Original Research Article

Microscopic and Molecular Diagnosis of Intestinal Schistosomiasis among Patients in Primary Healthcare Centres in Keffi, Nigeria

ABSTRACT

Aims: This study was conducted to detect *Schistosoma mansoni*among patients in primary healthcare centres in Keffi, Nigeria using microscopic and molecular techniques.

Study Design: The study was a cross sectional study.

Place and Duration of Study: Keffi, Nasarawa State, between March 2021 and September 2021.

Methodology: Stool samples were collected from 200 (29 each from Gidan Zakara, Sabon Gida, Jigwada, Angwan Jaba and 28 each from Kowa, KaiboMada and Yarkadai PHCs) patients and information were obtained by structured questionnaires. The ova of *S.mansoni*were microscopically detected from the samples using the formol ether stool sedimentation technique.The *S. mansoni* DNA was extracted from the samples and detected by conventional PCR technique using type-specific primers. Data collected were analysed using Smith's Statistical Package (version 2.8, California, USA) and *P* value of ≤ 0.05 was considered statistically significant.

Results: Of the 200 patients screened, 2(1.0%) and 11(5.5%) were positive for intestinal schistosomiasis using microscopy and PCR respectively. Although age, gender and occupation of the patients were not significantly associated with the parasitic infection (*P*>0.05), however, higher prevalence was recorded among males (12.8%) pupils/students (22.7%) aged ≤14 (20.0%).

Conclusion: Our findings indicated a notable discrepancy between the two diagnostic methods, with PCR identifying a higher prevalence of intestinal schistosomiasis suggesting it to be a more sensitive tool for detecting this infection. However, the choice between these methods should consider their respective strengths and limitations, as well as the practical implications for disease control and treatment.

Keywords: Schistosomiasis; Microscopy; PCR; Keffi; Nigeria

1. INTRODUCTION

Schistosomiasis, caused by parasitic worms of the *Schistosoma* genus, remains a significant public health concern in many parts of the world, particularly in sub-Saharan Africa which account for about 80-85% of the parasitic infection (Hotez *et al*., 2012; Barakat, 2013;Adenowo *et al*., 2015). Among the various species, *Schistosoma mansoni* is prevalent in regions with inadequate sanitation and limited access to clean water (Mohammed & Buhari, 2020;Oyeyemi, 2020;WHO, 2023). Nigeria, being one of the most populous countries in Africa, bears a substantial burden of schistosomiasis a Neglected Tropical Disease (NTD), with an estimated 101.3 million at risk of infection and 29 million of the people being infected (Hotez *et al.*, 2012;Mohammed & Buhari, 2020; WHO, 2022). In the pursuit of effective control and elimination strategies, understanding the prevalence and distribution of the parasite becomes imperative.

Primary healthcare centres (PHCs) play a crucial role in the healthcare landscape of Nigeria, serving as the first point of contact for many individuals seeking medical attention (Idemudia and Victor, 2010; Paul, 2023), and provides the most viable route towards achieving health related sustainable development goals (SDGs) (Alonge, 2020). These centres serve diverse communities, including those in remote and underserved areas such as those found in Keffi, Nasarawa State where schistosomiasis is often endemic (Aregbeshola & Khan, 2017; Mohammed & Buhari, 2020). However, most PHCs in Nigeria lack basic medical laboratory facilities mostly due to poor funding to carryout standard laboratory diagnosis. Hence, most tests conducted are presumptive and their sensitivities and specificities are not guaranteed(Oyekale, 2017; Paul, 2023).

Microscopic examination of stool samples has long been the standard method for diagnosing intestinal schistosomiasis. It involves identifying parasite eggs under a microscope, providing valuable information about the presence and intensity of infection (Utzinger et al., 2015). However, this method has limitations, including the potential for false-negative results due to low parasite burden and the expertise required for accurate diagnosis (Nigo et al., 2019). Molecular techniques, such as polymerase chain reaction (PCR) which relies on selective detection of the parasitic nucleic acid, have emerged as promising tools for enhancing diagnostic accuracy and sensitivity (Nigo et al., 2019). Thetechniques, however, are not frequently used in countries with limited resources were the infection is endemic due to the cost of the reagents, equipment and the technical expertise required to run them (Utzinger et al., 2015; Nigo et al., 2019).

