Original Research Article

MALARIA AND INTESTINAL HELMINTH PARASITES INFECTIONS AMONG CHILDREN OF AGE UNDER 15 YEARS IN ISUANIOCHA COMMUNITY AWKA NORTH LGA ANAMBRA STATE

ABSTRACT

Malaria parasites and helminth infections have long been recognized as major contributors to anaemia in endemic countries. This study investigated the malaria and intestinal helminth infections among children of under fifteen years in 10 villages in Isuaniocha Community Awka North Local Government Anambra State Nigeria. The study was a cross-sectional survey of blood and faecal samples collected from 390 randomly selected under 15 years old children. The study was conducted between June and July 2018 in 10 villages in Isuaniocha. Blood and stool samples were collected from participants in a clean Ethylene Diamine Tetraacedtic Acid (EDTA) and dry capped containers respectively and stored in a ice packed for transportation to the laboratory for testing. The blood sample were analyzed using microscopy (thin and thick smears) for the test for the presence of malaria parasite while the stool samples were analyzed using Kato-Katz method for the presence of helminth. The overall prevalence of malaria and helminth co-infection was 9.49%. The prevalence of malaria parasites was 38.23% and the prevalence of helminth infections was (17.44%). The prevalence of co-infection of malaria parasites and helminth parasites among children under 15 years showed that there is no significant difference (P>0.05) among age under 15. Children below 5 years of age were more susceptible to malaria and anaemia..

Keywords: Malaria parasites; Intestinal Helminths; Co-infection; Ethylene Diamine Tetra Acetic Acid (EDTA); Endemic countries; Isuaniocha

1. INTRODUCTION

Malaria as been known over the years as one of the most intractable public health problems widely co-endemic with intestinal helminths infections, most especially in children who live in the endemic regions of sub-Saharan Africa resulting in a high rate of co-infection. Malaria epidemics have increased in frequency and intensity in most sub-Saharan African populations due to the emergence of drug resistance over the last two decades [1]. While the prevalence of symptomatic malaria infection is well documented [2], there seems to be a lack of data on asymptomatic malaria infection [2]. Many of these asymptomatic infections go undetected as well as untreated while causing little or no manifestation in affected individuals. The extent of asymptomatic parasitemia could be said to be inversely related to a population's susceptibility to clinical disease [3,4], as, asymptomatic or sub-clinically symptom people are major reservoirs of infection [4,5].

The co-infection of Malaria and intestinal helminths are commonly seen in sub-Saharan Africa because of the environmental factors that supports their coexistence and survival. A prominent factor that favour the transmission of soil-transmitted helminths (Geo-helminths) which makes them widely distributed in rural communities can be said to be the inadequacy or non-existent of sanitary facilities [6,7]. Some major

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predictors of malaria and helminth infection have been noted in previous studies, including greater susceptibility of females, lack of toilets, non-working status of parents, lower socioeconomic status, younger age, household crowding, lower level of education, religion, non-use of footwear, defecation practices, pig ownership, and lack of access to clean water [8,9]. Another possible factor is the subsistence farming, which is widely practiced in rural communities in Africa, which exposes populations to geo-helminths infections. It can be said that through a number of interventions, there has been a global decline in the prevalence of *Ascaris lumbricoides*, *Trichuris trichiura* and the hookworms (*Ancylostoma duodenale* and *Necator americanus*) in the Americas and Asia, the situation in sub-Saharan Africa remains stagnant [10]. The health outcomes of intestinal parasitic infections include malnutrition, growth stunting, intellectual retardation, and cognitive as well as educational deficiencies [11]. However, the interference to nutrient uptake by intestinal helminths can lead to anaemia, malabsorption, impaired physical and mental development which poses a serious threat to children's long-term health, their education, and productivity [12]. Developing countries are known to habour a considerable morbidity and mortality especially among children due to intestinal helminthiasis especially the soil transmitted helminths infections [12]

