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An assessment of the endemicity of *Plasmodium falciparum* and soil transmitted helminth infections in the Tombel health district of Cameroon: Implications for monitoring the progress of disease control programs

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Abstract: **Parasitic infections such as Soil transmitted heminths and Plasmodium falciparum remain a call for concern in Cameron and other parts of Sub-Saharan Africa despite the efforts of various control programs. These diseases show similar geographic distributions and co-infections are common. Due to lack of information on pre-control endemicity levels of these diseases in Tombel health district of Cameroon, evaluation of disease control programs became difficult. We therefore designed this study to provide the scientific world and control program evaluators a reference point for their activities in this area. In a cross-sectional study involving 1083 participants (580 males and 503 females), finger-prick blood and fresh stool samples were collected from 1083 and 532 participants respectively. Blood and stool samples were examined using Geimsa stain and the Kato-Katz faecal technique to respectively diagnose P. falciparum and STHs. Questionnaire was used to obtain demographic, behavioural and socioeconomic data of the participants. Correlation models were used to test for association. The prevalence of STHs, malaria and co-infections were 10.2%, 33% and 7.1% respectively. Significantly higher prevalences of P. falciparum were recorded in the thick canopied forest communities of Ebonji and Ndom health areas as compared to those from less density forest areas. Prevalence and intensities of P. falciparum and STHs were higher in children 15 years and below. Correlation models revealed that living in typical rural communities and belonging in the age group 15 years and below were strongly positively associated with STHs infection(r = 0.62 and r = 0.85 respectively)and P. Falciparum infection (r = 0.71 and r = 0.80 respectively). Tombel health district was found to be hyperendemic for P. falciparum, with relatively low prevalence of STHs. Thus, still remain a call for concern. These baseline data will therefore serve as a reference point for the evaluation of various control programs targeting these infections.**

Keywords: **Endemicity, Tombel health district, co-infection, prevalence, soil transmitted helminths, P. falciparum.**

1. INTRODUCTION

Neglected tropical diseases (NTDs) and malaria blight the lives of a billion people worldwide and threaten the health of millions more. These close companions of poverty weaken impoverished populations, frustrate the achievement of health in the Millennium Development Goals and impede global public health outcomes. Amongst these NTDs are soil transmitted helminths (STHs). Today, between one-quarter and one third of Sub-Saharan Africa's population is affected by one or more STH infections with children, especially school-aged children, disproportionately affected. These

neglected infections are most often found co-existing with malaria which causes 300-500 million cases of illness and 1-3 million deaths annually. A leading cause of morbidity and mortality, especially among the pregnant women and children under the age of five years, Africa accounts for 90% of the global burden of malaria [1]. Studies have shown that STHs and malaria parasites show similar geographic distribution with very common co-infections [2], [3]. Such areas are present in Cameroon and account for increased morbidities and associated outcomes in various risk groups [4], [5], [6]. Some environmental factors like stagnant water, bushes, unhygienic conditions, alternating wet and dry seasons, high temperatures and low altitudes favour the growth and transmission of malaria parasites and STHs [7], [8]. Since farming marks an undeniable source of livelihood for a majority of people living in such rural communities, agricultural activities have hence been found associated with the increase transmission of both malaria parasites and STHs, [9], [10]. Disease control programs under the auspices of the ministry of Public Health have set up strategies to combat these infections. Examples of such programs are free distribution of long-lasting insecticide treated mosquito nets to all age groups, as well as free treatment of malaria among children below 5 years of age and the national deworming program against STHs that distributes Mebendazole to pre-school-age and school-age children since 2007 [11]. It is therefore very crucial that information on the prevalence and intensities of malaria parasites and STHs be provided in Tombel health district so as to facilitate future assessment of the impacts of these control programs on the morbidities associated with these diseases and to identify areas of priority when resources are limited.

2. METHODS

Ethics statement:

Authorisation to carry out this research was obtained from the National Ethics Committee for research involving human participants, while administrative authorisation was obtained from the Regional delegation of Public Health for Southwest region. Additionally, permission to carry out the study was sought from the local authorities of Tombel Health District. Before visiting each health area, the district medical officer and local chiefs were informed about the aim and procedures of the study. For each adult participant/guardian of children, written informed consent was obtained while minors gave oral assent. To ensure optimum confidentiality, both questionnaire data and parasitological results were coded, and no individual names were used.