In this study, we assessed the prevalence of *S.mansoni* infection among patients visiting PHCs in Keffi, Nigeria, using both conventional microscopic examination and modern molecular techniques. The sociodemographic characteristics of the patients, including age, gender, and occupation were also considered, to explore potential associations between infection rates and these factors. This integrated approach holds promise for accurate diagnosis, precise prevalence estimation, and informed decision-making in the battle against schistosomiasis. Furthermore, the results of this study could contribute to the formulation of more effective control strategies, better allocation of resources, and targeted interventions to reduce the impact and eventually facilitate the eradication of this

neglected tropical disease in the year 2025 as contained in the United Nations global goals for sustainable development.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in seven (7) selected PHCs (Gidan Zakara, Sabon Gida, Jikwada, Yarkaddai, Angwan Jaba, Kawo and KaiboMada) in Keffi Local Government Area of Nasarawa State, Nigeria. Keffi is a town in North-Central Nigeria and is approximately about 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the capital of Nasarawa State. It is located geographically between latitude $8^0$3'N of the equator and longitude $7^0$50'E and situated on an altitude of 850m above sea level. The inhabitants mostly engage in trading, farming, schooling and petty jobs (Yakubu, 2013).

2.2 Study Population

The study population are male and female patients of all age groups attending the seven (7) selected PHCs in Keffi for treatment who agreed to participate in the study. These PHCs were selected because of their proximity to the stream, where the local people go to swim, bathe and do other domestic chores, which may expose them to infected fresh water snails which harbours the infective stage of the parasite. The socio-demographic information of the participants was obtained by the use of a designed questionnaire.

2.3 Sample Size Determination

In this study, 200 patients were recruited and this was calculated using the formula by Pourhoseingholi et al. (2013) for sample size calculation in medical studies at 0.05 level of precision.

2.4 Sample Collection and Processing

Following the administration of the questionnaire, each pupil was given a 30 ml sterile wide mouth, screw-capped plastic container carrying their identification number and was instructed on how to collect the stool sample aseptically in private (Cheesbrough, 2009). A total of 200 stool samples (29 each from Gidan Zakara, Sabon Gida, Jigwada, Angwan Jaba and 28 each from Kowa, KaiboMada and Yarkadai) were collected between 10:00 am and 2:00 pm. The samples were transported in a cold box to Parasitology Laboratory unit of Federal Medical Centre, Keffi and were stored at 4^oC in the refrigerator until ready for analysis.

2.5 Laboratory Analysis

2.5.1 Macroscopic Examination of Stool Samples

All the collected stool samples were examined macroscopically for colour, consistency, presence and absence of blood, presence of any adult worm as previously described by Cheesbrough (2009).

2.5.2 Parasitological Examination of *S. mansoni*

All samples collected were examined for the presence of *S. mansoni*ova using direct microscopic examination and formol ether stool sedimentation techniques.

2.5.3 Direct Microscopic Examination

The direct microscopic examination of *S. mansoni*ova in the stool samples was carried out using standard parasitological technique as earlier described byCheesbrough (2009). Briefly, a drop of Dobel's iodine was placed in the centre of a well labelled clean grease free microscope slide. Using an applicator stick; a pea size of well mixed stool sample was emulsified to make smooth thin preparation. It was covered using a cover glass. The preparation was examined using 10x and 40x objective of the microscope for the presence of *S. mansoni*ova.

2.5.4 Formol Ether Stool Sedimentation Technique

The formol ether stool sedimentation technique for the detection of *S. mansoni*ova was performed using the method described byCheesbrough (2009). In brief, approximately 2g of stool sample was emulsified in 7ml of formol saline and passed through wire gauze into a falcon tube to remove the large particles. Then 3ml of ether was added and the tube was covered with a plastic stopper. The tube was shaken vigorously to mix and was centrifuged at 3000rpm for 10minutes and the resultant supernatant was discarded before adding a drop of Dobel's iodine. The content of the tube was mixed and placed on a clean grease free microscope slide and covered gently with a cover slip. It was examined under 10x and 40x objectives of the microscope for the presence of *S. mansoni eggs.*

2.5.5 Molecular Detection of *S. mansoni* DNA

The *S. mansoni* DNA was detected by conventional PCR system previously described byPontes et al. (2002) using specific primers (SmP consensus primers) adopted from the work of Lodh et al. (2014). It was conducted at Nigeria Centre for Disease Control research laboratory, Gaduwa, Abuja-Nigeria.

2.5.6 *S. mansoni* DNA Extraction

Schistosoma DNA was extracted and purified from 10% faecal suspensions in phosphate-buffered saline using FastDNA SPIN kit (MP Biomedicals, Santa Ana, California, USA) according to the manufacturer's instructions.

