Overlapping distribution of Plasmodium falciparum and soil transmitted helminths in a malaria hyper-endemic region, North-Central Nigeria

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Abstract

Objective: Malaria and soil transmitted helminths (STH) are endemic in many resource poor communities in sub-Saharan Africa (SSA) and there appears to be a synergistic relationship among the duo culminating into an overlap in prevalence and intensities. Methods: Peripheral blood smears and fresh stool samples were obtained from consenting individuals in the study population. Routine microscopy examination was conducted on valid samples. Malaria parasitaemia in thick film was estimated by counting the number of parasites per 200 white blood cells (WBC) and the parasite count/μL was determined by a fixed value of 8000 WBC. Fresh stool samples collected were fixed in 10% forma-saline solution and immediately processed for intestinal parasite egg identification. Intensity of helminth eggs in stool samples was assessed using the Kato-Katz technique. Prevalence and intensity of infections between ages and sexes were tested using the Chi-square (χ²) and One-way analysis of variance (ANOVA) respectively. For each value, the 95% confidence interval (95% CI) (P < 0.05) was calculated. The association between STH prevalence and malaria mean parasitaemia load was assessed with student independent-t-test.

Results: Seven hundred and thirty seven (737) individuals comprising 287 (38.9%) males and 450 (61.1%) females participated in the study. Ascaris lumbricoides determined the increase prevalence of Plasmodium falciparum (198) (OR = 2.59; 95% CI: 1.894 – 3.545). The intensities in the associations were highly significant (P < 0.001). Malaria, ascariasis and trichuriasis prevalence decreased with age and therefore exhibited marked age dependency patterns. However, only hookworm spread and prevalence increased with age. Overlapping distribution occurred in all infections with respect to the different age groups.

Conclusions: In endemic communities like the present study area, a stable but mild infection intensities is observed all year round. Intervention and mass awareness are however advised to forestall continuous cycling and transmission of malaria and STH.

1. Introduction

Human infections resulting from Plasmodium falciparum (P. falciparum) and soil transmitted helminths (STH) has been reported in several literatures to exhibit synergistic associations[1-4]. STH infects more than one third of the continent’s population at any one time[5]. Intestinal helminth infection and malaria places much burden on its victims and has been identified to be most endemic in rural and urban regions of sub-Saharan Africa (SSA)[6,7]. The duo of malaria and helminthiasis has been widely reported to be the most prevalent infectious diseases affecting humans in the tropics and it is estimated that globally, 40% of the world’s population is at risk of malaria, and about 90% of the malaria infected population live in SSA[8-10] while helminths rank among the most common human infections responsible for disability, morbidity and mortality[11,12]. Disability adjusted life years (DALYs) for malaria is estimated at 35.4 million[13]. Globally, hookworms (Necator americanus and Ancylostoma duodenale) are estimated to affect 1.300 million people[5], 1.472 million persons harbour Ascaris lumbricoides (A. lumbricoides), and about 1.049 million have Trichuris trichiura (T. trichiura).
inflicts the largest burden[7] and hookworm infection is amongst the outcomes and antagonistic interactions [25-27]. In Lagos, Nigeria cognitive impairment, iron-deficiency, anemia, growth retardation, are particularly vulnerable, with heavy infections associated with women usually fall victim of immune suppression[34-36]. The present susceptibility to malaria infection during pregnancy, because gravid infection. Helminths can therefore either improve or heighten malaria or malaria may influence the clinical consequences of helminths infection may alter susceptibility to clinical malaria which may work in either direction usually occurs, i.e. helminths infection may alter susceptibility to clinical malaria or malaria may influence the clinical consequences of helminths infection. Helminths can therefore either improve or heighten malaria severity and therefore share the same spatial distribution[4,24,30-33]. Recent studies suggest that helminth infections may increase susceptibility to malaria infection during pregnancy, because gravid women usually fall victim of immune suppression[34-36]. The present study will explore if synergy does exist and identify points of overlap between P. falciparum infections and STH infections among individuals attending basic health centres in a malaria hyper endemic region of Nigeria.

2. Materials and methods

2.1. Data and sample collection

The study was conducted between October 2014 and May 2015 (8 months). Samples for this study were collected from voluntary donors in two major state government owned health centres in Ilorin viz; Civil Service hospital, GRA Ilorin and Children Specialist hospital Centre Igboro, Ilorin, North-Central, Nigeria. The two health centres selected for this study are strategically located in the heart of the town to provide primary health facilities to civil servants and their dependants as a government funded project. Study participants were randomly selected using questionnaire to identify patients who have not had episodes of malaria infections and anthelmintic drugs intervention in the last three months respectively (inclusion and exclusion criteria). Peripheral blood and fresh faecal samples were collected from volunteer patients through the clinic laboratories beginning from 8.00 a.m. to 11.00 a.m. daily except on Saturdays and Sundays. Pre-labeled sterile plastic bottles and instructions on how and when sample should be taken were given to volunteers for stool samples. Peripheral blood samples of respectful donors were taken on sterile slides. Items in the questionnaire includes; biodata, duration of last visit to the hospital, complaint then, type of medication prescribed, reasons for coming for treatment etc.

