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ASYMPTOMATIC MALARIA AND INTESTINAL PARASITES CO-INFECTION AMONG NON-BENEFICIARIES OF THE NATIONAL DEWORMING PROGRAM IN NGIE: A PRELIMINARY STUDY OF A RURAL COMMUNITY IN NORTHWEST REGION, CAMEROON

Epidemiology

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ABSTRACT

Background: Malaria and intestinal parasitic infections (IPIs) are among the most prevalent of human infections in developing countries. This study assessed the prevalence of asymptomatic malaria and intestinal parasites coinfection among non-beneficiaries of the national deworming population in Ngie.

Methods: A community-based cross section study was used. Blood and stool samples were collected from those participants who gave consent to take part in the study and assayed for the presence of malaria and IPIs according to standard procedures. Data were analyzed using Pearson's Chi-square and Logistic Regression analysis and were considered significant at $P \leq 0.05$.

Results: The prevalence rates of malaria parasite, IPIs and malaria and intestinal parasites co-infection were 40.1%, 31.5%, and 10.9%, respectively. Ascaris lumbricoides (13.2%) was the most prevalent intestinal parasites identified. Helminths infections (19.2%) were 1.8 times (OR=1.84,95% CI:1.45-8.62) more prevalent than protozoa infections (12.3%). IPIs were 2.7 times (OR = 2.71, 95% CI: 1.34 – 5.49) more prevalent in Teze village than any other villages. No significant association was observed between co-infection with malaria and intestinal parasitic infection (P=0.863).

Conclusion: This study revealed that malaria and IPIs still constitute a major public health problem in Ngie despite a lack of any significant association between them.

KEYWORDS

Prevalence, Asymptomatic malaria, Intestinal parasitic infections, Co-infection, Ngie

Introduction

Malaria and intestinal parasitic infections (IPIs) constitute the most prevalent diseases that are impediment to development in Sub-Saharan African (SSA) countries including Cameroon [1]. Although significant progress has been recorded in malaria control efforts that has led to a 29% reduction in malaria mortality rates globally since 2010, the prevalence remains high especially in SSA and children under five years of age are the most affected [2]. In 2016, an estimated 216 million cases of malaria with 90% in the African Region and 445000 deaths with approximately 91% in the WHO African Region was reported [2]. In Cameroon, over 90% of the population is at risk of malaria infection and approximately 41% having at least one episode of the disease each year [3]. Moreover, malaria accounts for 50–56% of morbidity and 40% of the annual mortality among less than five-year old [4]. Malaria also imposes a heavy economic burden on both individuals and governments, with an estimated direct cost of at least US $12 billion per year [5]. The emergence of resistant strains to current chemotherapeutics couple with patient non-compliance to medication are the contributing factor to the high prevalence recorded [6,7].

Intestinal parasitic infections on the other hand caused either by protozoa or helminths or both are among the most widespread of human infections worldwide accounting for about 25% of human infectious diseases [8]. The incidence of IPIs is disproportionately high in sub-Sahara African countries carrying up to 95% of the disease burden [9,10]. IPIs have very negative effects on the survival, growth, appetite, school attendance and cognitive performance of infected persons [11]. The infections also affect child health and development and slow down growth, while reducing adults' productivity and work capacity. Global infections reported for some of the most common intestinal parasites are Ascaris (20%), hookworm (18%), Trichuris trichiura (10%), and Entamoeba histolytica (10%) [12]. Populations in different parts of the world face diverse parasitic challenges. For example, Enterobius vermicularis is more common in temperate zones [13] and Ascaris lumbricoides is more prevalent in Tropical areas [14] In Cameroon, common intestinal parasites reported in adult populations include Entamoeba coli, Ascaris lumbricoïdes, Entamoeba histolytica, Cryptosporidium spp, Isospora belli, Trichuris trichiura, Strongyloïdes stercoralis, Ancylostoma duodenale, Necator americanus, and Ankylostoma duodenalis [15,16].