2.5.7 Polymerase Chain Reaction (PCR)

The 28S rDNA region of *S. mansoni* was amplified and detected from the extracted DNA with MJ Research PTC-100 programmable thermal cycler (MJ Research Inc., Water- town, USA), using the following sets of oligonucleotide primers adopted from the previous work of Lodh et al. (2014).

SmPF Forward (5'-GATCTGAATCCGACCAACCG-3')

SmPR Reverse (5'-ATATTAACGCCCACGCTCTC-3')

The PCR cycling parameters consisted of 3 min at 94 °C followed by 35 cycles of 30 sec at 94 °C, 20 sec at 65 °C, and 20 sec at 72 °C with a final extension at 72 °C for 7 min.

2.5.8 Agarose Gel Electrophoresis

The PCR products were analysed by running a 1.5% agarose gel stained with ethidium bromide. The sizes of PCR products were estimated in relation to the migration pattern of a 100bp to 1000bp increments plus DNA molecular marker (BIONEER Daejeon, North Korea).

2.6 Data Analysis

The data obtained were analysed using Smith's Statistical Package (version 2.8, California, USA). Chi-square test was conducted at 95% confidence interval and *P* values ≤ 0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

This present study uses microscopic and molecular approaches to diagnosed intestinal schistosomiasis among patients in some selected Primary Healthcare Centres in Keffi, Nigeria. A total of 200 patients (29 each from Gidan Zakara, Sabon Gida, Jigwada, Angwan Jaba and 28 each from Kowa, KaiboMada and Yarkadai) majority of which were females (114/200), civil servants (87/200), andaged 15-34 years (84/200) were recruited and their stool samples screened for the presence of *S. mansoni*using microscopic and PCR techniques. From the results, 2(1.0%) and 11(5.5%) patients were positive for intestinal schistosomiasis using microscopy and PCR respectively (Figures 1, 2 and 3).

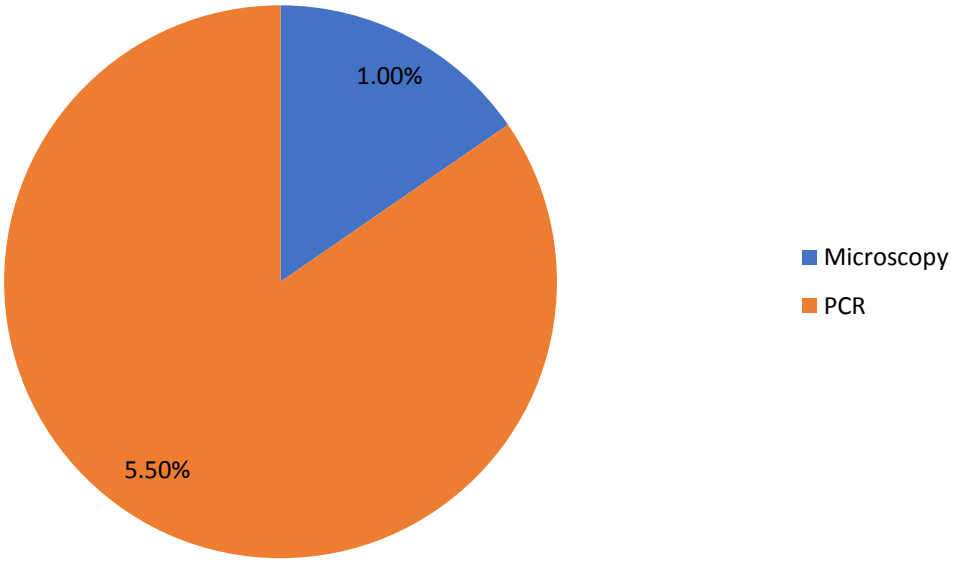

Figure 1: Prevalence of *S. mansoni*infectionamong Patients in Primary Healthcare Centres in Keffi, Nigeria Using Microscopy and PCR

Microscopy has been the conventional method for diagnosing schistosomiasis. However, there is high chance of false-negative results especially due to low parasite intensity or when it is not conducted by an expert (Nigo et al., 2019). This is confirmed by the results of this current study as only 2(1.0%)

patients were positive for intestinal schistosomiasis using microscopy as compared to the 11(5.5%) who were positive using PCR method. The higher prevalence observed using PCR suggests it accuracy and robustness in the detection of schistosomiasis even at low levels, which may have gone undetected by microscopy.