Study area and design:

The study was conducted in Tombel health district, which is a town and community in the Southwest region of Cameroon, located in the northern part of the Mungo Valley at $4^{o}44^{'}47^{"}$N $9^{o}40^{'}13^{"}$E .The town is traditionally a 'Bakossi' people's land but has a great deal of the Bamileke population among other ethnic groups. Thirteen communities under five health areas were randomly selected. These health areas include; Ebonji, Tombel, Nyasoso, Ndibendjock and Ndom. These areas are made up of a population of farmers who depend largely on what they cultivate for their livelihood with their main cash crops being cocoa, plantains and cocoyam.

This was a cross-sectional study carried out between the months of April and July 2014 to assess the endemicity of malaria and soil-transmitted helminthes in Tombel health district of Cameroon. In each community, a social mobilizing team was organized with community health workers and community members to sensitize the people on the aim and the benefits of the research. The research methods included the use of questionnaire for socio-demographic information, and collection of blood and stool samples for laboratory investigations. The sample size was determined using the formula $n=z^2p(1-p)/m^2$ [12].Where n = required sample size, z = confidence level at 95% (standard value of 1.96) p = estimated prevalence 1-p = expected non-prevalence m = margin of error at 5% (standard value of 0.03) or maximum tolerable error. From the formula, the optimum sample size estimated was 1,067 participants but due to a higher turnout of participants a total of 1,083 were sampled.

Study procedures:

Local district authorities were briefed on the aims, procedures and indirect benefits of the research and which of the health areas had been selected to be included in the survey. Social mobilizing teams ('Town Criers') were contacted to inform community members to assemble in their various community halls on the day of the study. The entire research team visited one health area per day during which geographical coordinates of each community were recorded using a GPS (Global Positioning System). All consented participants were subjected to a questionnaire as well as parasitological examination of their blood and stool samples. Children were assisted by their parents or guardians in providing the necessary information.

Well-labeled screw-cap stool containers with individual identification numbers were distributed to all study participants to provide fresh stool samples. These stool samples were placed in sample collection boxes containing ice bags to avoid hookworm egg degeneration. Also, finger-prick blood samples were collected for malaria parasites identification. Both thick and thin blood films were prepared on the same microscope slide. Two slides of blood films were prepared for each participant in case of breakage or loss. The slides were then air-dried and transported to REFOTDE (Research Foundation for Tropical Diseases and the Environment), while the stool samples were transported to a field laboratory for analysis.

Sample processing and Examination:

Stool samples were prepared using the Kato-Katz faecal thick smear technique according to a previous study [13]. The stool samples were analyzed one hour after collection, slides were prepared according to WHO guidelines and helminth eggs identification done based on their morphology **[**14]. Since the template used had a capacity of 41.7 mg (0.04g), the number of eggs counted per slide was subjected to a multiplying factor of 24 to get the number of eggs per gram of stool. That is, Number of eggs per slide (0.04g) X 24 = eggs per gram of stool (epg). The intensity of STH infections was categorized based on the WHO standard [15].

Blood films for *P. falciparum* detection and quantification were prepared using the standard Geimsa staining procedures for malaria parasite detection [13]. Blood films were examined under the microscope and parasites were quantified by counting the number of trophozoites against 200 white blood cells and gametocytes against 500 white blood cells. The parasites were then reported as per µl of blood by subjecting the number of parasites counted to a multiplying factor of 40 **[**16]. Malaria parasitaemia was classified as Low: <1000 parasites/ µL, Moderate: 1000-4999 parasites/ µL and High: ≥5000 parasites/ µL [17].

Statistical Analysis:

Data were entered into EpiInfo version 3.5.3 and exported to SPSS version 20.0 for analyses and statistical significance threshold was set at $p<0.05$. Chi-square test was used to compare proportions of individuals positive for parasites with respect to age group, gender and health area and Mann-Whitney test was used to compare means between age groups and gender. Pearson correlation was also used to test the strength of association between variables.

