment was transferred to a slide and covered with a cover glass. Then the preparation was examined microscopically using the $\times 10$ and $\times 40$ objective lenses. Presence of lateral spine indicates *S. mansoni*. Procedures developed by WHO (2011) were used to determine the intensity of

the *S. mansoni* infection. Two slides per individual stool sample were prepared by filling a Kato-Katz template on 2 separate slides with stool (Kato-Katz kit, Vestergaard-Frandsen, Lausanne, Switzerland), levelling and covering each sample with a cellophane slip pre-stained with methylene blue (Katz *et al.*, 1972). The stool slides were read within 60 minutes for detection of *Schistosoma mansoni*, eggs. The number of eggs per gram (EPG) of stool was calculated by multiplying the crude egg number per slide by 24 in line with producer instructions (WHO, 2011). Based on egg per gram (EPG) of faecal samples, three classes of severity of the infection were classified as follows: Light: 1 to 99; Moderate: 100 to 399; Heavy: > 400. The infected individuals were treated with praziquantel, anti schistosomal drugs which are used for the treatment of schistosomiasis.

Data Analyses:

Descriptive statistics was used to determine the frequency of distribution and percentage prevalence of *Plasmodium* and *Schistosoma* species. Chi-Square was used to test the association of *Plasmodium* and *Schistosoma* species with demographic and socio-economic factors. Odds ratio was used to test association

between risk factors and prevalence of infection. Differences in the mean variables of the *Plasmodium* and *Schistosoma* species were analyzed using t-test and one-way ANOVA. Values were also considered to be significant at $P < 0.05$.

RESULTS

The result presented in Table 1 is the intensity of *Plasmodium* and *Schistosoma* species among primary school pupils in Makurdi, Benue State, Nigeria. *Schistosoma mansoni* (17.26) had the highest intensity, followed by *P. falciparum* (14.6), the least intensity was *S. haematobium* (13.32). Species intensity revealed that highest intensity (21.09) of *P. falciparum* was recorded at C.A.C Wadata, while the least intensity (11.75) was recorded Al-Ihsan International Academy. Highest intensity (19.00) of *S. haematobium* was recorded in Al-Ihsan International Academy, while the least intensity (9.71) was recorded in Holy Ghost. Highest intensity (26.00) of *S. mansoni* was recorded in LGEA primary school north bank, while the least intensity (12.60) was recorded in Holy Ghost.

Table 1: Intensity of *Plasmodium* and *Schistosoma* Species among Primary School Pupils in Makurdi, Benue State, Nigeria

Schools	Number examined	<i>Plasmodium falciparum</i>			<i>Schistosoma haematobium</i>			<i>Schistosoma mansoni</i>		
		Infected	Parasite counts	Intensity	Infected	Egg counts	Intensity	Infected	Egg counts	Intensity
CAC Wad Prim. Sch.	20	11	232	21.09	6	85	14.17	3	48	16.00
Holy Ghost Prim. Sch.	20	9	128	14.22	14	136	9.71	5	63	12.60
LGEA ghr Prim. Sch.	20	10	132	13.20	9	117	13.00	3	55	18.33
Arabic ghr prim. Sch.	20	7	96	13.71	7	97	13.86	3	59	19.67
Root Succ Prim. Sch.	20	8	103	12.88	3	49	16.33	2	34	17.00
NomadicNprim.Sch.	20	6	86	14.33	4	71	17.75	1	23	23.00
LGEANB prim. Sch.	20	6	78	13.00	2	33	16.50	1	26	26.00
Al-Ihsan I Prim. Sch.	20	8	94	11.75	2	38	19.00	1	20	20.00
Total	160	65	949	14.60	47	626	13.32	19	328	17.26
T-test P-value		P = 0.28			P = 0.27			P = 0.04		

The result presented in Table 2 is the intensity of *Plasmodium* and *Schistosoma* species among secondary school students in Makurdi, Benue State, Nigeria. *Schistosoma haematobium* had the highest intensity (17.30), followed by *S. mansoni* (13.56), the least intensity was *P. falciparum* (13.09). Species intensity revealed that highest intensity (22.36) of *P. falciparum* was recorded at Al-Irshad model academy, while the least intensity (8.17) was recorded in

Salam Academy Wadata. Highest intensity (15.33) of *S. haematobium* was recorded in Al-Irshad model academy, while the least intensity (11.44) was recorded at Al-Burhan International Academy Wadata. Highest intensity (22.00) of *S. mansoni* was recorded in Salim Progress School Wadata, while the least intensity (9.00) was recorded in Al-Irshad model academy.