2. MATERIAL AND METHODS

2.1. The Study Area

The Study Area is Isuaniocha in Awka North Local Government Area of Anambra State, southeast Nigeria. Isuaniocha community is a rural town located at the outskirts of Awka, the capital of Anambra State. It is located approximately at latitude N6°16'14" and longitude E7°02'37". The population census of the community of study with respect to the creation of Anambra state in 1991, as projected by 3% to 2013 was 7,631 [13]. The study area is within the tropical rain forest zone and has marked wet and dry seasons. The wet season (March-October) is about 8 months while the dry season (October-February) is about 4 months. The relative humidity averages 70%, reaching 80% during rainy season with an annual rainfall of about 2000-3000 mm. The temperature ranges from 26ºC-35ºC in the dry season and 22ºC-30ºC in wet season. The community has 10 villages which are; Umuomite, Umudunu, Umueze, Otoko, Adama, Umuneri, Umuelom, Agwu Albert, Ekeagba and Ifite-isu. Isuaniocha is predominated mostly by the tribe called Igbos with few people from other ethnic groups in Nigeria like the Kogi's. Most of the inhabitants are farmers, with some petty traders, civil servants, students and technicians. The community has streams which serve as a source of domestic and drinking water supply to them. Isuaniocha is contingent with Mgbakwu, Awka, Achalla, and Urum.

2.2. Study Population

The study population comprised of children under 15 years. The sample size was 390 children which was determined using the formula as cited by [14] as shown below:

$$n = \frac{N}{1+Ne2}$$

n=Size of Sample
N=Size of population
e=level of precision (usually 0.05)

2.3. Study Design

The study was a cross-sectional community-based survey and involved collection of blood and stool samples from children below 15 years to determine malaria and helminth co-infection respectively in the 10 villages in Isuaniocha community, Awka North LGA Anambra. The population sizes of the 10 villages, presence of markets, churches and the topographical nature of the communities inform the selection distribution across villages. For every household, a maximum of 2 children were sampled. Children that showed signs of fever and and frequent stooling were selected for the study. The services of the nurses from the primary health care centers in the communities were engaged in sample collection.

2.4. Collection of Blood Samples

Blood were collected with a 5ml syringe from the participants and stored in an EDTA container and were used for the preparation of thin and thick blood films on a microscopic slide and in filling of a heparinized capillary

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tube for the determination of packed cell volume. Thin and thick blood smears was made on a slide and allowed to air-dried [15], and viewed under the microscope to determine the presence of malaria parasite.

2.5. Collection of Faecal Samples

A clean and well labeled stool containers were given to the caregivers with instructions on how to collect the children stool sample the next morning. This stool samples were collected between 8.00 and 10.00am and using the Kato-katz method to determine the presence of intestinal helminths eggs [16, 17].

2.6. Examination of Blood Samples for Malaria Parasites

2.6.1. Preparation of thick blood film

Two drops of the participant's blood was placed on a well labelled grease-free microscopic slide. The blood was homogenously spread in a circular motion using the edge of a spreader slide to make an even smear. The smear was allowed to air dry and placed in a slide box away from flies, dust and heat.

2.6.2. Preparation of thin blood film

A drop of the participant's blood was placed at the center of a well labeled grease-free microscopic slide. A smooth edged spreader slider was placed at an angle of 45˚C with the horizontal slide in front of the drop of blood. The drop of blood was allowed to run along the edge of the spreader slider. The spreader was pushed gently but firmly along the horizontal slide to make a thin smear of the blood. Care was taken to ensure even contact of the spreader and the surface of the horizontal slide. The film was allowed to air dry on a slide rack and later placed in a slide box away from flies, dust and heat.

2.6.3. Staining of the Blood films

Thick blood films was stained by holding the slide with the dried thick film facing downwards and dipped into Field's stain A for 5 seconds. The excess was drained off by touching a corner of the slide against the side of the container. The slide was washed gently in clean water for about 5 seconds and dipped into Field's Stain B for 3 seconds. The excess was drained off and the slide washed in clean water. The back was wiped clean and placed in an upright position on a draining rack for the film to air dry. The thin blood films was first fixed in absolute methanol for two minutes. Using a pipette, each film was covered with diluted Fields stain B and an equal volume of Field's stain A was added immediately and mixed well using the pipette. The film was left to stain for one minute, washed off with clean water, and placed upright on a draining rack to air dry.