2.2. Ethical consideration

We sought the consent and approval of the ministry of health ethical review committee before undertaking the study. Each volunteer’s permission was requested and approved and in cases where minors were involved, their care-givers and parents’ consent was also obtained before sample collection.

2.3. Malaria gold standard technique

Thick and thin blood films preparation as described by Gilles (1993)[37] was employed for the study. The ball of the middle finger i.e. the third finger is raised up horizontally and gently palpatated and wiped clean with an alcohol-lightly-pre-soaked cotton wool (The big toe was used in infants). A sterile lancet was used to puncture the ball of the finger or big toe (infants) and gentle pressure is applied to squeeze out a drop of blood on the slide for thin smear and two to three drops on another part of the same slide (about 1 cm away) to make thick smear. A clean alcohol-lightly-pre-soaked cotton wool is then applied to the punctured finger ball to allow blood clot. The thin blood film was prepared by placing the smooth edge of a spreader slide on the drop of blood, adjusting the angle between slide and spreader to 45°, then allowing the blood to spread along the entire width of the spreader slide and gently smearing the blood with a swift and steady sweep along the surface. The slides were then air-dried, and then the thin film was washed in absolute methanol. Thick and thin malaria smears were stained with 3% Giemsma stain for an upward of 45 min in a staining trough. The slides were then rinsed under mild running tap water and allowed to air-dry before they were examined under oil immersion microscope. The presence of any asexual blood stage parasite was declared as malaria positive. Smears were declared negative after reading 200 fields.

2.4. Stool sampling technique

Pre-labeled, wide-mouthed screwed capped plastic containers were distributed to the consenting blood donors for stool samples. Individuals were taught how to collect fresh urine-free faeces. Fresh stool samples collected were fixed in 10% forma-saline solution and immediately transported to the Parasitology Laboratory of the Zoology Department, University of Ilorin where they were processed for parasite identification. Intensity of STH parasite infection was assessed using the Kato-Katz technique[38]. Stool samples of donors who met the inclusion criteria were screened for the presence of STH using standard procedure for the identification of eggs. For each stool sample, two slides were prepared and examined by different laboratory scientist blinded to each other’s results. From both results, an average of total egg counts was determined and recorded. Intensity of infection was estimated from the number of eggs per gram of faeces (epg). Egg count of parasite species was classified as light, moderate or heavy infections in accordance with WHO (2002) criteria[39]. Samples with A. lumbricoides > 50 000 eggs per gram (epg), hookworm > 4000 and T. trichiura >10000 epg, were considered as heavy infections.
2.5. Intensity of infection

2.5.1. Malaria intensity
Malaria parasitaemia was estimated by first counting the number of parasites per 200 white blood cells (WBC) in a thick blood film and then calculating the parasite count/μL from the total white blood cell count/μL. The value of 8000 WBC was generally assumed [13]:

\[
\text{Number of observed asexual parasites} \times \frac{\text{total WBC count}/\mu L}{200}
\]

2.5.2. STH Intensity
The intensity of the triads of soil transmitted helminths was estimated by multiplying counted eggs for each parasite by 24 to obtain the number of eggs per gram of faeces after the methods of Cheesbrough [40] and Endriss et al. [41].

2.6. Data analysis
All statistical analysis were performed using Epi Info Database Package (Centers for Disease Control and Prevention, Atlanta, GA) and Statistical Package for Social Sciences, version 16.0 for Windows (SPSS Inc. Chicago, IL, USA) version 16. *P. falciparum* infection was used as the exposure variable and the outcome variable was determined by the egg load of STH stratified with categorical thresholds. The prevalence, intensity of malaria and infection between ages and sexes were tested using the \(\chi^2\) and One-way ANOVA tests respectively; the \(P\)-value level of significance was assigned at \(P < 0.05\). The association between prevalence of STH and mean parasitaemia load of malaria was assessed with student independent-t-test.

3. Results
Seven hundred and thirty seven (737) individuals were targeted for the study, but only 700 blood samples were valid. Thirty seven (37) individuals did not provide valid blood samples and were therefore excluded from the study. Only 696 individuals with correctly filled questionnaire and urine-free stool samples were examined for STH. Forty one (41) submitted questionnaires but provided no stool samples and were also excluded from the study (Figure 1).