The type and frequency of the incidence of malaria as well as intestinal parasitosis in humans depends on the climate, socio-economic conditions, education, personal and public hygiene practices and nutritional habits [7,9,10] Malaria is most prevalent in Africa since the continent provides suitable climate for victor to thrive [17]. Poor environmental sanitation and personal hygiene have shown to be associated with the high prevalence of parasitic disease observed in SSA [18].

To curb the spread of intestinal parasites in our communities, a holistic approach involving all actors and tools is recommended [8]. In Cameroon, the government started the national deworming of school children in 2006 [19], through mass drug administration with mebendazole/albendazole (1-5 years) for soil transmitted helminths (STHs) and praziquantel (5-14 years) for schistosomiasis in schools. However, this control program does not involve school drop outs and the elderly persons in the community who might serve as a reservoir for rapid re-infestation. We herein investigated the occurrence of malaria and intestinal parasites, as well as their co-infection among non-beneficiaries of the national deworming program in Ngie, a rural community in the Northwest Region of Cameroon.

Materials and methods

Study area

This study was conducted in the Ngie Sub-division, Momo Division of the Northwest region, Cameroon. Ngie Sub-Division also known as Ngie clan, is one of the five subdivisions of Momo Division with a population of about 18 000 inhabitants and a total surface area of about square kilometers or the 2 should be superscript for the 4000Km2 and 5persons/km2. The clan is made up of 19 autonomous villages predominantly of a sub-group of the Widikum ethnic group with a few Mbororos (Fig 1) [20]. Ngie is located between latitude 50 43' and 60 10'N and longitude 90 43' and 90 55'E [21]. It is bounded to the north, west, south and east by Mbengwi, Njikwa, Widikum and Batibo subdivisions, respectively [20]. The area could be described as a transition zone between the wet tropical and Sudan climate. The annual rainfall ranges between 2.200mm and 3.000mm while the mean annual temperature is The temperatures are; 21 degree Celsius, 15 degree Celsius, and 30 degree Celsius. [21]. The natural vegetation is made up of the tropical rain forest, which has been significantly modified by human activities especially agricultural practices. Farming is the most important occupation in the communities. The cash crops produced in the area include kola nut, cocoa, coffee and palm oil while yam, maize, cocoyam, beans, vegetables and plantains are the major food crops [21].

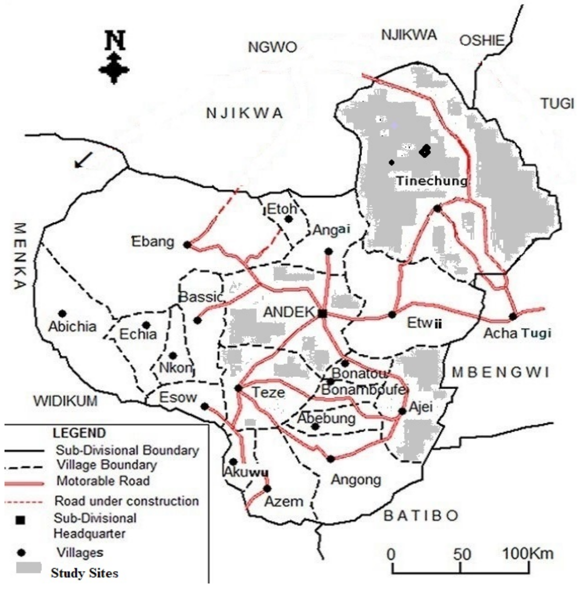

Fig. 1 Map of Ngie clan showing the study sites (Source: Andek Council, 2014)

Study population and design

The study conducted between March and May 2011 was a community-based cross-sectional study. The participants of the study were people who had lived in the communities for at least two years, aged ≥10 years old, with no sign of fibril and were non-beneficiaries of the government annual deworming program.

