Seroepidemiology of helminths and the association with severe malaria among infants and young children in Tanzania

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Abstract

The disease burden of Wuchereria bancrofti and Plasmodium falciparum malaria is high, particularly in Africa, and co-infection is common. However, the effects of filarial infection on the risk of severe malaria are unknown. We used the remaining serum samples from a large cohort study in Muheza, Tanzania to describe vector-borne filarial sero-reactivity among young children and to identify associations between exposure to filarial parasites and subsequent severe malaria infections. We identified positive filarial antibody responses (as well as positive antibody responses to Strongyloides stercoralis) among infants as young as 6 months old. In addition, we found a significant association between filarial seropositivity at six months of age and subsequent severe malaria. Infants who developed severe malaria by one year of age were 3.9 times more likely (OR = 3.9, 95% CI: 1.2, 13.0) to have been seropositive for filarial antigen at six months of age compared with infants who did not develop severe malaria.

Author summary

In this paper, we used a multiplexed, serologic assessment to identify children with previous or current exposure to or infection with filarial parasites or S. stercoralis (a soil transmitted helminth), enhancing our understanding of co-infections in early childhood. We identified an increasing prevalence of filarial antibodies over time in a population of children as young as 6 months old. In addition, we found a significant association between filarial seropositivity at six months of age and subsequent severe malaria.
Introduction

Parasitic helminths and malaria are both highly prevalent globally and overlap extensively in tropical areas [1–3]. In 2016, more than 216 million cases of malaria were estimated to occur with 89 percent of cases occurring in Sub-Saharan Africa [4]. Nearly all cases of severe malaria are due to infection from *Plasmodium falciparum* [5], although *P. vivax* is increasingly regarded as a potential cause of severe malaria infection [6].

Lymphatic filariasis, caused exclusively by the helminth *Wuchereria bancrofti* in Africa, affects an estimated 120 million to 130 million persons globally [7]. *W. bancrofti* is highly endemic throughout Tanzania and especially in the northeast region [8–10], with an estimated 34 million people at risk of filarial infection and 6 million people affected by filariasis [11]. Filariasis has an overlapping geographical distribution with malaria in Tanzania [12–14] where it shares the same Anopheles vector as *P. falciparum* [15]. Co-infection is frequent [16, 17], with between 0–11% of school age children co-infected, depending on local ecology, in one study from Mvomero District, Tanzania [16]. In 2000 the Tanzanian National Lymphatic Filariasis Elimination Programme (NLFEP) was launched to distribute ivermectin and albendazole, highlighting the disease burden in this region [18], however the program is limited to individuals greater than 5 years of age.

The interaction of helminth and malaria co-infections is not well understood, and studies have had contradictory conclusions related to the inter-infection effects [19–23]. Differences in *W. bancrofti* prevalence by age have been previously described [16, 24, 25], but few studies have focused on filarial infection among infants, who are most likely to suffer severe malaria in the context of high malaria endemicity. The effects of *W. bancrofti* infection on the risk of malaria [12], especially severe malaria among infants, are largely unknown despite the findings of filarial-induced immune modulation on malaria-specific responses [26–28].

To address this gap, we used the remaining serum samples from infants and children in the Mother-Offspring Malaria Study (MOMS) Project, a large cohort study conducted from 2002 to 2006 in Muheza, Tanzania, to estimate the effect of exposure to filarial parasites on subsequent severe malaria infections. We tested the sera for reactivity to crude filarial antigens using a well-established immunoassay. We hypothesized that coinfection with filarial and *Plasmodium* species will modify immune responses and impact the risk of severe malaria.

Materials and methods

Details for the cohort have been previously described [29]. Briefly, starting at birth, serum samples were taken at 3 and 6 months (+/- 2 weeks) of age and then at 6-month intervals, and malaria smears were collected at 2-week intervals during infancy and at 4-week intervals thereafter. All data analyzed were de-identified and anonymized. We used the remaining samples from this study to perform the assays listed below, along with the participant data already collected to perform this exploratory study. A comparison of participant characteristics between the previously published study and the current study is provided in Table 1.