### Table 1
Overall prevalence of malaria and STHs in the study (%).

| Sex | Malaria (700) | Ascaris (696) | Hookworm (696) | Trichuris (696) |
|-----|---------------|---------------|----------------|----------------|
|     | No | Yes | No | Yes | No | Yes | No | Yes |
| Male | 123 (37.5) | 148 (39.8) | 151 (39.2) | 120 (38.6) | 146 (42.6) | 124 (36.0) | 142 (38.2) | 128 (39.5) |
| Female | 205 (62.5) | 224 (60.2) | 234 (60.8) | 191 (61.4) | 259 (64.0) | 167 (57.4) | 230 (61.8) | 196 (60.5) |
| Total | 328 (47.0) | 372 (53.1) | 385 (55.3) | 311 (44.7) | 405 (58.2) | 291 (41.8) | 372 (53.4) | 324 (46.6) |
| \(P\)-value | 0.538 | 0.864 | 0.080 | 0.719 |

No: Uninfected; Yes: Infected.

### Table 2
Parasitaemia load of *P. falciparum* infections stratified with respect to age and sex

| Age (years) | Sex | No. Exam | No. infect | Mean ± S.E.M | Total Parasitaemia across age groups |
|-------------|-----|----------|------------|--------------|-------------------------------------|
| 5           | Male | 106      | 32         | 4251.25 ± 642.513 | 4795.85 ± 367.568 4067.03 5524.67 |
|             | Female | 74      |           | 5031.35 ± 447.259 |
| 6-10        | Male | 95       | 41         | 5743.41 ± 665.763 | 5818.11 ± 436.827 4950.78 6685.44 |
|             | Female | 54      |           | 5874.81 ± 584.174 |
| 11-20       | Male | 112      | 48         | 4333.33 ± 642.513 | 3921.79 ± 370.952 3186.72 4656.85 |
|             | Female | 64      |           | 3613.13 ± 542.444 |
| 21-30       | Male | 126      | 53         | 3680.75 ± 624.837 | 4105.40 ± 373.653 3365.89 4844.90 |
|             | Female | 73      |           | 4413.70 ± 458.818 |
| 31-40       | Male | 228      | 84         | 2345.71 ± 395.748 | 1988.95 ± 231.311 1533.16 2444.74 |
|             | Female | 144     |           | 1780.83 ± 283.955 |
| 41-50       | Male | 13       | 3          | 0.000 ± 0.000    | 1944.62 ± 1380.877 -1064.06 4953.29 |
|             | Female | 10      |           | 2528.00 ± 1772.369 |
| 51          | Male | 20       | 10         | 3516.00 ± 1758.084 | 3874.00 ± 1257.601 1241.81 6506.19 |
|             | Female | 10      |           | 4232.00 ± 1886.361 |
| Total       | Male | 700      | 148        | 3715.13 ± 251.874 | 3676.91 ± 157.077 3368.52 3985.31 |
| Female | 224 |           |           | 3652.77 ± 201.161 |
| \(F\) value | 0.037 | 0.847 |           | 13.788 | < 0.001 |
The distribution of the prevalence patterns of malaria and STH with respect to sexes is as depicted (Table 1). None of the associations were statistically significant with respect to sexes (P > 0.005). This further shows that malaria and STH infections are not sex biased.

The intensity of *P. falciparum* infections stratified with age and sex of the donors indicated that stratification across age groups and sex were comparable, hence their association were not statistically significantly different (F = 0.037, P = 0.847). On the other hand, the quantification of total parasitaemia across age groups showed a significant statistical difference (F = 13.788, P < 0.001). However, there was uneven mean parasitaemia load across age groups, for instance, age 6–10 years had the highest total parasitaemia mean (5818.11 ± 436.827; 95% CI 4950.78–6685.44) and age 41–50 years had the lowest mean parasitaemia load (1944.62 ± 1380.877; 95% CI –1064.06–4953.29) (Table 2).

A significant association between malaria infection, ascariasis, hookworm and *Trichuris* infections exhibited marked age dependency in infection patterns (Figure 2). Only hookworm spread and prevalence increased with age. There was a significant drop in infection rate as age increases for malaria, *Ascarisis* and *Trichuriasis*.

| Parasite species | Variable | N | Positive | OR (95% CI) | P-value |
|------------------|----------|---|----------|-------------|---------|
| *A. lumbricoides* | Uninfected | 215 | 155 | -- | -- |
|                  | Light    | 106 | 198 | 2.591 (1.894–3.545) | < 0.001 |
|                  | Moderate | 0   | 0   | -- | -- |
|                  | Heavy    | 0   | 0   | -- | -- |
| Hookworm         | Uninfected | 203 | 187 | -- | -- |
|                  | Light    | 99  | 102 | 1.118 (0.796–1.572) | 0.519 |
|                  | Moderate | 18  | 64  | 3.860 (2.206–6.753) | < 0.001 |
|                  | Heavy    | 0   | 0   | -- | -- |
| *T. trichiura*   | Uninfected | 210 | 147 | -- | -- |
|                  | Light    | 81  | 108 | 1.905 (1.333–2.722) | < 0.001 |
|                  | Moderate | 29  | 98  | 4.828 (3.033–7.684) | < 0.001 |
|                  | Heavy    | 0   | 0   | -- | -- |