Table 2: Intensity of *Plasmodium* and *Schistosoma* Species among Secondary School Students in Makurdi, Benue State, Nigeria

Schools	Number examined	<i>Plasmodium falciparum</i>			<i>Schistosoma haematobium</i>			<i>Schistosoma mansoni</i>		
		Parasite			Egg			Egg		
		Infected	counts	Intensity	Infected	counts	Intensity	Infected	counts	Intensity
Forward E. Acad.	20	6	68	11.33	3	40	13.33	1	19	19.00
Extensive college	20	10	116	11.60	4	53	13.25	1	15	15.00
Salim progress. S	20	6	52	8.67	3	38	12.67	1	22	22.00
Al-burhan I Acad.	20	7	64	9.14	9	103	11.44	3	31	10.33
Salam academy	20	6	49	8.17	2	27	13.50	1	16	16.00
Al-Irshad model	20	11	246	22.36	6	92	15.33	3	27	9.00
Gov't. girls Coll.	20	10	117	11.70	9	107	11.89	3	39	13.00
Gov't. College.	20	7	106	15.14	7	96	13.71	3	41	13.67
Aliyu jama'a M	20	7	98	14.00	3	41	13.67	2	34	17.00
Total	180	70	916	13.09	46	796	17.30	18	244	13.56
t-test P-value				P = 0.28			P = 0.27			P = 0.04

DISCUSSION

There was no statistically significant difference ($P > 0.05$) in intensity of *P. falciparum*, *S. haematobium*, however, there was statistically significant difference ($P < 0.05$) in intensity of *S. mansoni* in the different secondary schools, thus implicating intestinal schistosomiasis

Similarly, there was no statistically significant difference ($P > 0.05$) in intensity of *P. falciparum* and *S. haematobium*, but statistically significant difference ($P < 0.05$) in intensity of *S. mansoni* in the different primary school was obtained.

The highest intensity could be due to the proximity of the area to water bodies infested with the parasites as reported by Rasoamanahaja *et al.* (2016), Oniya and Olofintoye, (2009) which gives an alarming high prevalence and intensity of schistosomiasis. As such this neglected tropical disease is still endemic in tropical countries as reported by Sulyman *et al.* (2009). The prevalence and intensity of the *Plasmodium* and *Schistosoma* Species among the school-aged children around riverine communities could be results as a results of their lack of safe water for domestic purposes which resulted them in visitation to riverine, outdoor activities and open well for source of water, also could be as a results of having multiple breeding sites of mosquitoes around homes and non-using of mosquito nets or stay outdoor overnight, or not known the causes of blood in urine and not listing to health talks to know the causes of infections. This finding is in similar with the findings of these following researchers: Bigwan, *et al.* (2012) who carry out similar research on Prevalence of Schistosomiasis among secondary school boarding students in potiskum metropolis, Yobe State, North eastern Nigeria. Duwa, *et al.* (2009) with a similar research on prevalence and intensity of urinary scistosomiasis among Primary School Pupils in Minjibir local government area of Kano State. Sarkinfada, *et al.* (2009) with a similar research also on Urinary Schistosomiasis in the danjarima community in Kano State. Akeh, *et al.* (2010) with also a similar research on Urinary schistosomiasis and treatment-seeking behavior in Sankwala, Cross River State, south eastern Nigeria. Sulyman, *et al.* (2009) has also carried out two similar research on *Schistosoma haematobium* and concurrent parasitic infections in

school-aged children. And Schistosomiasis and anthropometric indices of children in Abeokuta North Local Government Area, Ogun State, Nigeria. Ezeadila, *et al.* (2015) has a similar research also on Prevalence of urinary schistosomiasis among community primary school pupils in Amagunze, Enugu State, Nigeria. Oniya and Olofintoye (2009) which has also this similar research on the Prevalence of urinary schistosomiasis in two endemic Local Government Area in Ondo State, Nigeria respectively. Nyarko, (2016) carry out a similar research of one aspect of this particular research i.e. *Schistosoma haematobium* and *Plasmodium falciparum* concomitant infection and haemoglobin level in children of school going age.

Conclusion

The overall intensity of *Plasmodium falciparum*, *Schistosoma haematobium* and *Schistosoma mansoni* among school-aged children in Makurdi, Benue State, Nigeria was 949 (14.60), 796 (17.30), and 328 (17.26) respectively. This shows that malaria and schistosomiasis are still endemic and with high intensity in riverine area of Benue State, Nigeria, and on account of this it is important that the infected participants treated with anti-malarial drugs and praziquantel for schistosomiasis should be properly monitored for progression or cure of the infections. Similarly, continuous follow up treatment and control intervention should be frequently done by health authorities to prevent re-infection, due to subsequent exposure to risk factors.

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