Sample Size determination

The minimum sample size required for this study to yield a statistically significant result was estimated to be 198 participants using the f   l

$$n = \frac{Z\alpha^2 pq}{d^2}$$

Where n = sample size, Z_α = standard normal deviate, set at 1.96 (for 95% confidence level), d = desired degree of accuracy 5% (0.05) and p = the estimate of our target population having intestinal parasites = 15.2% [22]. A total of 302 participants enrolled for the study.

Sample collection and investigation

Four villages within Ngie clan namely: Ajei, Andek, Teze and Tinechung were randomly selected for sample collection. Prior to sample collection, inhabitants of the selected communities were sensitized in churches, markets and meeting groups on the nature and importance of the study. Participants who voluntarily reported to the different health facilities in each health area were recruited for the study. Once participants gave signed informed consent, their stool and blood samples were collected. They were instructed to put a teaspoon of stool into sterile leak-proof wide-neck stool containers. Capillary blood was also collected by means of finger prick as described by Cheesbrough [23] for malaria microscopy. Short structured questionnaire was also designed to capture socio-demographic variables such as age, gender and community of origin.

Parasitological analysis

Detection of malaria parasites

Thick blood films were prepared, stained with 10% Giemsa and examined for *Plasmodium* parasite by microscopy using the oil immersion (100X) objective [23]. If parasites were observed (either trophozoites, schizonts or gametocytes), the density was then determined by counting the number of parasites against 200 leucocytes. Slides were considered positive when asexual forms (trophozoites and schizonts) and or gametocytes of any *Plasmodium* species were present in the blood film. Slides were declared negative after observing 100 high powered fields without identifying any parasite. The parasite density was obtained by dividing the number of parasites by 200 and multiplying the result by the actual number of white blood cell count of the patient [24].

Stool processing and detection of intestinal parasites

A wet mount identification technique as described by Cheesbrough [23] was used in the processing of stool samples and examined for the presence of intestinal parasites. Samples containing at least one form of parasite were recorded as positive.

Ethical considerations

This study was approved by the Regional Delegation of Public Health (006/L/NWR/RDPH/CEA-3).

Only individuals who voluntarily accepted to participate in the study by signing a written informed consent, after adequate sensitization on the project objectives, risks and possible benefits were enrolled. Children below 18 years of age were registered for the study after signing an assent form in addition to their signed parental/guardian authorization. All participants' information collected during the course of the research were entered into a password protected database and were strictly confidential. In addition to the individual consent, the village chiefs gave their authorisation for the study to be carried in their communities.

Statistical Analysis

Data were entered in Epi Info™ 3.1 statistical package and analyzed using Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, Atlanta, GA, USA). The outcome variables measured included malaria prevalence and parasitaemia, intestinal parasites and co-infection with malaria and intestinal parasites. Descriptive statistics was carried out to measure percentages, averages, and relative frequencies of the variables. Categorical data were compared using the Pearson's Chi-Squared (χ^2) test at 95% confidence interval (CI). The variables that were significantly associated with intestinal parasites and malaria were analyzed using Logistic Regression, and only variables with a significance threshold of less than 0.05 were included in the final model. Results were reported as adjusted odd ratios (OR) together with their confidence intervals. Statistical level of significance was set at P≤0.05.

Results

Characteristics of the study population

A total of 302 participants who were non-beneficiaries of the deworming program and accepted to provide stool and blood samples were recruited for this study. The age distribution of participants ranged from 10-87 years old with a mean age distribution of 27.67 ±14.04SD. Majority were female (63.2%), aged 20-39 years (48.3%), and from Andek village (39.4%), (Table 1). The prevalence rates of intestinal parasitic infections (IPIs) as well as malaria are shown on figure 2.