3. RESULTS

Table I: Demographic characteristics of study participants

Age group	**Sex**		**Total (%)**
	Male (%)	Female(%)	
≤ 15	240 (56.1)	188 (43.9)	428 (39.5)
16 – 25	66 (48.2)	71(51.8)	137 (12.7)
26 – 35	69 (44.5)	86 (55.5)	155 (14.3)
36 – 45	70 (55.1)	57 (44.9)	127 (11.7)
>45	135 (53.6)	101 (46.4)	236 (21.8)
Total	**580**	**503**	**1,083**

Out of the 1,083 participants, 532 stool samples were provided and the prevalence of STH was 10.2%. The STH species found were *Trichuris trichiura* (7.1%); *Ascaris lumbricoides* (3.9%) and *N. americanus* (0.6%). The mean egg counts for these parasites were 398.9epg, 180epg and 64epg for *A. lumbricoides, T. trichiura and N. americanus* respectively. Nyasoso health area (17.4%) was the most significantly infected ($p < 0.001$) (Fig. 1), with a strong positive association ($r = 0.62$). Also age group was found to be strongly positively associated ($r = 0.85$) with infection, and children below 15 years of age were most infected (7.0%) than the other age groups ($p < 0.001$) (Fig. 2). This age group related prevalence was found to be significantly reduced (2.1%) among children who regularly take anthelminthic drugs during de-worming campaigns ($p<0.001$). Prevalence in males (6.2%) was also significantly higher ($p = 0.003$) than in females (3.6%). Infections with *Ascaris* species were of low intensity (1 - 4999epg), Trichuriasis ranged from low (1-999epg) (7.1%) to moderate infection (1000-9999epg) (0.2%), while all hookworm infections were of relatively low intensity (1-1999epg). Parasite burden was higher in males than in females but the difference did not reach statistical significance.

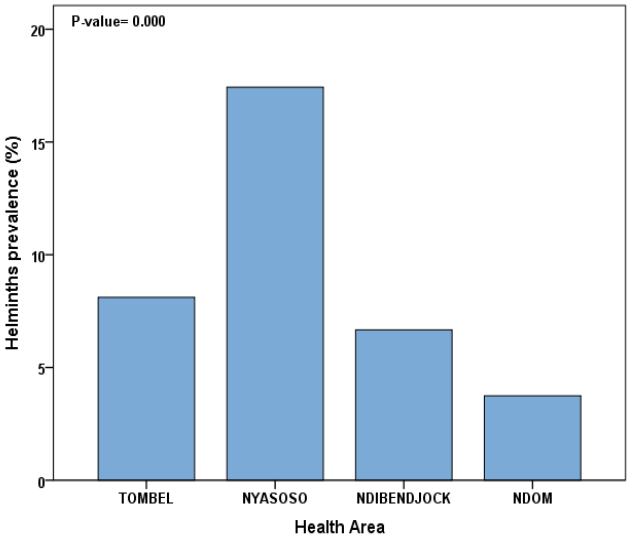

Fig 1: Health area prevalence of STH

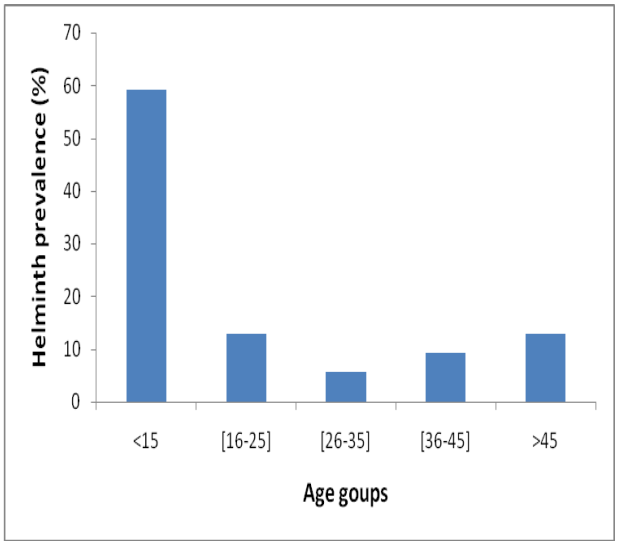

Fig 2: Age-group prevalence of STH

Out of the 1,083 participants under survey, 358 (33%) were positive for *P. falciparum*. Health area was found to be strongly positively associated (r = 0.70) with *P. Falciparum* infection, with Ebonji health area being significantly (p <0.001) most affected (48.5%) (Fig.3). Also the prevalence of *P. falciparum* was higher in the male population (34.2%) than in the female population (31.6%) and children below 15 years (17.6%) were found to be significantly (r = 0.85, p < 0.001) most infected (Fig.4), and there was a strong positive association (r = 0.85) between age group and infection. The mean parasite load was 849.1 and was higher in females (973.3) than in males (749) but with no statistically significant difference (P = 0.876). This prevalence was found to be significantly lower (13.6%) among those who use mosquito nets.

