Original Research Article

Epidemiological Studies of Polyparasitism and Gender Risk Factors in Women in Igede Land

ABSTRACT

Introduction: Parasitic infections and polyparaisitism remain one of the most pressing public health problems with one third of the world population generally estimated to be infected.
Aim: to epidemiologically study of polyparasitism in Women Igede land of Benue State, Nigeria.
Study Design: a cross-sectional study design was adopted for this study.
Materials and methods: Fresh stool, urine and blood samples were collected from women and processed for internal parasites, urine parasites and malaria parasite respectively. Well-structured questionnaires were further utilized to elicit sociodemographic and risk-factor information as well as knowledge and perception of the women towards polyparasitism. Positive samples were afterwards processed molecularly using PCR technology and the results documented.
Results: Results obtained revealed an overall prevalence of 33.50% for single parasitic infection and 3.50% prevalence for polyparasitism. The dominant parasite was *Plasmodium* spp. (52.16%) while the least was *Strongyloides stercoralis*(1.44%). Parasite combination involving *Schistosomahaematobium*and Malaria parasite recorded highest prevalence (36.11%) while least prevalence was recorded for coinfection with Hookworm, *Entamoeba histolytica* and *Asccaris lumbricoides*. (8.33%). Also, risk factors of excretion and water source were significant (P<0.05) while meat consumption and sanitation were not significant P>0.05).
Conclusion: polyparasitism is prevalent in Igedeland of Benue State and its detection by molecular method is of a higher specificity than other methods of diagnosis of parasites.

Keywords: Polyparaisitism, risk factors, Plasmodium, women, Igede.

1. INTRODUCTION

There have been a general renaissance in the epidemiological investigation of polyparasitism, with a particular focus on multiple helminth species and more recently, on *Plasmodium*-helminth co-infections [1, 2, 3]. Studies across multiple epidemiological settings have shown that polyparasitism is the norm rather than the exception and occurs at different frequencies than would be expected under assumptions of independence [4,5,6]. Interactions between parasites in humans can be synergistic or antagonistic. For example, studies have demonstrated a positive association between intensity and concurrent infection of helminth species, suggesting that individuals harbouring multiple helminth species also harbour the most intense infections [4,5,6]. It is conceivable therefore that polyparasitism may have a greater impact on morbidity than single species infections since morbidity is typically related to infection intensity for most parasite species. Multiple species infections may also increase susceptibility to other infections [3,7].

Although accurate estimate of the magnitude of polyparasitism is unknown, the case is distributed widely in the tropics and sub-tropics [8]. It was found that individuals with polyparasitic infection also harbor the most intense infections [1, 6]. Even low-intensity of polyparasitic infections may result in clinically significant morbidity [9, 10]. Therefore, polyparasitism may have a greater impact on morbidity than single species infection. It may also increase susceptibility to other infections and adversely affect the clinical outcome of the concomitant diseases such as tuberculosis, HIV/AIDS, and malaria [7,11].

Several findings from different parts of the world have revealed tangible information pertaining to the impact of polyparasitim on general health. Menendez *et al.,* [12] revealed that infants and pregnant women are the group of individuals most likely to harbour multiple parasite infections; Stephenson *et*

Comment [WU1]: The Title should be SMART. With a cross-sectional study design and X^2-value it is imposibel to study risk factors rather it is better to say associated factors.

Comment [WU2]: I found the abstract is too poor. Because of the following issues
1.The introduction part is not inline to the objective. The problem should better specifically to the study population.
2.Material and methods part should clearly mention the study design, study period area, sampling technique and study population including eligibility criteria and method for analysis
3.Result part should narrates the overall prevalence of poly-parasitism
4.Conclusion part is not in line to the result as well as the objective of the study.

Comment [WU3]: Which type of women....is that symptomatic or asymptomatic one?

Comment [WU4]: It should be a part of Material and methods

Comment [WU5]: The introduction part is not informative as of my understanding as per the objective of the study.
1. It doesn't correlate the burden of poly-parasitism with gender particularly women
2. It doesn't narrate about the possible factors for co-infections (poly-parasitism)
3. I couldn't understand, what was the gap that initiates the author to do so?
4. The reference style is not appropriate and it semis like both Harvard and Vancouver style.

al., [13] opined that Malaria, hookworm, schistosomiasis and, to a lesser extent, *Trichuristrichiura* are associated with iron loss and imbalances predisposing to anaemia among nutritionally vulnerable populations, with risk being closely correlated with infection intensity. Hotez*et al.* [14] revealed the high iron demands of infant growth and pregnancy means that anaemia is most common and severe among children and pregnant women. The gut protozoa such as *Entamoeba histolytica*, *Giardia lamblia* and *Cryptosporidium* spp. are also highly prevalent and of major importance in children, pregnant women and in HIV-infected patients. Al-Delaimy [15] also revealed that polyparasitism is higher among younger children than adults. They attributed this to factors such as environment and personal hygiene.