1. Positive for malaria is the reference category. Classification of helminth intensities of infection: *A. lumbricoides* – light, 1–4999 epg; moderate, 5000–9999 epg; severe, 10000 epg. Hookworm – light, 1–1999 epg; moderate, 2000–3999 epg; severe, 4000 epg. *T. trichiura* – light, 1–999 epg; moderate, 1000–9999 epg; heavy, 10000 epg.[42]

| Malaria Intensity | No. Infected | Mean ± SEM | 95% CI | P-value |
|-------------------|--------------|------------|--------|---------|
| Malaria           | 372          | 3676.91 ± 157.077 | 3368.52–3985.33 | < 0.001 |
| Ascaris + Malaria | 198          | 7264.44 ± 215.941 | 4544.810–5606.543 | < 0.001 |
| Hookworm + Malaria| 168          | 7346.19 ± 221.701 | 4301.085–5558.391 | < 0.001 |
| Trichuris + Malaria| 206         | 213.73 ± 213.384 | 4600.274–5728.440 | < 0.001 |
| Mal+Asc+Hkw+Tric  | 149          | 7481.07 ± 232.581 | 7036.677–7925.471 | < 0.001 |

### 4. Discussion

A significant association between malaria infection, ascariasis, hookworm and *Trichuriasis* were found; intensity of malaria increased with multiplicity of STH infections. The observed co-occurrence of parasite species in the same individuals may be attributed to similar adaptation of parasites to a common environmental niche.[43,44] Mwangi et al.[2] also noted co-infections of *Plasmodium* and *P. falciparum* infections and that it has supposed clinical importance. Brooker et al.[45] reported that age-stratified epidemiologic studies in several malaria endemic communities indicate that the prevalence of asymptomatic *Plasmodium* infections increases in early childhood as it was also observed in the present study, and probably begins to decline as a result of gradual acquisition of immunity[8,11,46]. Ashford et al.[47] and Brooker et al.[45] insinuated that the precise rate and age at which immunity is acquired is exposure dependent, but in areas of stable transmissions, (like the area for the present study) infections in adulthood are generally low. Earlier studies in several malaria and STH endemic communities asserted that the occurrence of co-infection is usually predicated on the overall prevalence of individual species and the degree of association between different species. The aforementioned trend was observed in the present study[21,26,48].
Therefore, if infection with *P. falciparum* and helminths are independent, occurrence of co-infection is simply determined by the relative frequency of individual species. Thus, the age patterns of co-infection will depend on the age-specific prevalence rates which can be predicted by simple probability[25]. However, with co-infection being either synergistic or antagonistic, the occurrence of both parasites may appear significantly different from that predicted by individuals’ chance with either malaria or STH infection[24,49,50]. Biologic associations may enhance the survival of both infections (Brooker et al. 2007), whereby the presence of one species promotes or inhibits the establishment and/or survival of the second species. The most significant is the effect of chronic helminthiasis and malaria on PCV leading to anemia and iron deficiency[45]. From our study, we observed varying overlap interplay between malaria and STH in the studied populations. The concept of overlap does occur in distribution, ecological transmission risks and clinical presentation leading to populations being at increased risk of co-infections[44,45,51]. Our findings also lay emphasis on the complex nature of interactions between STH and malaria despite variability in predilection site. In another study, the authors remarked that such interactions may promote significant associations between infection status, socio-ecological settings, and host inflammatory and micronutrient status with some form of benefits to the host[33].

The downregulation of Th1 immune response during STH infection has been substantiated as it may hamper the development of vaccine-induced protective immunity against malaria[8,46]. In the present situation, increasing prevalence of the trio STH led to an increase in mean plasmodium parasitaemia. Degarege et al.[8] in their review stressed that *Plasmodium* infection induces pro-inflammatory cytokines which subsequently lower the production of erythropoietin that is responsible for red blood proliferation, again another reason probably for the interplay observed in our study, while on the other hand STH induces anti-inflammatory cytokine IL-10 that will downregulate the pro-inflammatory cytokines[52-54].

In view of recent findings, we recommend that the treatment of malaria should go parry-par-sue with that of soil transmitted helminths in order to achieve effective control. Since the prevalence of hookworm infection increases with age, awareness programmes should be focused on older-age groups in order to intimate them with the risk factors that can predispose them to further re-infection. Further studies are recommended to establish age-dependent risks associated with this overlap.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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