2.6.4. Microscopic Examination of the Blood films

When the stained thin and thick films were completely dried, a drop of immersion oil was added on each slide and examined under the microscopic using x100 objective lens. The thick film was used to detect the presence of malaria parasites while the thin film was used to identify the species. The results were recorded against the names and number of the participants. The intensity of malaria was recorded using the plus sign thus: Mild infection (+) or 1-10 parasites per 100 high power fields. Moderate infection (++) or 11-100 parasites per 100 high power fields. Heavy infection (+++) or 1-1000 parasites per high power fields [17].

2.6.5. Determination of Packed Cell Volume

Blood filled microhematocrit tubes was collected and centrifuged using a microhematocrit rotor for 5 minutes at 10,000g. PCV values ≤ 31% to determine anaemia, which was classified as mild (21–30%), moderate (15-20%), or severe (≤15%) [18, 2]

2.7. Examination of Faecal Samples for Helminths Parasites

Kato-Katz technique was used for the detection of helminths in the stool samples. A well labeled glass slide with the sample number and a template placed on top of it was used. 1 Gram (1.0g) of faecal sample was placed on a newspaper with a piece of nylon screen mesh pressed on top. A spatula was used to scrape the sieved faecal material through the screen mesh so that only the debris remained. Some of the sieved faeces were scrapped up to fill the hole in the template (disc). Air bubbles were avoided and excess faeces removed. Care was taken while lifting off the template (disc). After this, a piece of cellophane which had been soaked

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overnight in methylene blue glycerol solution was placed over the faecal sample. Then a clean slide was placed over the top and pressed evenly downwards to spread the faeces in a circle and then the slide was carefully removed and observed under microscope using x10 and x40 objectives [19].

2.8. Determination of the Risk Factors

A structured questionnaire were administered to all the 390 study participant with consent from their parents. The information contained include; their demography and behavioural hygiene, the occupation of the parents, the use of long lasting insecticide nets, household sanitary facility, licking of fingers, walking barefooted, hand washing after visit to toilet, foot wearing habit and frequency of deworming were the variables determined using the structured questionnaire.

2.9. Data Analysis

The data collected was entered into EPI data 3.1 statistical packages and analyzed using Statistical Package for the Social Sciences (SPSS) version 16.0. The outcome of the variables measured were; malaria, parasitaemia, helminthiasis and malaria and intestinal helminths co-infection. Descriptive statistics such as frequencies, proportions, means and confidence intervals were used to summarize the data. While the categorical data were compared using the Chi-square test or the Fisher's exact test, as appropriate, a p-value of < 0.05 of the study was considered for statistical significance.

3. RESULTS

3.1. Prevalence of Co-infections of malaria and intestinal helminth infection among children of under fifteen years in Isuaniocha Community Anambra

The overall prevalence of malaria and helminth parasites co-infection was (9.49%) while the prevalence of malaria parasites was (38.23%) and the prevalence of helminth infections was (17.44%). Of the 390 participants for the study, (9.14%) of the male had malaria and intestinal helminth parasites co-infection and about 26.34% was anaemic. About (9.80%) of the female participants had malaria and intestinal helminth parasites co-infection with (52.49%) of the female been anaemic. The Age 11 - 15 years had the highest malaria and helminth co-infection of 11.38% with anaemia of (24.55%) (TABLE 1). There is no significant difference ($p=0.41$, $p>0.05$) in the prevalence of malaria and helminth parasite co-infection among under fifteen children of Isuaniocha Community.