The statements made by the above researchers as well as findings from previous research have formed a good background to the study of polyparasitism over the years. However, little is known about the impact of polyparasitism on the health of women in the world, North central Nigeria and Benue State in particular. This is therefore the driving force behind this study.

2. MATERIAL AND METHODS

Comment [WU6]: Material and Methods is also poorly presented.
1. the study period is not clearly stated
2.the sampling method is not clearly known.
3.the population (source and study population) is not mentioned
4.Iligebility criteria is not clearly stated.

The study was carried out in Oju and Obi local government areas in the southern part of Benue State. Oju is located between latitude $6^{o}6^{1}$N and $6^{o}9^{1}$E and Longitude $8^{o}10^{1}$E and $8^{o}25^{1}$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 widely disputed result of 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.

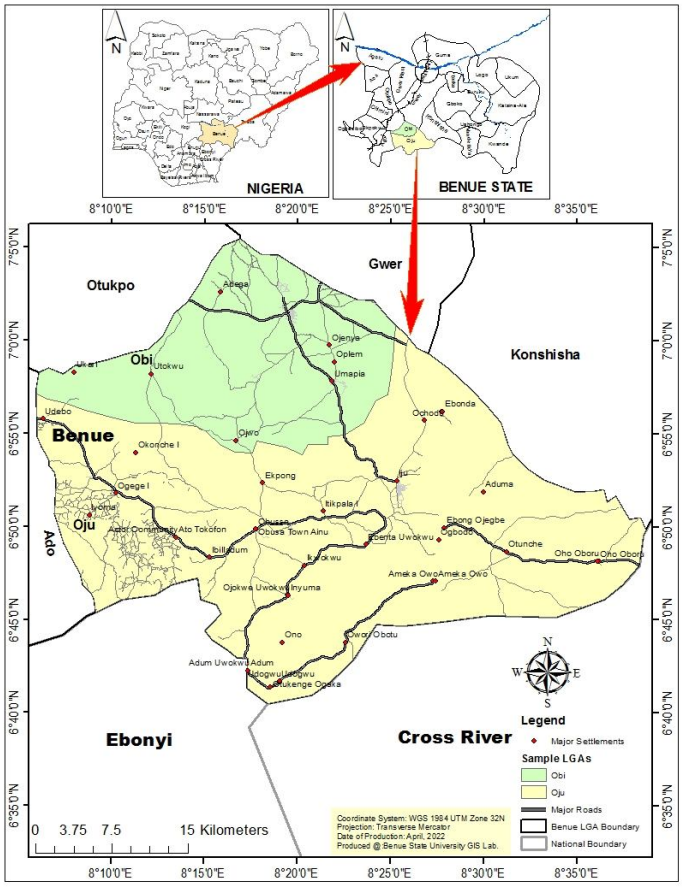

Figure 1: Map of Benue State showing Location of Oju and Obi Local Government Area[16]

Experimental Design

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 used for the study (1037) was determined using the formula below [17].

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

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)

Comment [WU7]: Sample size calculation is not clear.
1. what mean "p = expected prevalence" and what is its exact value.
2.What about non-responce rate?
3.sample collection tool is not informative and pre-test was not conducted.

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 their local languages. 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 consenting women who participated in the study. They return the containers with urine and stool in them. Blood was however collected intravenously by laboratory technicians from women using appropriate aseptic techniques. Blood samples were examined immediately, while stool and urine samples were taken to the laboratory for microscopic examination.

Comment [WU8]: Why don't you take capillary blood instead?

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

Examination of Faecal samples

Faecal samples collected during the study were examined according to the formol ether concentration technique [18]. Using an applicator stick, an estimated 1g (pea-size) of faeces was emulsified in about 4 ml of 10% formol water contained in a screw-cap tube. A further 3–4 ml of 10% v/v formol water was added, the bottle capped, and mixed well by shaking. The emulsified faeces were sieved, collecting the sieved suspension in a beaker and the suspension was transferred to a conical (centrifuge) tube made of strong glass, and about 3–4 ml of ethyl acetate was added. The tube was stoppered and mixed for 1 minute and with a tissue or piece of cloth wrapped around the top of the tube, the stopper was loosened. The content of the tube was centrifuged immediately at 750–1000 g (approx. 3000 rpm) for 1 minute. Using a stick, the layer of faecal debris from the side of the tube was loosened and the tube inverted to discard the ether, faecal debris, and formol water while the sediment remained. The tube was returned to its upright position, and the fluid from the side of the tube was allowed to drain to the bottom. The bottom of the tube was tapped to re-suspend and mix the sediment, and the sediment was transferred to a slide, and covered with a cover glass. The preparation was examined microscopically using the 10x objective with the condenser iris closed sufficiently to give good contrast. The 40x objective was used to examine cysts and eggs. To assist in the identification of cysts, a small drop of iodine was run under the cover glass [18].