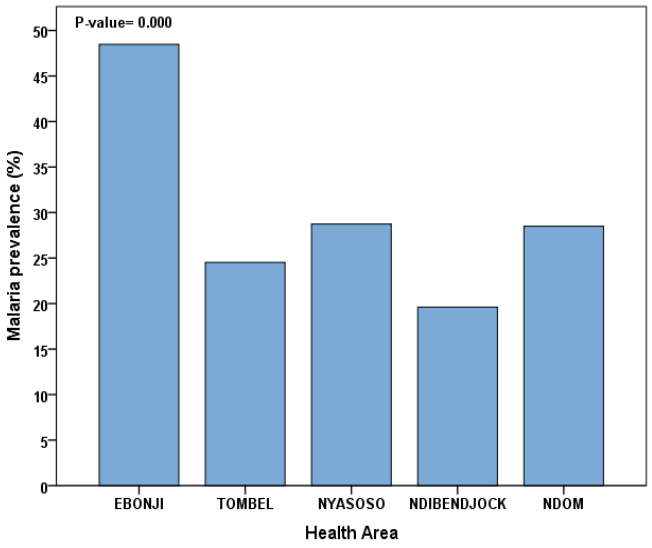

Fig 3: Health area prevalence of *P. falciparum*

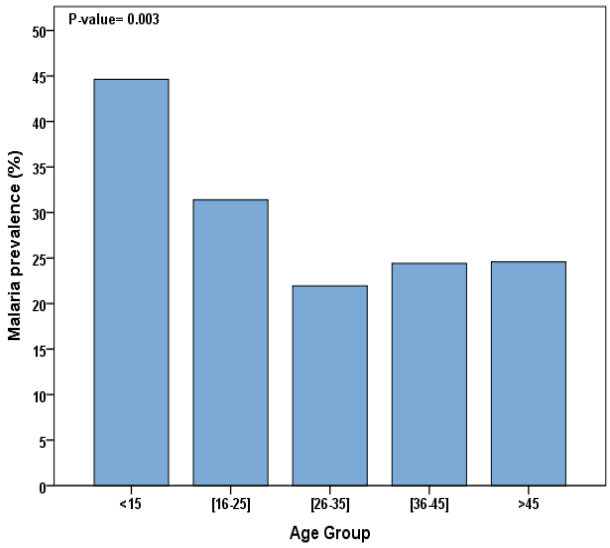

Fig 4: Age group prevalence of *P. falciparum*

Prevalence of Co-infection:

The prevalence of *P. falciparum* and STHs co-infection was 3.8%, and was higher in males (4.8%) than in females (2.5%) but with no statistical significance (p = 0.166). Co-infection was highest among the ≤15 years age group (4.3%) but the difference was not also statistically significant (p = 0.854).

Entering the geographical coordinates unto MapInfoPro™, a GIS (Geographic Information Systems) program produced an epidemiologic map of the parasitic infections under survey in Tombel health district of Cameroon (Fig.5).