Table 1: Association of demographic variables and prevalence rates of parasitic infections

	Frequency	Gender		p-value	Age group (years)			p-value	Village				p-value
		Male	Female		10-19	20-39	>40		Ajei	Andek	Teze	Tinechung	
Sample size		111(36.8)	191(63.2)		101(33.4)	146(48.3)	55(18.2)		29(9.6)	119(39.4)	75(24.8)	79(26.2)	
Protozoa+helminths	98(31.5)	38(34.2)	57(29.8)	0.428	38(37.6)	42(28.8)	15(27.3)	0.257	7(24.1)	39(32.8)	32(42.7)	17(21.5)	0.032
Protozoan infections													
Entamoeba histolytica	22(7.3)	17(8.9)	5(4.5)	0.156	7(6.9)	13(8.9)	2(3.6)	0.434	1(3.4)	10(10.9)	4(5.3)	4(5.1)	0.265
Entamoeba coli	16(5.3)	11(5.8)	5(4.5)	0.639	4(4.0)	8(5.5)	4(7.3)	0.671	3(10.3)	6(5.0)	3(4.0)	4(5.1)	0.626
Trichomonas hominis	1(0.3)	1(0.5)	0(0.0)	0.445	1(1.0)	0(0.0)	0(0.0)	0.368	0(0.0)	0(0.0)	1(1.3)	0(0.0)	0.386
With protozoan infections	37(12.3)	10(9.0)	27(14.1)	0.19	11(10.9)	20(13.7)	6(10.9)	0.761	3(10.3)	18(15.1)	8(10.7)	8(10.1)	0.675
Helminth infections													
Ascaris lumbricoides	40(13.2)	18(16.2)	22(11.5)	0.246	17(16.8)	15(10.3)	8(14.5)	0.321	3(10.3)	15(12.6)	13(17.3)	9(11.4)	0.664
Shistosoma mansoni	1(0.3)	1(0.5)	0(0.0)	0.445	1(1.0)	0(0.0)	0(0.0)	0.368	0(0.0)	0(0.0)	1(1.3)	0(0.0)	0.386
Stronyloides Stercoralis	8(2.3)	5(2.6)	3(2.7)	0.97	6(5.9)	2(1.4)	0(0.0)	0.036	0(0.0)	0(0.0)	8(10.7)	0(0.0)	0.001
Trichuris trichiura	3(1.0)	0(0.0)	3(2.7)	0.022	1(1.0)	2(1.4)	0(0.0)	0.683	0(0.0)	1(0.8)	1(1.3)	1(1.3)	0.926
Taenia spp	5(1.7)	2(1.8)	3(1.6)	0.879	3(3.0)	1(0.7)	1(1.8)	0.382	0(0.0)	2(1.7)	3(4.0)	0(0.0)	0.226
Ancylostoma duodenale	17(5.6)	9(4.5)	8(7.2)	0.364	5(5.0)	10(6.8)	2(3.6)	0.635	3(10.3)	6(5.0)	7(9.3)	1(1.3)	0.109
With helminth infections	58(19.2)	28(25.2)	30(15.7)	0.043	27(26.7)	22(15.1)	9(16.4)	0.061	4(13.8)	21(17.6)	24(32.0)	9(11.4)	0.008
Malaria	121(40.1)	44(39.6)	77(40.3)	0.908	35(34.7)	58(39.7)	28(50.9)	0.14	10(34.5)	51(42.9)	24(32.0)	36(45.6)	0.285
Malaria+IPIs co-infection	33(10.9)	16(14.4)	17(8.8)	0.139	8(7.9)	17(11.6)	8(14.5)	0.416	4(13.8)	13(10.9)	6(8.0)	10(12.7)	0.765

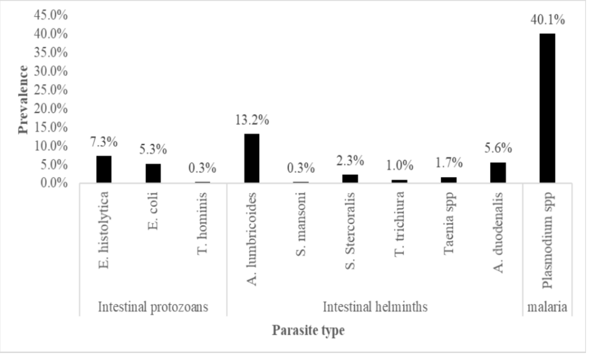

Fig. 2. Distribution of parasite types in the study population

Prevalence of MP and IPIs

The prevalence rates of malaria as well as IPIs according to demographic variables are shown in table 1.