Table 1.Prevalence of Co-infections of malaria and intestinal helminth infection among children of under fifteen years in Isuaniocha Community Anambra

Variables: Gender/Age (in years)	Number Examined (%)	Number for malaria Positive (%)	Number for helminth Positive (%)	Number Co-infected with malaria and helminths Co-infection (%)	Number Anaemic (%)	X^2	P-value	df
Male	186 (47.69)	69(37.10)	32 (17.20)	17 (9.14)	49 (26.34)	0.679	0.410	1
Female	204 (52.31)	84 (41.20)	36 (17.65)	20 (9.80)	52 (52.49)			
Total	390	153 (39.23)	68 (17.44)	37 (9.49)	101 (25.90)			
≤5 years	45 (11.54)	18 (40 00)	14 (31.10)	4 (8.89)	18 (40.00)	0.558	0.756	2
6-10 years	178 (45.64)	73 (41.00)	21 (11.80)	14 (7.87)	42 (23.60)			
11-15 years	167 (42.82)	62 (37.13)	33 (19.80)	19 (11.38)	41 (24.55)			
Total	390	153 (39.23)	68 (17.44)	37 (9.49)	101 (25.90)			

3.2. Prevalence of Co-infection of Malaria and Helminth Parasites infections among children below fifteen years by Occupation

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Occupation of parents of participant showed a high co-infection of malaria and helminth parasite infections (12.60%) with the least among parent of participants that were public servants (5.80%) (TABLE 2). The prevalence of co-infection of Malaria parasites and Helminth infections among children below fifteen years by parent's occupation shows that there is a significant difference (p=0.001, P<0.05)

Table 2. Prevalence of Co-infection of Malaria and Helminth Parasites infections among children below fifteen years by Occupation

Parent's occupation	Number Examined (%)	Number Positive for co-infection of Malaria parasite and helminth infection (%)	X^2	P-value	df
Trader	139 (35.64)	10 (7.19)	13.080	0.001	2
Farmer	182 (46.67)	23 (12.6)			
Public Servant	69 (27.54)	4 (5.80)			
Total	**390**	**37**			

3.3. Prevalence of Co-infection of Malaria Parasites and Helminth infections among children below fifteen years by Villages in Isuaniocha Community Anambra

Among villages in Isuaniocha, Adama village had the highest number of children below fifteen with co-infection of malaria and helminth parasite infections (16.22%) while the least co-infection of malaria and helminth parasites infections were recorded in Umueze village (4.00%) (TABLE 3). The prevalence of co-infection of Malaria parasites and Helminth infections among children below fifteen years by Villages shows that there is no significant difference (p=0.685, P>0.05) among children below fifteen years in Isuaniocha community.

Table 3. Prevalence of Co-infection of Malaria Parasites and Helminth infections among children below fifteen years by Villages in Isuaniocha Community Anambra

Villages	Number Examined (%)	Number of Positive Malaria Parasite and Helminth infection (%)	X^2	P-value	df
Adama	37 (9.49)	6 (16.22)	6.538	0.685	9
Agwu Albert	27 (6.92)	2 (7.41)			
Ekeagba	36 (9.23)	2 (5.56)			
Ifite Isu	44 (11.28)	3 (6.82)			
Otoko	34 (8.72)	4 (11.76)			
Umudunu	43 (11.03)	4 (9.30)			
Umuelom	43 (11.03)	4 (9.30)			
Umueze	50 (12.82)	2 (4.00)			
Umuneri	34 (8.72)	5 (14.71)			
Umuomite	42 (10.77)	5 (11.90)			
Total/	**390**	**37**			

3.4 Prevalence of Anaemia

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nd co-infection of Malaria and Helminth parasites infections among children under fifteen years in Isuaniocha Community Anambra

A high percentage of the participant who were anaemic (54.14%) had a co-infection of Malaria parasites and Helminth infections (43.53%) (Table 4). There is a significant difference in the prevalence of Anaemia and co-infection of Malaria and Helminth parasite infections among children below fifteen years by age (p=0.000, P<0.05) in Isuaniocha community.