Filarial-specific antibody levels were measured using a multiplex array system modified for filarial and Strongyloides antigen from a technique published by Fouda et al [30] for *P. falciparum*. Briefly, crude soluble lysates from *Brugia malayi* adults (BmA) or *S. stercoralis* larvae were coupled to fluorescently labeled beads. *B. malayi* antigen is used for filarial assays owing to its high cross-reactive antigenicity with *W. bancrofti* [31], and its amenability to in vitro culture to generate assay antigen. Ten positive control sera were collected from parasitologically proven infections with *W. bancrofti* (n = 5) and *S. stercoralis* (for Strongyloides) (n = 5). Negative control sera were from 19 non-exposed adults in the United States. Samples were assayed in duplicate. Discrepancies between duplicates were evaluated by the coefficient...
of variance: the highest result was dropped from pairs with a coefficient of variance greater than 0.4. The mean value of the duplicates was used where the coefficient of variance was less than 0.4. The positive cutoff was defined using a receiver operating characteristic (ROC) curve to identify the cutoff point that produced the highest sensitivity and specificity based on positive and negative controls (Fig 1).

Available serum samples from children ≤2.5 years of age were assayed for antibodies to filarial antigen and to *S. stercoralis* larval antigen. Subsequent risk factor analysis was further limited to visits at 6 months and 1 year of age because this was the age window during which most severe malaria infections occurred. Children were excluded from this analysis if they: 1) had HIV or sickle cell anemia; 2) were twins or triplets; 3) had moved away from the study area since enrollment. Severe malaria was defined using WHO criteria [32].

### Risk factor analysis

Logistic regression models were used to assess the risk of severe malaria in the 6 months following sample collection for measurement of filarial serologies. We estimated the risk of severe malaria in the 6-month period following serologies to determine if children who were seropositive were at higher risk of severe malaria than those who were seronegative. To assess confounding, we estimated the association of specific variables known to be related to severe malaria (maternal parity, placental malaria, village, infant anemia, presence of an insecticide treated bed net in the household and malaria transmission season during birth) for their association with both filarial sero-status and severe malaria in this cohort. Only variables that were significantly associated with both severe malaria and filarial sero-status in this cohort were considered confounders. All models included only children who had a positive malaria blood smear in the six months following the visit when filarial serology was assessed, because development of severe malaria requires infection from *P. falciparum*. Statistical significance was assessed at \( p < 0.05 \).

### Cytokines

We quantified plasma cytokine levels at 6 months and 1 year of age to assess associations with filarial serology and severe malaria risk, after stratifying by malaria blood smear positivity. We assessed pro-inflammatory (IL1, IL6, IFN\( \gamma \) and TNF\( \alpha \)) and anti-inflammatory cytokines (IL4,
IL5, and IL10), based on the hypothesis that the balance of pro- and anti-inflammatory cytokine levels may influence severe malaria risk [33]. Cytokine assays were performed as previously described [34, 35]. The detection limits for the different analytes were as follows: TNF-α, 0.10 pg/ml; IFN-γ, 0.04 pg/ml; IL-1β, 0.01 pg/ml; IL-4, 0.3 pg/ml; IL-5, 0.02 pg/ml; IL-6, 1.45

Fig 1. Receiver operating curves for filaria (a), Strongyloides (b) using sera from US naïve donors as negative controls and from parasitologically proven infected donors as positive controls. The analysis suggested good performance characteristics for both assays, with optimal cutoff values for seropositivity of 107 for filaria and 170 for Strongyloides.

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pg/ml; IL-10, 0.02 pg/ml. Values were log transformed after adding one to all values to avoid log transformation of zero and the geometric means were compared using ANOVA. IL4 was analyzed as detectable vs. non-detectable using the detectable limit of 0.3. We accounted for multiple comparisons by using a Bonferroni correction. Statistical significance was assessed at $p < 0.0125$.

Ethics
Data for the MOMS study were collected under protocols approved by the International Clinical Studies Review Committee of the Division of Microbiology and Infectious Diseases at the US National Institutes of Health, and ethical clearance was obtained from the Institutional Review Boards of Seattle BioMed and the National Medical Research Coordinating Committee in Tanzania.

Results
A total of 746 serum samples were selected for risk factor analysis as outlined by the flowchart in Fig 2. The proportion of children with positive serology to filarial antigens ranged from 16.8%-60% with the highest proportion seropositive at 2.5 years. The percentage of children

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**Fig 2. Flowchart of children and samples included in the study.** A total of 471 children were assessed for eligibility in the study. Children were excluded from the study due to HIV or sickle cell anemia, if it was a multiple birth, or if they moved from the study area. Children with blood samples at 6 months (n = 261 samples) and 1 year (n = 196 samples) were used for the risk factor analysis, of which 180 children had serum for the assays and a positive blood smear between 6 months and one year of age, and 125 children had serum and a positive blood smear between one year and 18 months of age.