Malaria prevalence

One hundred and twenty-one (121) participants were diagnosed positive for *Plasmodium* species, resulting in a prevalence of 40.1%. The parasite density ranged between 38 and 130,215 with a mean distribution of 9,285 ± 14,320.4SD. Although there was no significant difference between prevalence of malaria and gender (P=0.908), females (40.3%) tended to be more prone to malaria infections than males (39.6%), (Table1). A steady increase in the prevalence rate of malaria was recorded from 34.7% in the 10-19 years age group to 50.9% in ≥ 40 years old participants, though not statistically significant (P = 0.14). Participants from Tinechung (45.6%) tended to be more prone to malaria than those from Andek (42.9%), Ajei (34.5%) or Teze (32.0%) villages (P=0.285).

Intestinal parasitic infections prevalence

Among the 302 participants, 98 were positive for intestinal parasitic infections, resulting in a prevalence of 31.5% (Fig 3). IPIs prevalence was higher in males (34.2%) compared to females (29.8%) however, no significant difference was observed. Results further revealed that there was no significant difference between IPIs and age group (P=0.257), the prevalence decreased with increased age from 37.6% in the 10- 19 years age group to 17.3% in ≥ 40 years old group (Table 1). IPIs were more prevalent (P=0.032) in Teze (42.7%) village than Andek (39.4%), Ajei (24.1%) or Tinechung (21.5%) villages. In fact, participants from Teze village were 2.7 times (OR = 2.71, 95% CI: 1.34 – 5.49, P = 0.006) more likely to have IPIs compared to their counterparts from Tinechung village. Both protozoans and helminths were found in stool analysis.

Of the intestinal parasites infections (IPIs), helminths infections (19.2%) were 1.8 times (OR=1.84,95% CI:1.45-8.62, P<0.002) more prevalent than protozoa infections (12.3%). Of the nine (9) species of IPIs identified in the study, infection with *Ascaris lumbricoides* species was the most common IPI (35.9%), followed by *Entamoeba histolytica* (19.4%), *Ancylostoma. duodenale* (hookworm) (15.0%), *Entamoeba coli* (14.2%), *Strongyloides. stercolaris* (7.1%), *Taenia spp (4.4%), Trichuris.trichiuria* (2.7%) while <1% of *Trichomonas hominis*, *and Schistosoma mansoni* were identified. The association between de[illegible] 1.

Fig. 3 Variation of parasite coinfections with age, gender and village of participants

Co-infection with malaria and IPIs

Among the three hundred and two (302) participants, 18 (6.0%) had mixed infections of two or more IPIs (Fig.3) mostly *E. histolytical* + *A. duodenale*, E. coli+ *A. lumbricoides* and *A. duodenale* + *Taenia spp* associations. Moreover, mixed infections of malaria and IPIs occurred in 10.9%(33) of participants.

There was no association between co-infection with malaria and IPIs (P=0.863). Although no statistically significant relationship was observed between malaria co-infection with IPIs and demographic variables (Table 1), males (14.4%) tended to be more infected than their females (8.9%) counterparts (P=0.139). Malaria co-infection with IPIs increased with age group from 7.9% to 14.5 % observed in the 10-19years and ≥40 years old age groups, respectively (P=0.416). Participants from Ajei village (13.8%) tended to be more prone to malaria and IPIs coinfections than their Tinechung (12.7%), Andek (10.9%) and Teze (8.0%) counterparts (P=0.765).