Table 4. Prevalence of Anaemia and co-infection of Malaria and Helminth parasites infections among children under fifteen years in Isuaniocha Community Anambra

Anaemia	Number Anaemic (%)	Number Positive for co-infection of Malaria parasite and helminth infection (%)	X^2	P-value	df
Yes	85 (54.14)	37 (43.53)	37.733	0.000	1
No	72 (45.86)	1 (1.39)			
Total	**157**	**38**			

4. DISCUSSION

This study reported a prevalence of 38.23% of malaria parasites, which is lower than the high 91.3% and 50.6% prevalence rate reported by Efunshile et al. (2015)[20] in Ilero and Kunihya et al. (2016)[21] in Yola respectively. The study shows a prevalence of 17.44% for helminth infections which was higher than the observed prevalence of 6.02% in Anambra state [Aribodor et al 2021][22] but lower compare to 52.50% gotten from Ogbaru Local Government Area of Anambra State (Uchenna et al., 2018)[23] and the recorded prevalence rates of 89% [Okereke et al 2015] [24], 44.7%[Onyido et al 2016][25] and 10.18% [Mbanugo et al 2015][26] all in Anambra State.

The study reported that female had the highest prevalence of malaria parasites than male, 41.20% and 37.10% respectively. This disagrees with Ezeigbo et al. (2014) [27] and Kiggundu et al., 2013[28] in Uganda, which showed that confirmed malaria was lower among girls 52.3% than boys 57.3% and also the findings of Benisheikh et al. (2014)[29] as well as Umaru and Uyaiabasi (2015)[30] which reported higher prevalence rate in males than their female's counterparts but had no bearing on the effect of helminth co-infection. This difference was of significance and had no borderline. This varied finding may be explained by Abdullahi et al. (2009)[31] and Okonkwo et al. (2012) [32] who stated that there is no scientific evidence to prove higher prevalence being related to gender as susceptibility to malaria infection is not influence by gender. This implies that malaria infection depends on the person's exposure to infectious bites of mosquito vectors. However, children and pregnant women are biologically more susceptible to malaria; there are compounded gender differences due to sociocultural norms and expectations that influence patterns of exposure, decision making, and economics (Ministry of Health Malaria Control Unit, 2015)[33].

This study shows that a higher prevalence of malaria parasites among age 6 years and above contradicts the usual trends of malaria epidemiological findings (WHO, 2012)[34]. Children 6 and above often show low prevalence of malaria with high prevalence of malaria found among under 5 years (Efunshile et al., 2015)[20], but corroborates with the work of Aina et al. (2013)[35] in Lagos which shows high prevalence of malaria parasite among age 5-14 years, this may be as a result of a range of factors such as fewer numbers of under 5 years that showed up for the exercise and their clinical stage at the time of conducting the test. Also, the high helminth infections 31.10% from the study among the under 5 years may have conferred immunity against malaria (Efunshile et al., 2015) [20]. These findings strengthen the worries that regular deworming of children in endemic areas could potentially be putting the children at increased risk of malaria (Specht and Hoerauf, 2007) [36] as it was particularly seen that the protective effect of helminth co-infection was seen in the youngest children under 5 years who are at the highest risk of malaria compare to other ages, as deworming is now a routine biannual public health intervention in Nigeria (Strunz, 2011) [37]. It is very unlikely that permission would be granted to conduct a randomized trial of the effect of deworming on malaria risk. Furthermore, historical controls cannot be used to compare malaria risk before and after deworming in a

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treatment series, because malaria transmission changes with season and annual variation. However, this study raises the question of whether deworming should routinely be combined with intermittent preventive treatment of malaria in children (IPTc) (Midzi et al., 2011) [38] or other interventions aimed at reducing malaria risk.

With regards to parent's occupation of the children who participated in the study, their knowledge on the use of nets, a high prevalence of malaria parasites among children whose parent's occupation were farmers 43.96% which was expected considering the low educational level of the group with a high significant difference (p=0.000). Although it was expected that children of parents working as public servants should be more knowledgeable on the cause and prevention of malaria parasites.