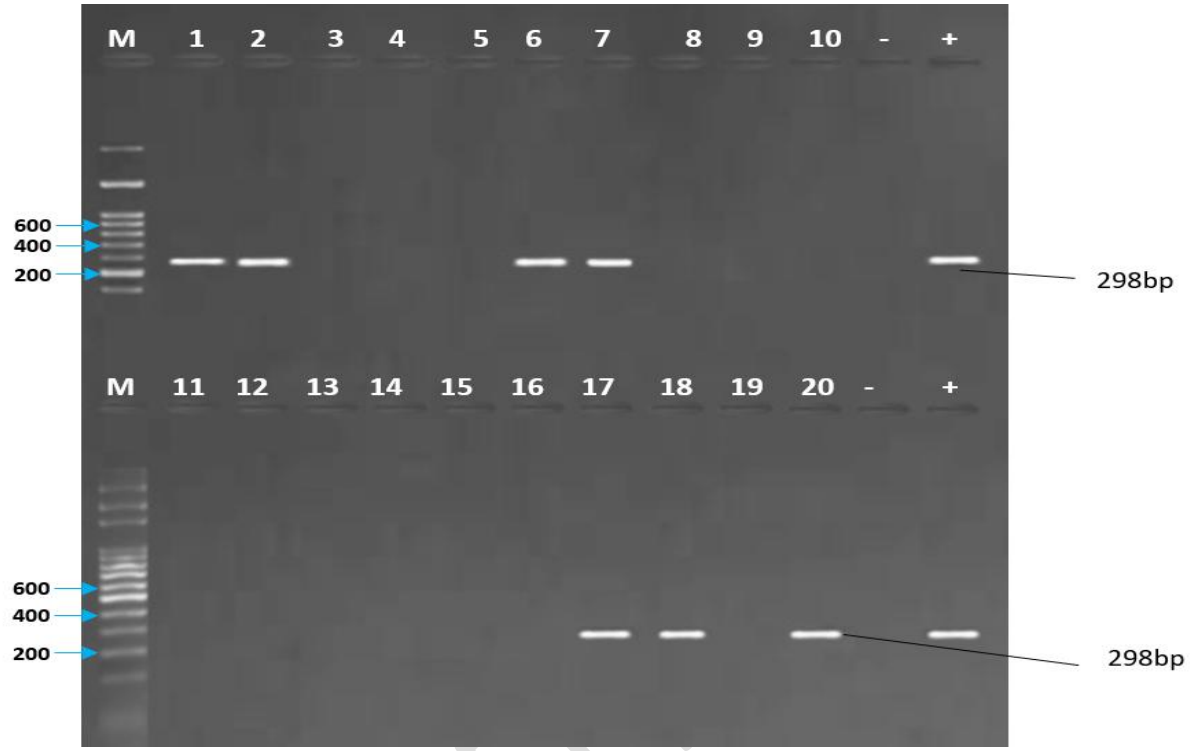

Figure 2: Agarose gel electrophoretogram of *S. mansoni*28S rDNA amplified gene. Samples 1, 2, 6, 7, 17, 18, and 20 were positive for *S. mansoni*DNA while samples 3, 4, 5, 8, 9, 10, 11, 12, 13 14, 15, 16 and 19 were negative. *M represents the molecular ladder, '−' is the negative control while '+' is the positive control.