Discussion

This is the first study designed to investigate asymptomatic malaria and intestinal parasites Co-infection among non-beneficiaries of the national deworming program in Ngie. Our study revealed that the prevalence of asymptomatic malaria was 40.1%. This indicates that this area is highly endemic for malaria. However, the malaria prevalence in this study population is lower when compared to studies conducted in Cameroon [1] and Nigeria [25] among children in selected rural communities. This higher prevalence observed in these areas could be attributed to the fact that the study populations were children who are more prone to malaria attacks compared to adults. It was further observed that malaria prevalence was higher in females aged 10-19 years old though not statistically significant. Farming is the main occupation of Ngie indigenes and is mostly done by females. This explain the fact that females tended to be more prone to malaria infection due to exposure. Furthermore, the higher prevalence observed in participants aged 10-19 years could be attributed to the fact that the incidence of malaria increases with age [26].

The prevalence of intestinal parasites in the study population stood at 31.5% with nine (3 protozoans and 6 helminths) different intestinal parasite species identified. The 31.5% prevalence is far lower than the 56.7% in street food vendors in Buea [15] and 57.48% among HIV patients in Yaounde [16]. This lower prevalence could be because of the lack of facilities for more sensitive and advanced techniques to detect all the parasites in this study. However, the overall prevalence (31.5%) is very close to the 34.5% among HIV/AIDS patients attending Bamenda Regional Hospital in North West, Cameroon [27] and higher than the 15.2% reported in Douala urban city [22]. Compared to the overall STHs prevalence of 32.5% in school age children of the four Regions of Cameroon as reported by Tchuem-Tchuente and others [28], it clearly indicates the need to include those not presently benefiting from the annual MDA in the national deworming program.

The common intestinal parasites identified in this study were like those reported in other studies in Cameroon [15, 16, 28] but the dominant species differ from those identified among HIV/AIDS patients attending Bamenda Regional Hospital [27]. Compared to the average figures of four Cameroon regions reported in 2013 [28], the prevalence of common intestinal parasites such as *Ascaris*, Hookworm, and *Trichiuris trichiura* are lower in this study. Also, the highest prevalence of *Ascaris lumbricoides* in this area is in accordance with the report of Stepek and colleagues [14] which says that Ascaris is the most common intestinal parasite in the tropics. The results also show a significant variation in intestinal parasite prevalence between different communities as was the case between schools, villages, districts and regions of Cameroon reported in 2013 [28]. The significantly higher prevalence of IPIs in Teze could be attributed to observed characteristics such as lack of portable water, farmers eating without washing their hands while in the farm, uncontrolled pig raring and the presence of favorable environmental conditions for the survival of parasite infective stages. Also, Teze is found at the lowest altitude of all the four villages and having the highest number of streams flowing very close to human settlements.

The 10.9% of malaria and intestinal parasites co-infection identified in this study is very close to the 11.9% reported in some rural communities in Cameroon [29] but very different from the 57.1%

reported in asymptomatic children in Ibadan, Nigeria [25].

Conclusions

This study showed that, malaria, IPIs and co-infection of both are common among non-beneficiaries of the national deworming program in the study area. These preliminary results will help to guide future research on prevention and control of parasitic infections in Ngie rural communities. Also, the results indicate that government efforts of malaria control through insecticidal mosquito treated bed nets and annual deworming of pre-school and primary school pupils may not attain its goal, if a holistic approach that involves the entire population is not taken. Therefore, there is need for alternative control measures, good potable water supply, proper education of the rural communities on the importance of public and personal hygienic practices, regular use of chemotherapy/antimalarial (intermittent preventive treatment) and deworming of the entire rural populations to drastically reduce these parasitic infections to the least possible levels. Community based malaria and IPI integrated control programs and health education for all is seriously needed in this area.

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Competing Interests

The authors declare that they have no competing interests

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Authors' contributions

RAA designed the study, coordinated the field activities, participated in sample collection and processing, interpreted the results and wrote the manuscript. MUA participated in sample collection and processing, data analysis and proof read the manuscript. EAA participated in sample collection, processing and edited the manuscript. PUA, participated in sample collection and processing, proof read the manuscript. DNA designed the study, supervised the entire research and edited the manuscript. All authors read and approved the final manuscript.

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