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 500–1000 g 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 [18].

Examination of Blood Samples

Comment [WU9]: Blood Sample was processed for only diagnosis of malaria. What about other hemoparasities?

1.malaria diagnosis process via RDT is not the golden standared
2.the role of performing PCR is not clearly stated
3.which sample type was processed via PCR , is that positive or negetaive sample?

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 [18].

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 .05 (P<.05) was considered statistically significant.

3. RESULTS AND DISCUSSION

The parasites encountered include: *Entamoeba histolytica,* Malaria parasite, *Schistosomahaematobium*, Hookworm, *Ascaris lumbricoides*, and *Trichuris trichuria* with prevalence of 9.51%, 6.05%, 5.47%, 1.44%, 1.72%, 52.16%, 18.15% and 5.47% respectively.

In terms of parasite combinations, coinfection with malaria parasite and *Schistosomahaematobium*recorded the highest prevalence (36.11%). Also, Idelle community recorded the highest prevalence of polyparasitism while Oju centre recorded the least prevalence (Fig 2).

In terms of risk factors of infection, excretion and water sources were observed to have a significant association with rate of polyparasitism (P<0.05) while meat consumption and sanitation were not statistically significant. Results obtained showed that women who utilize pit toilet recording the highest prevalence of infection (7.10%) while those who utilize water system recorded the least prevalence (2.00%).

Women who utilize rainwater recorded highest prevalence rates (10.60%) while the least prevalence was recorded among women who use well other sources of water (1.70%). For meat consumption, women who consume pock the most recorded the least prevalence of infection (2.10%) while those who consume goat meat recorded the highest prevalence of infection (4.60%). Also, women who use other means of sanitary disposal recorded least prevalence of infection with polyparasites while those who utilize garbage pits recorded the highest prevalence of infection (4.80%).

Table 1: Distribution Patterns of Polyparasites among Women of Igede Land, Benue State, Nigeria.

Parasite Combination	Frequency	Percentage
E.H+MP+S.H	4	11.11
HW+A.L+MP	3	8.33
MP+SH+AL	3	8.33
HW+EH+SH	3	8.33
MP+SH	13	36.11
MP+TT	6	16.67
MP+TT+HW	4	11.11
Total	**36**	**100.00**

E.H= *Entamoeba histolytica,* MP= Malaria parasite, SH= *Schistosomahaematobium*, HW: Hookworm, AL= *Ascaris lumbricoides*, TT= *Trichuris trichuria*.

Comment [WU10]: Results and discussion part is not also clear.

1. Socio-demographic part is not state basic information about women (age, residence, occupation academic status, monthly income, pregnancy, etc.)

2.The overall poly-parasitism is not stated very well

3.The overall infection rate as per each Socio-demographic characteristics is not mentioned.

4.The diagnosis discrepancy between conventional (microscopic and RDT) and PCR is not clearly stated.

5.The discussion is not informative.

A.The authors should calculate the 95% CI of the overall prevalence of poly-parasitism to compare and contrast with previous findings.

B.The comparison articles should in line to the current study unless comparison is in possible

C.The justification to the discrepancies of the finding is too general.

D.The prevalence of each parasitic infection is not markedly discussed.

E.The association of the poly-parasitism is not also discussed very well

6. The conclusion should better to re-write again in line to the finding with considering recommendation.

Figure 2: Polyparasitism in the selected Communities Surveyed

Comment [WU11]: The figure should have data value.