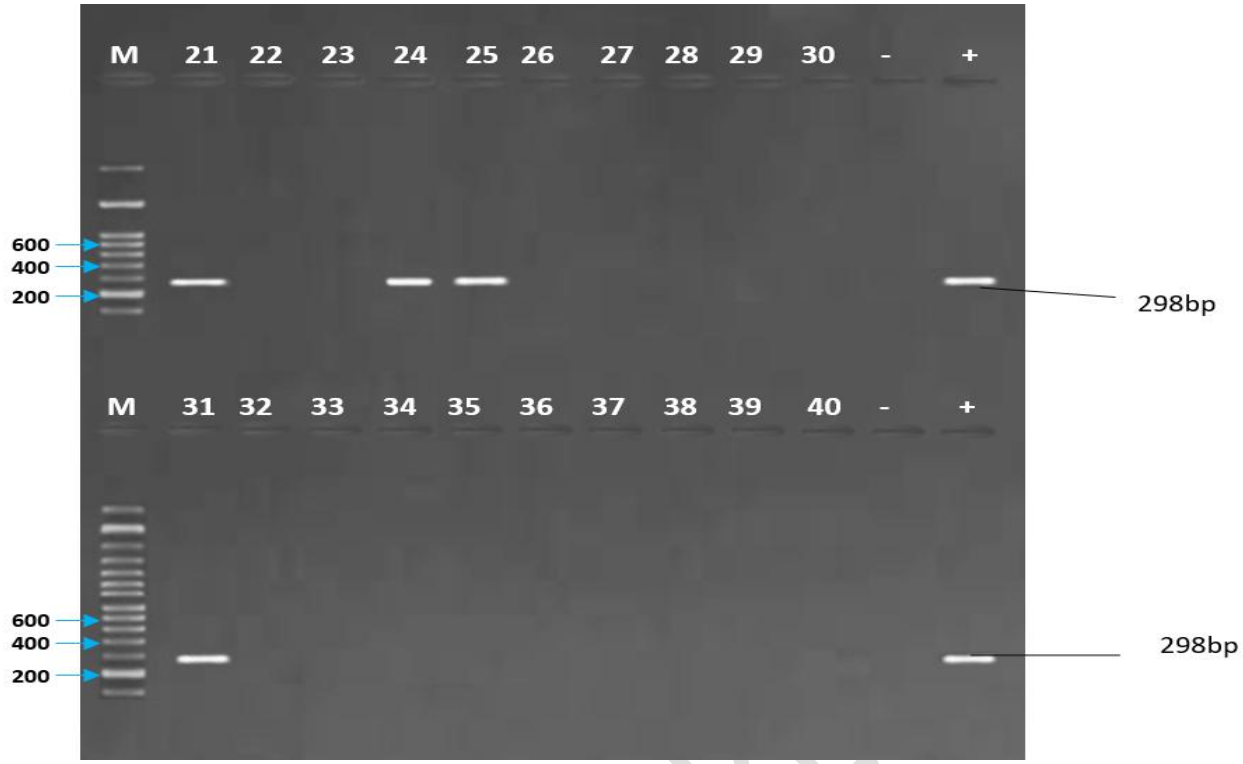

Figure 3: Agarose gel electrophoretogram of *S. mansoni*28S rDNA amplified gene. Samples 21, 24, 25 and 31 were positive for *S. mansoni*DNA while samples 22, 23, 26, 27, 28, 29, 30, 32, 33, 34, 35, 36, 37,38, 39 and 40 were negative. *M represents the molecular ladder, '−' is the negative control while '+' is the positive control.

The overall 5.5% prevalence of *S. mansoni* infection using PCR recorded in this current study was higher than the 2.7% reported among school-aged children in Jigawa State (Dogara et al., 2020), 2.9% in poor communities in Sokoto State (Singh et al., 2016) and 3.2% among primary school pupils in Keffi, Nasarawa State (Pam et al., 2016). It was however lower than the 8.0% among school children in Osun State (Alade et al., 2023), 8.9% in Hausa communities in Kano State (Dawaki et al., 2016) and 9.0% among South-west Nigerian school going children (Ojo et al., 2021). Similarly, researchers from Africa and other parts of the World also reported varying estimates of the parasitic infection. For instance, it was 2.9% among school children in Khartoum, Sudan (Hajissa et al., 2018), 9.3% among Yemeni children (Sady et al., 2013), 24.0% among school children in South-west Ethiopia (Bajiro et al., 2016) and 30.5% in a local Brazilian population (Santos et al., 2020). The differences observed in the prevalence rates from different studies may be attributed to testing methods used, peculiar ecological characteristics, level or contact of individuals with water bodies and the degree or exposure to infective *Schistosoma* cercariae in different locations.

Table 1: Prevalence and Distribution of *S. mansoni*infection in relation to Sampling Areaamong Patients in Primary Healthcare Centres in Keffi, Nigeria Using Microscopy and PCR

Sampling Area	**No. Examined (N=200)**	**Prevalence (%)**	
		Microscopy	**PCR**

PHC Gidan Zakara	29	1(3.4)	1(3.4)
PHC Sabon Gida	29	1(3.4)	8(27.6)
PHC Jigwada	29	0(0.0)	0(0.0)
PHC Angwan Jaba	29	0(0.0)	1(3.4)
PHC Kowa	28	0(0.0)	1(3.6)
PHC Kaibo Mada	28	0(0.0)	0(0.0)
PHC Yarkadai	28	0(0.0)	0(0.0)
Total	**200**	**2(1.0)**	**11(5.5%)**

At any age, coming into contact with fresh water harbouring cercariae can lead to *Schistosoma* infection (Hotez et al., 2012). However, School-aged-children (SAC) which are usually under 14 years of age are known to be the most vulnerable groups for schistosomiasis (Sady et al., 2013; Pam et al., 2016; Dogara et al., 2020; Mohammed & Buhari, 2020; Ojo et al., 2021) because they are more likely to engage in activities that may expose them to contaminated fresh water sources, including playing in infested water, swimming, washing etc (Hajissa et al., 2018; Alade et al., 2023). This may likely explain why intestinal schistosomiasis was found with the highest prevalence among participants aged ≤14 years (20.0%) compared to other age groups in this study (Table 2). However, this current study did not record significant association between *S. mansoni* infection and age of the participants (*P*>0.05). Other previous studies also reported the same findings (Sady et al., 2013; Bajiro et al., 2016; Pam et al., 2016).