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with positive serology to Strongyloides antigens ranged from 3.1–8.1% (Fig 3). Because of the low numbers of children with positive serology for Strongyloides, we did not include these in subsequent risk factor analysis (Table 2).

Children with remaining serum samples collected between 6 months and 1.5 years of age (n = 612) were assessed for the occurrence of severe malaria and serologic evidence of filarial infection, with the highest proportion of severe malaria events occurring in the first year of life (Fig 4).

In order to assess risk associated with filarial serology, we used the data for children with samples at 6 months and 1 year who also had blood smear data. To better understand the risk of progressing to severe malaria among those already infected, we further limited the sample to children with at least one positive blood smear: 236 children had at least one serum sample at 6 months or 1 year of age and subsequent or concurrent positive malaria blood smear. 180

Fig 3. Proportions of children seropositive for filaria and for Strongyloides by age. The proportion of children with filarial antibodies increased with age: 16.8% at 6 months, 18.9% at one year, 32.9% at 1.5 years, 39.2% at 2 years to 60.0% at 2.5 years. In contrast, the proportion of children with antibodies to Strongyloides stayed fairly constant: 8.1% at 6 months, 3.1% at 1 year, 4.5% at 1.5 years, 5.4% at 2 years and 8.0% at 2.5 years.

Table 2. Seroprevalence and blood smear status of children in the study, n (%).

| Serology and Blood Smear Status by Age n (%) | 6 months | 12 months | 1.5 years | 2 years | 2.5 years |
|----------------------------------------------|----------|-----------|-----------|---------|----------|
| Filaria +                                    | 44 (16.8)| 37 (18.9) | 51 (32.9) | 40 (39.2)| 30 (60.0) |
| Strongyloides +                              | 21 (8.1) | 6 (3.1)   | 7 (4.5)   | 6 (5.4) | 4 (8.0)   |
| Blood smear positive (%)                     | 27 (10.3)| 23 (11.8) | 16 (10.7) | 14 (16.7)| 4 (8.7)   |
| Average Parasites (SD)*                     | 244.6 (767.8)| 318.5 (559.4)| 1492.0 (4002.9)| 172.0 (301.8)| 480.7 (688.2) |

*Positive samples only. Parasite count is per 200 WBCs.
children had a serum sample at 6 months of age and a subsequent or concurrent positive malaria blood smear, and 125 children had a serum sample at 1 year of age and a subsequent positive malaria blood smear. Overall, of the 236 children, 70% (n = 166) had a report of treated bed net use at some period during the observation period and 16% (n = 38) had missing information for this variable.

**Risk factor analysis**

None of the tested potential confounding variables were associated with both severe malaria and filarial antibody positivity in this cohort. We assessed the risk of severe malaria in the 6 months after filarial seropositivity at 6 months and 1 year. We found that infants who developed severe malaria between 6 months and 1 year of age were 3.9 times more likely (OR = 3.9, 95% CI: 1.2, 13.0; p-value = 0.02) to have had positive filarial serology at 6 months of age compared with infants who did not develop severe malaria. Children with severe malaria between 1 and 1.5 years of age were not significantly more likely to have positive filarial serology at 1 year of age than children who did not develop severe malaria (OR = 1.4; 95% CI: 0.27, 7.6; p-value = 0.67).

**Cytokine analysis**

We did not identify a difference between IL1, IL5, IL6, IL10, TNF-alpha, and IFN-gamma levels by filarial serology at 6 months or 1 year of age when using a significance value of p<
0.0125 among infants who were blood smear positive or negative. Among infants who subsequently developed severe malaria, no significant difference in IL1, IL5, IL6, IL10, TNF-alpha, or IFN-gamma levels by filarial serology was found at either 6 months or 1 year of age (Figs 5 and 6). Likewise, the presence of detectable IL4 was not associated with severe malaria risk during the 6 months after assay (Table 3).

Discussion

We describe an age-specific increase in prevalence of filarial antibodies beginning in infancy in Tanzania. Although previous studies have identified filarial infection in young children, this study identifies an increasing filarial seropositivity with age starting at 1 year. Additionally, we found that filarial seropositivity at 6 months of age was significantly associated with severe malaria by 1 year of age.