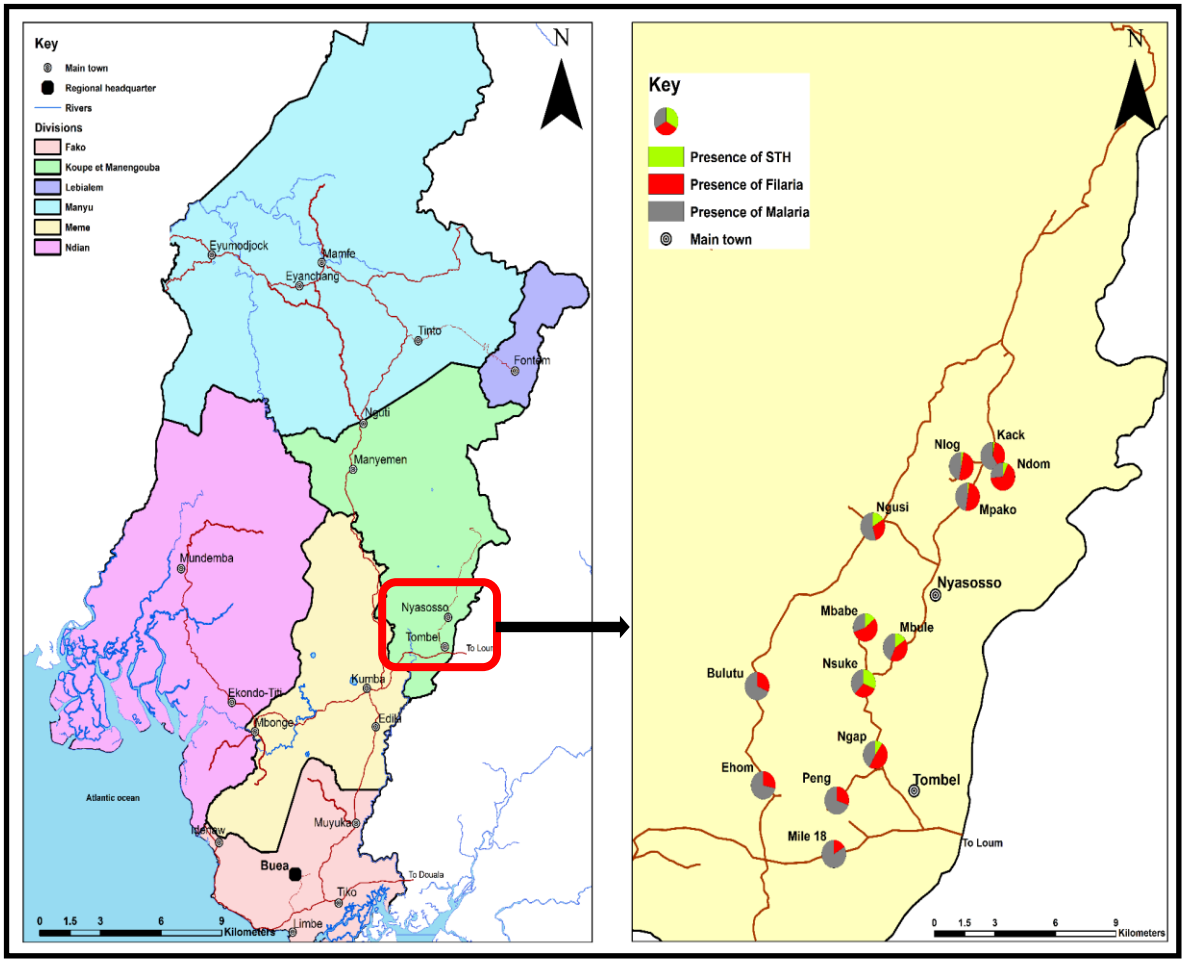

Fig 5: Epidemiologic map of major parasitic infections in the Tombel health district of Cameroon