All the 11(12.8%) participants infected with intestinal schistosomiasis in this present study were males (Table 2). This may be due to the fact most males especially those in local settings engage in outdoor jobs such as farming and fishing which involve contact with freshwater sources, thereby increasing their risk of infection. This is consistent with the findings of most other previous researchers who equally reported significantly higher rates of the parasitic infection among males compared to their female counterparts (Dawaki et al., 2016; Pam et al., 2016; Singh et al., 2016 Santos et al., 2020).

Table 2: Prevalence and Distribution of *S. mansoni*infection in relation to Socio-demographic Factorsamong Patients in Primary Healthcare Centres in Keffi, Nigeria Using Microscopy and PCR

Socio-demographic	**No. Examined (N=200)**	**Prevalence (%)**	
Microscopy	**PCR**		
Age (Years)			
≤14	50	2(4.0)	10(20.0)
15-34	84	0(0.0)	0(0.0)
35-64	43	0(0.0)	1(2.3)
≥65	23	0(0.0)	0(0.0)
P-value		**0.0000**	**0.3101**
Gender			
Male	86	2(2.3)	11(12.8)
Female	114	0(0.0)	0(0.0)
P-value		**0.3101**	**0.3101**

Occupation			
Pupil/Student	44	2(4.6)	10(22.7)
Farmer	87	0(0.0)	1(1.2)
Civil Servant	41	0(0.0)	0(0.0)
Others	28	0(0.0)	0(0.0)
P-value		**0.0000**	**0.0000**

Interestingly, occupation of patients is also not associated with the prevalence of intestinal schistosomiasis in this study (p>0.05). However, pupils/students were more infected with the parasite (22.7%) than farmers (1.2%). In contrast however, most previous studies reported higher prevalence of the infection among farmers (Yapi et al., 2005; Okoli et al., 2006; Dogara et al., 2020; Jibril et al., 2020; Gruninger et al., 2023) which they attributed with their frequent contact with contaminated freshwater source. The higher prevalence of the infection observed among pupils/students in this current study may probably be because the schools in the study areas are located near *Schistosome* contaminated water bodies and the students/pupils might engage in play activities that involve contact with the water.

4. CONCLUSION

This current study reveals that, the prevalence of intestinal schistosomiasis varies depending on the diagnostic method used, with PCR indicating a higher prevalence compared to microscopy. The varying prevalence estimates from these two methods have important implications for public health strategies and therefore, the choice between these methods should consider their respective strengths and limitations, as well as the practical implications for disease control and treatment.

Ethical Approval and Consent

Ethical approval to conduct this study (KLG/WELL/227/VOL.I/XXX) was obtained from the Keffi Local Government Council Ethics Committee. Approval was also obtained from the heads of each PHC where samples were collected. In addition, all individuals included in this study willingly completed and signed an informed consent form.

REFERENCES

Adenowo, A. F., Oyinloye, B. E., Ogunyinka, B. I., &Kappo, A. P. (2015). Impact of human schistosomiasis in sub-Saharan Africa. The Brazilian Journal of Infectious Diseases, 19(2), 196–205. https://doi.org/10.1016/j.bjid.2014.11.004

Alade, T., Ta-Tang, T.-H., Nassar, S. A., Akindele, A. A., Capote-Morales, R., Omobami, T. B., &Berzosa, P. (2023). Prevalence of *Schistosoma haematobium* and Intestinal Helminth Infections among Nigerian School Children. Diagnostics, 13(4), 759. https://doi.org/10.3390/diagnostics13040759

Alonge, S.K. (2020). PRIMARY HEALTH CARE IN NIGERIA: AN APPRAISAL OF THE EFFECT OF FOREIGN DONATIONS. *African Journal of Health, Safety and Environment.* Vol: 1 (2): 86-100

Aregbeshola, B. S., & Khan, S. M. (2017). Primary Health Care in Nigeria: 24 Years after Olikoye Ransome-Kuti's Leadership. Frontiers in Public Health, 5, 48. https://doi.org/10.3389/fpubh.2017.00048

Bajiro, M., Dana, D., Ayana, M., Emana, D., Mekonnen, Z., Zawdie, B., Garbi, A., Kure, A., &Zeynudin, A. (2016). Prevalence of *Schistosoma mansoni* infection and the therapeutic efficacy of praziquantel among school children in Manna District, Jimma Zone, southwest Ethiopia. Parasites & Vectors, 9(1), 560. https://doi.org/10.1186/s13071-016-1833-6

Barakat, R. M. R. (2013). Epidemiology of Schistosomiasis in Egypt: Travel through Time: Review. Journal of Advanced Research, 4(5), 425–432. https://doi.org/10.1016/j.jare.2012.07.003

Cheesbrough, M. (2009). District Laboratory Practice in Tropical Countries Part 1, Second Edition.