Locations with much bushes around its surrounding tend to have high prevalence of malaria parasite and helminth co-infections owing to the conducive breeding environment for the female mosquitoes and the helminth parasites as seen in the study which showed that Adama, Otoko, Umuneri and Umuomite villages has 16.22%, 11.76%, 14.71% and 11.90% respectively

From the study by Uchenna et al. (2018) [23], the effect of malaria parasitaemia on anaemia using the mean and standard deviation of Hb and PCV of the children with malaria parasite showed no significant difference between malaria which conforms with this study which also did not shows significance difference (p=0.510) among under 15 years. Okafor et al. (2001) [39] and Cheesbrough (2010) [40] reportedanaemia as the commonest complication of malaria among 1-5 age groups contrary to what this study reflects which shows 33.33%, of severe parasitaemia level across the various age range. This study shows that anaemia can be as a result of other causes other than malaria since confirmed malaria cases is often recorded in age 1-5 years. By gender, female had the most severe parasitaemia level (1000-10,000parasites/ul) 66.67% against male 33.33%.

This study recorded an overall prevalence of 17.44% for helminth infections, which is low and corroborate with the findings from Mirisho et al. (2017) [41] in Ghana which also showed a low prevalence of 17.33% compare to other studies done among children in different countries with a prevalence of 41.46% in a district in Northern Ethiopia (Abera and Nibret, 2014) [42] and 31% from rural Kenya (Nguhiu, et al., 2009)[43]. The result shows that helminth parasites are highly prevalent in rural communities due to poor sanitary conditions prevailing in most of these areas (Hotez and Kamath, 2009)[44]. This low intensity and prevalence of intestinal parasites in Isuaniocha community could be attributed to the bi-annual deworming campaigns that the government implemented in the region.

5. CONCLUSION

Children less than 5 years old were more susceptible to malaria and anaemia as well as helminth infections. Multiple risk probably contributed to the prevalence of co-infection of malaria and helminth. In addition, nutritional factors probably also contributed especially to the anaemia observed. Studies assessing the effect of helminth infections on the course of malaria are very important in target malaria vaccine trial communities. The recent biannual vaccination exercise in the country may probably be a result of previous research findings on their co-infection. Well-structured longitudinal studies investigating the underlying immune mechanisms involved in malaria-helminths co-infection are very important in this regard. Effort should be made to develop a behavioural change model for communication on the use of LLINs and other interventions that will forestall absolute compliance. These results also indicate that employing the proper intervention in a combined malaria-helminth cost effective approach will yield timely results in endemic communities.

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CONSENT

Authors declare that 'written informed consent was obtained from the patient and parents.

ETHICAL APPROVAL

The study was approved by the ethical review committee of Chukwuemeka Odimegwu Ojukwu Teaching Hospital, Awka Anambra, Nigeria. An advocacy visit to the State Ministry of Health with a letter of introduction from the head of Department of Parasitology and Entomology, Nnamdi Azikiwe University, Awka, Anambra State was made to secure their approval. An advocacy visit with a copy of the approval letter from the State Ministry of Health was conducted to the Medical Director of the Health facilities, the community heads and parents to get their support and co-operation.

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Asymptomatic (sub-clinical) malaria infection: refers to the presence of malaria parasites in the blood without symptoms, usually provides a reservoir for transmission, and is an antecedent to symptomatic malaria

Parasitemia: Parasitaemia is the quantitative content of parasites in the blood. It is used as a measurement of parasite load in the organism and an indication of the degree of an active parasitic infection

Morbidity and Mortality: Morbidity is when you have a specific illness or condition. Some examples of common morbidities are heart disease, diabetes, and obesity while mortality is the number of deaths due to a specific illness or condition

Ethylene Diamine Tetra Acetic Acid (EDTA): It is an anticoagulant for blood samples for complete blood count (CBC), also known as a full blood count (FBC), where the EDTA chelates the calcium present in the blood specimen, arresting the coagulation process and preserving blood cell morphology.

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