Table 2: Parasite Distribution in Women of Igede Land in Relation to Location

Location	Communities	Number examined	Number Positive (%)	Parasites (%)							
				E.hystolytica	*A. lumbricoides*	Hookworm	*S. stercoralis*	*Taenia* spp,	*Plasmodium* spp.	*S. haematobium*	*Trichuristrichura*
Oju	Uje	140	43 (30.71)	3 (6.97)	5 (11.62)	2 (4.65)	0 (0.00)	0 (0.00)	20 (46.51)	13 (30.23)	0 (0.00)
	Uwokwu	30	12 (40.00)	0 (0.00)	0 (0.00)	0 (0.00)	4 (33.33)	2 (16.66)	3 (25.00)	3 (25.00)	0 (0.00)
	Ega	50	30 (60.00)	4 (13.33)	4 (13.33)	1 (3.33)	0 (0.00)	0 (0.00)	9 (30.00)	6 (20.00)	6 (20.00)
	Oju center	34	23(67.64)	3 (13.04)	1 (4.34)	2 (8.69)	0 (0.00)	0 (0.00)	10 (43.47)	7 (30.43)	0 (0.00)
	Idelle	178	58(32.60)	5 (8.62)	4 (6.89)	6 (10.34)	0 (0.00)	0 (0.00)	30 (51.72)	10 (17.24)	3 (5.17)
Obi	Adiko	220	75 (34.54)	9 (12.00)	3 (4.00)	3 (4.00)	0 (0.00)	1 (1.33)	44 (58.66)	12 (16.00)	3 (4.00)
	Obarike	150	33 (22.00)	3 (27.27)	2 (6.06)	0 (0.00)	0 (0.00)	3 (9.09)	18 (54.54)	5 (15.15)	2 (6.06)
	Itogo	150	49 (32.70)	4 (8.16)	1 (2.04)	3 (6.12)	0 (0.00)	0 (0.00)	36 (73.46)	2 (4.08)	3 (6.12)
	Adum	85	24 (28.23)	2 (8.33)	1 (4.16)	2 (8.33)	1 (4.16)	0 (0.00)	11 (45.83)	5 (20.83)	2 (8.33)
Total		**1037**	**347 (33.50)**	**33 (9.51)**	**21 (6.05)**	**19 (5.47)**	**5 (1.44)**	**6 (1.72)**	**181 (52.16)**	**63 (18.15)**	**19 (5.47)**

χ^2=17.05; P=.003

Table 3: Distribution Patterns of Polyparasites among Women in Igede Land, Benue State in Relation to Location

Location	Communities	Number examined	Number Positive	Parasites Combinations						
				E.H+MP+S.H	HW+A.L+MP	MP+SH+AL	HW+EH+SH	MP+SH	MP+TT.	MP+TT+HW
Oju	Uje	140 (13.50)	4 (2.86)	0 (0.00)	1 (25.00)	0 (0.00)	1 (25.00)	2 (50.00)	0 (0.00)	0 (0.00)
	Uwokwu	30 (2.89)	2 (6.67)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (50.0)	0 (0.00)	1 (50.00)
	Ega	50 (4.82)	3 (6.00)	0 (0.00)	0 (0.00)	1 (33.30)	2 (6.60)	0 (0.00)	0 (0.00)	0 (0.00)
	Oju center	34 (3.28)	1 (2.94)	0 (0.00)	1 (100.0)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
	Idelle	178 (17.16)	7 (3.93)	2 (28.60)	0 (0.00)	1 (14.30)	0 (0.00)	3 (42.90)	1 (14.30)	0 (0.00)
Obi	Adiko	220 (21.22)	6 (2.72)	2 (33.33)	0 (0.00)	0 (0.00)	0 (0.00)	2 (33.33)	2 (33.33)	0 (0.00)
	Obarike	150 (14.46)	5 (3.33)	1 (20.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (20.00)	1 (20.00)	2 (40.00)
	Itogo	150 (14.46)	5 (3.33)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (40.00)	2 (0.00)	1 (20.00)
	Adum	85 (8.20)	3 (3.53)	0 (0.00)	1 (33.33)	1 (33.33)	0 (0.00)	1 (33.33)	0 (0.00)	0 (0.00)
Total		**1037(100.00)**	**36(3.50)**	**4(11.11)**	**3(8.33)**	**3(8.33)**	**3(8.33)**	**13(36.11)**	**6(16.67)**	**4(11.11)**

χ^2=16.92 P=.02

E.H= *Entamoeba histolytica,* MP= Malaria parasite, SH= *Schistosomahaematobium*, HW: Hookworm, AL= *Ascaris lumbricoides*, TT= *Trichuris trichuria*.