Dawaki, S., Al-Mekhlafi, H. M., Ithoi, I., Ibrahim, J., Abdulsalam, A. M., Ahmed, A., Sady, H., Atroosh, W. M., Al-Areeqi, M. A., Elyana, F. N., Nasr, N. A., & Surin, J. (2016). Prevalence and risk factors of Schistosomiasis among Hausa communities in Kano State, Nigeria. Revista Do Instituto de Medicina Tropical de São Paulo, 58, 54. https://doi.org/10.1590/S1678-9946201658054

Dogara, M. M., Ahmad, S., Balogun, B. J., Dawaki, S. S., Mustapha, M. B., Abdurrahman, A. U., Bala, L., Zakari, A., & Livingstone, D. U. (2020). Schistosomiasis and Associated Risk Factors Among School-Aged Children in Northern Nigeria. International Journal of Translational Medical Research and Public Health, 4(2), Article 2. https://doi.org/10.21106/ijtmrph.146

Gruninger, S. K., Rasamoelina, T., Rakotoarivelo, R. A., Razafindrakoto, A. R., Rasolojaona, Z. T., Rakotozafy, R. M., Soloniaina, P. R., Rakotozandrindrainy, N., Rausche, P., Doumbia, C. O., Jaeger, A., Zerbo, A., von Thien, H., Klein, P., van Dam, G., Tannich, E., Schwarz, N. G., Lorenz, E., May, J., … Fusco, D. (2023). Prevalence and risk distribution of schistosomiasis among adults in Madagascar: A cross-sectional study. Infectious Diseases of Poverty, 12(1), 44. https://doi.org/10.1186/s40249-023-01094-z

Hajissa, K., Muhajir, A. E. M. A., Eshag, H. A., Alfadel, A., Nahied, E., Dahab, R., Ali, S. M., Mohammed, M., Gaafar, M., & Mohamed, Z. (2018). Prevalence of schistosomiasis and associated risk factors among school children in Um-Asher Area, Khartoum, Sudan. BMC Research Notes, 11(1), 779. https://doi.org/10.1186/s13104-018-3871-y

Mohammed, H.I., & Buhari, S. (2020). Prevalence and Determining Factors of Urinary Schistosomiasis among Primary School Pupils in North Central Nigeria. 61175.

Hotez, P. J., Savioli, L., & Fenwick, A. (2012). Neglected tropical diseases of the Middle East and North Africa: Review of their prevalence, distribution, and opportunities for control. PLoS Neglected Tropical Diseases, 6(2), e1475. https://doi.org/10.1371/journal.pntd.0001475

Idemudia, E. & Victor, O. (2010) The role of Primary Healthcare in Nigeria. Healthcare Delivery Systems: Problems and Prospects. *Knowledge Review Volume* 21 No. 1

Jibril, H., Mohammed, Y., Idris, A., Manu, A., Umar, A., & Ahmad, I. T.-. (2020). Incidence of urinary schistosomiasis among rice farmers in some selected villages of kura local government area, Kano, Nigeria. Nigerian Journal of Experimental and Clinical Biosciences, 8(2), 65–65.

Lodh, N., Naples, J. M., Bosompem, K. M., Quartey, J., & Shiff, C. J. (2014). Detection of Parasite-Specific DNA in Urine Sediment Obtained by Filtration Differentiates between Single and Mixed Infections of *Schistosoma mansoni* and *S. haematobium* from Endemic Areas in Ghana. PLoS ONE, 9(3), e91144. https://doi.org/10.1371/journal.pone.0091144

Nigo, M. M., Salieb-Beugelaar, G. B., Battegay, M., Odermatt, P., & Hunziker, P. (2019). Schistosomiasis: From established diagnostic assays to emerging micro/nanotechnology-based rapid field testing for clinical management and epidemiology. Precision Nanomedicine, 3(1), 439–458. https://doi.org/10.33218/prnano3(1).191205.1

Ojo, J. A., Adedokun, S. A., Akindele, A. A., Olorunfemi, A. B., Otutu, O. A., Ojurongbe, T. A., Thomas, B. N., Velavan, T. P., &Ojurongbe, O. (2021). Prevalence of urogenital and intestinal schistosomiasis among school children in South-west Nigeria. PLOS Neglected Tropical Diseases, 15(7), e0009628. https://doi.org/10.1371/journal.pntd.0009628

Okoli, C. G., Anosike, J. C., &Iwuala, M. O. E. (2006). Prevalence and Distribution of Urinary Schistosomiasis in Ohaji/Egbema Local Government Area of Imo State, Nigeria.