4. DISCUSSION

This study was conducted between the months of April and July 2014. To the best of our knowledge, this is the first survey targeting Malaria parasites and STH infections in Tombel health district of Cameroon. A STH prevalence of 10.2% was registered in this area and is much lower than that observed from previous studies conducted in other health districts like Dschang, Mfou and Mbonge [18], [19], [13]. From the above studies especially that conducted in Dschang, the inhabitants of these areas have the habit of rearing pigs near their homes which help in shedding more parasites in the environment, and also they have shallow latrines with the waste products continuously being discharged into streams during the rainy season, and the water is in turn used for agricultural and domestic purposes [18]. Conversely, the inhabitants of Tombel health district have high access to pipe-borne water. Trichuriasis was the most prevalent STH infection followed by Ascariasis which corroborates with previous reports in some parts of Cameroon [20], [18]. Trichuriasis has also been reported second most prevalent STH infection in surveys carried out in the Littoral, North-west, South and Southwest regions of Cameroon [21], [22]. Trichuriasis is thus considered an urban-area infection in the tropics and sub-tropics [23]. The relatively high prevalence of trichuriasis and ascariasis in this study and previous studies could be due to the fact that these two helminths have similar modes of transmission and can be favoured by behavioural factors [21] so their infections may be due to promiscuous defecation and contamination of the soils by individuals. Eggs of these helminths can persist for some time in the environment in dust, soils and on vegetables resisting harsh conditions. This therefore implies that factors that predispose individuals to one species will increase the risk of being infected with the other species [24]. This study recorded a very low prevalence of ancylostomiasis while other studies in some parts of the country recorded no case [19], [13] but other studies as well recorded higher prevalence [18], [20], [25]. Low prevalence or absence of ancylostomiasis could be associated with the soil type of the area which may not be favourable for larval development or to the time between stool processing and microscopy, because hookworm eggs start to degenerate after about 45 minutes of Kato-Katz preparation [26]. It could also be due to the fact that prevalence of hookworm infection is generally low in the Southwest region of Cameroon [13]. STH infection was significantly higher in children below 15 years.

The prevalence of *P.falciparum* in the study area was 33% and was higher in children below 15 years. This is consistent with results obtained in Buea health district of Cameroon, Ethiopia, and the Mount Cameroon area [27], [25], [17]. This is because children in this age group have not developed a stronger immunity against the malaria parasites as well as their poor awareness of malaria prevention measures. On the other hand this prevalence is lower than that reported from previous studies in the South West region of the country [7], [28], [29]. This decrease in malaria parasite prevalence could be due to the recent implementation by the Cameroon Ministry of Public health of control and preventive measures such as distribution of insecticide-treated bed nets (ITNs) and long-lasting insecticide-treated bed nets (LLINs) throughout the country [28]. This might therefore imply that the prevalence of malaria was higher in this area before the mass distribution of mosquito nets as studies have shown that the use of LLINs reduces the incidence of malaria [30], so repeated distribution of LLINs and increasing compliance to the use of mosquito nets in Tombel health district and all over the country would reduce incidence and mortality from malaria. A lower prevalence of *P. falciparum* could also be due to the shift from treatment of malaria with sulphadoxine-pyrimethamine due to increasing resistance to the more effective artemisinin-based combination therapies as of 2004 in Cameroon [31]. Ebonji health area had the highest prevalence because it is characterised by a good number of marshy areas, stagnant water bodies and bushes near homes which serve as good breeding sites for mosquitoes. The semi-urban nature of some health areas like Ndibendjock and Tombel could be accountable for their low malaria parasites prevalence. Urbanization leads to improved infrastructure, good-quality mosquito-protected houses, increased access to medical care and reduced mosquito breeding sites [32]. Important to note is that some of these health areas have a good number of modern block houses with screens on doors and windows which reduce the number of crevices for mosquito entry and exit. Studies have shown that prevalence of malaria parasites among people living in wooden plank houses is significantly higher than among those living in non-plank houses [7]. Also, as urbanisation emerges there is an influx of people from the rural areas which leads to a higher ratio of humans to mosquitoes and is thought to reduce human biting rates [33]. This survey also reported significantly higher malaria parasites prevalence in the male population than in the female population and this could be explained by differences in behaviour as men often more involved in long night activities, spend long hours of work in areas like construction sites and plantations while women are more involved with near-home activities and domestic chores. Apart from these activities that expose men to mosquito bites, studies have shown that their susceptibility could be dependent on the male sex hormone- testosterone [34].

5. CONCLUSION

With the efforts and resources put in place by the Cameroon's Ministry of Public Health for the fight against parasitic infections, *P. Falciparum* and soil transmitted helminthes infections still remain the focal points for high priority interventions. Tombel health district was thus found to be hyper-endemic for *P. Falciparum* with relatively low prevalence of STHs. These data reveal that environment related factor and age group are strongly associated with both *P. Falciparum* and STHs infections. Since School aged children are the most affected, a scale up on the control and intervention strategies among this age group could go a long way to reduce prevalence and parasite burden. These data will therefore serve as the baseline for the evaluation of various control programs put in place for the control of these parasitic infections in Tombel health district of Cameroon.

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