Table 4: Polyparasitism in Women of Igede Land in relation to Possible Risk Factors

Risk factor	Number Examined (%)	Number Positive (%)	Polyparasitism (%)	χ^2	P-value
Excretion					
Water System	488	124 (25.40)	10 (2.00)		
Pit Toilet	239	116 (48.50)	17 (7.10)		
Bucket System	222	74 (33.30)	7 (3.20)		
Open defecation	88	33 (37.50)	2 (2.30)	12.848	.005
Water Source					
Rain	85	49 (57.60)	9 (10.60)		
Borehole	468	149 (31.80)	14 (3.00)		
Tap	295	102 (34.60)	10 (3.40)		
Well	119	38 (31.90)	2 (1.70)		
Others	70	9 (12.90)	1 (1.40)	15.185	.004
Meat Consumption					
Beef	353	103 (29.20)	9 (2.50)		
Chicken	168	53 (31.50)	5 (3.00)		
Goat	194	73 (37.60)	9 (4.60)		
Pork	48	28 (58.30)	1 (2.10)		
Fish	270	90(32.80)	12 (4.40)	2.758	.599
Sanitation					
Garbage pit	84	25 (29.80)	4 (4.80)		
Outside the compound	384	141 (36.70)	13 (3.40)		
Waste bin	547	176 (32.20)	19 (3.50)		
Others	22	5 (22.70)	0 (0.00)	1.217	.749
Total	**1037**	**347(33.50)**	**36 (3.50)**		

Findings from the study revealed a 33.50% prevalence of parasitic infection among the study population with 3.50% of them suffering from polyparasitism. This prevalence of polyparasitism recorded among women of Oju and Obi LGAs, Benue State is comparatively low compared to what was reported in past by Omudu*et al.* [19] in Naka area of Benue State where a cumulative prevalence of 9.8% was reported. It was also lower than the report of Akor*et al.* [20] who reported a prevalence of 13.86% for polyparasitism in Oju Local Government Area of Benue State. Differences between the findings of this study and that of Omudu*et al.* [19] might be attributed in part to the differences in the geographical conditions of the various study location since Naka regions are dominated mainly by the Tiv people whose practices and beliefs are quite different from that of the Igede people. A possible reason for the variation experienced between the findings of this study and that of Akor*et al.* [20] may be due to the gender bias of this study which was limited to women alone unlike what was obtainable in the study of Akor*et al.* where the study population encompassed both males and females.

The prevalence of polyparasitism among women of Oju and Obi LGAs as observed in this study is lower than that of Akor*et al.* who reported a prevalence of 10.3% in Okpokwu area of Benue State [20]. It is also lower than a 12.2% prevalence reported for polyparasitism among children in Nassarawa State, Nigeria [21]. A prevalence of 3.50% is also lower than an 8.66% prevalence reported for polyparasitism inn Kwasa village in Makurdi Nigeria [11]. Differences in the prevalence rates between the retrospective studies highlighted above and that of this study might be attributed in part to the study population examined, the methods of diagnosis and the geographical location which is a predisposing factor to the prevalence of parasitic infection in Nigeria.

Risk factors of infection with parasites was shown to play a significant role in determining the prevalence of polyparasitism among women of Igede land, Benue State. Findings from the study showed that Excretion place and source of drinking water had a significant association with prevalence of polyparasitism among the study population ($P<0.05$). Higher prevalence of polyparasitismwere recorded for people who utilize pit toilet than those who utilize other forms of toilet. This finding is in agreement with the report of Onah*et al.* who reported a higher prevalence of polyparasitism among people who utilize pit toilet than other forms of toilet [11]. People who utilize pit toilets are usually at risk of coming into direct contact with parasites associated with stool since the pits are usually exposed and parasites can easily infect the women when they attempt to defaecate. This finding is also in agreement with the findings of Akor*et al.* who reported higher polyparasitism rates among people utilizing pit toilet [20]. Higher prevalence recorded for people who utilize rain water could however be attributed to the ability of parasites to be carried in rain water and on surfaces during rain. Also, those who treat rain water recorded a higher prevalence of polyparasitism than those who don't treat water. According to Omudu*et al.,* the major reasons behind the high prevalence of polyparasitism can be blamed on the sanitary and environmental condition which they described as deplorable [19]. These conditions in most regions including the study location were below standard and as such might promote the proliferation of parasitic organisms.

4. CONCLUSION

Polyparsitism among women of Igede land was relatively low in comparison to previous rates by previous authors. Nevertheless, occurrence of single parasite infection is high with malaria parasite being the most dominant parasite species. Also, several risk factors of infection have a significant impact on polyparasitism among women of Igede land, Benue State. These risk factors of infection include: type of toilet utilized and source of drinking water.The parasites encountered which included *Plasmodium* species, *Schistosomahaematobium, Ascaris lumbricoides, Entamoeba histolytica, Taenia species, Trichuris trichuria* and Hookworms suggests the existence of a significant public health hazard in the study area.

CONSENT (WHEREEVER APPLICABLE)

All authors declare that 'written informed consent was obtained from the subjects for publication of this case report and accompanying images.

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