Oyekale, A. S. (2017). Assessment of primary health care facilities' service readiness in Nigeria. BMC Health Services Research, 17(1), 172. https://doi.org/10.1186/s12913-017-2112-8

Oyeyemi, O.T., Jeremias, W.J., Rafaella. (2020). Schistosomiasis in Nigeria: Gleaning from the past to improve current efforts towards control. *Elsevier*, *One Health*100183.

Pam, D. D., Pam, V. A., & Nuhu, E. I. (2016). Prevalence of *Schistosoma mansoni* infection among primary school pupils in Keffi Town, Keffi Local Government Area, Nasarawa State, Nigeria. Nigerian Journal of Parasitology, 37(2), Article 2. https://doi.org/10.4314/njpar.v37i2

Paul. (2023, August 14). Factors militating against effective implementation of primary health care (PHC) system in Nigeria. Annals of Tropical Medicine and Public Health (ATMPH). https://www.atmph.org/factors-militating/

Pontes, L. A., Dias-Neto, E., & Rabello, A. (2002). Detection by polymerase chain reaction of Schistosoma mansoni DNA in human serum and feces. The American Journal of Tropical Medicine and Hygiene, 66(2), 157–162. https://doi.org/10.4269/ajtmh.2002.66.157

Pourhoseingholi, M. A., Vahedi, M., & Rahimzadeh, M. (2013). Sample size calculation in medical studies. Gastroenterology and Hepatology From Bed to Bench, 6(1), 14–17.

Sady, H., Al-Mekhlafi, H. M., Mahdy, M. A. K., Lim, Y. A. L., Mahmud, R., & Surin, J. (2013). Prevalence and Associated Factors of Schistosomiasis among Children in Yemen: Implications for an Effective Control Programme. PLOS Neglected Tropical Diseases, 7(8), e2377. https://doi.org/10.1371/journal.pntd.0002377

Santos, I. G. de A., Bezerra, L. P., Cirilo, T. M., Silva, L. O., Machado, J. P. V., Lima, P. D., Bispo, M. R. S., Gomes, S. da C., Silva, G. I. L. da, Alencar, V. J. B., Damasceno, I. A., Carvalho, M. M. V. de, Gomes, D. S., Ramos, R. E. S., Santos Júnior, E. G., Alves, L. C., & Brayner, F. A. (2020). New epidemiological profile of schistosomiasis from an area of low prevalence in Brazil. Revista Da Sociedade Brasileira de Medicina Tropical, 53, e20200335. https://doi.org/10.1590/0037-8682-0335-2020

Singh, K., Muddasiru, D., & Singh, J. (2016). Current status of schistosomiasis in Sokoto, Nigeria. Parasite Epidemiology and Control, 1(3), 239–244. https://doi.org/10.1016/j.parepi.2016.08.003

Utzinger, J., Becker, S. L., Lieshout, L. van, Dam, G. J. van, & Knopp, S. (2015). New diagnostic tools in schistosomiasis. Clinical Microbiology and Infection, 21(6), 529–542. https://doi.org/10.1016/j.cmi.2015.03.014

World Health Organization (2022). Schistosomiasis: Retrieved August 18, 2023, fromhttps://scholar.google.com/scholar_lookup?title=Schistosomiasis:+progress+report+2001 2011+and+strategic+plan+2012-2020&publication_year=2013&

World Health Organization (2023). Soil transmitted helminth infections: Retrieved August 18, 2023,

from https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections

Yakubu, I. O. (2013). Geography of Keffi, Nassarawa State, Nigeria (Published online in 2013). Retrieved August 20, 2023, from https://www.academia.edu/13014831/Geography_of_Keffi_Nassarawa_State_Nigeria_Published_online_in_2013_

Yapi, Y. G., Briët, O. J. T., Diabate, S., Vounatsou, P., Akodo, E., Tanner, M., & Teuscher, T. (2005). Rice irrigation and schistosomiasis in savannah and forest areas of Côte d'Ivoire. Acta Tropica, 93(2), 201–211. https://doi.org/10.1016/j.actatropica.2004.11.005