Although transplacental maternal antibodies may play a role in the observed prevalence, particularly at the 6-month measurement, the increasing seroprevalence after 1 year is noteworthy. Weerasooriya et al. described a decline in urinary antigens after 1 year of age among infants born to mothers who were Brugia pahangi antibody-positive [36]; antibodies from breast milk are not known to enter the infant’s circulation [37] suggesting that our results in children 1 year and older indicate the presence of antibodies acquired through filarial exposure rather than through maternal-infant transfer. Many public health studies focus on school age
children when describing infections among children. However, our results may indicate that pre-school age children are at increased risk of filarial exposure as well.

This study is also valuable in that it uses a multiplexed serologic assessment to identify children with previous or current exposure or infection for filariae and Strongyloides. The assay data appear consistent using the non-exposed controls, so we are confident that we are detecting both exposures. This obviously provides a framework for adding multiple (up to 50) antigens to gain a comprehensive assessment of seroreactivity in a single assay [38]. Other helminths contribute to polyparasitism in Tanzania including: *Onchocerca volvulus*, the causative agent of onchocerciasis [39]; *S. mansoni* and *S. haematobium* [40] [41]; and multiple species of soil-transmitted helminths. Several challenges exist with identifying the soil-transmitted helminth *S. stercoralis* infections in young children, a focus of this study. Very young children are incorrectly thought to be at lower risk based on the idea that they are not in direct contact with infective sources. Further, diagnosis may be hindered because measures of active infection (e.g. eggs in stool, parasites in the blood) may lag behind serologic measures of exposure [42].

Fig 6. Cytokine profiles of children in the study at 12 months by filarial serostatus, stratified by whether they experienced (“Severe Malaria”) or did not experience (“No Severe Malaria”) severe malaria between 12 and 18 months of age.

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|                | 6 months | 1 year |
|----------------|----------|--------|
|                | Severe Malaria | No Severe Malaria | Severe Malaria | No Severe Malaria |
| IL4 detectable | Filaria+ | Filaria- | Filaria+ | Filaria- | Filaria+ | Filaria- | Filaria+ | Filaria- |
| IL4 detectable | 1 (16.7) | 1 (16.7) | 5 (18.5) | 36 (24.2) | 2 (100) | 0 (0) | 11 (34.4) | 25 (17.9) |
| IL4 not detected | 5 (83.3) | 5 (83.3) | 22 (81.5) | 113 (75.8) | 0 (0) | 5 (100) | 21 (65.6) | 115 (82.1) |

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The significant association between filarial serostatus at 6 months and subsequent severe malaria infection highlights the need for further investigation to assess whether the increased risk is due to a shared vector or if immune modulation is occurring. As such, a primary limitation to this analysis is the potential for confounders in the relationship between severe malaria and filarial serology. Both malaria and filarial parasites are transmitted by mosquitoes, and previous studies have suggested that the same mosquito species may transmit both infections [17, 41].

The association between filarial seropositivity and severe malaria has been reported elsewhere in studies of older children and adults. Increased risk of severe malaria with helminth co-infection has been reported in children aged between 1–15 years in Senegal and Northern Senegal, and helminth coinfection has been associated with an increase in clinical malaria in children aged < 16 years in Zaire [23, 43, 44]. A study of adults in Thailand found an increase in clinical malaria associated with co-infection with intestinal helminths [19]. Conversely, studies in Senegal and Mali found decreases in malaria parasite densities associated with S. haematobium co-infection in cohorts aged 3–15 [21, 45], and no influence on malaria incidence was found in mixed age cohorts in Southwest Uganda and Northern Senegal [46, 47]. These apparent differences in findings may be explained by parasite differences, with S. haematobium co-infections having little or no influence on severe malaria, while Ascaris, S. mansoni, filarial and Strongyloides co-infection may confer increased risk. The differences may also be attributable to the age of the children in this study, as the children in this cohort are younger and the effect of coinfection may be different, or the antibodies present may reflect maternal antibodies.

We assessed potential confounding for a variable indicating the presence of an insecticide treated bednet in the household and this variable was not significantly associated with filarial serology at 6 months or 1 year of age. A modest proportion of children were missing information for this variable. Even among children who had an insecticide treated net in their household, we are unaware of actual utilization rates or integrity of the bed nets, so actual assessment of treated bed net use may be imprecise.

This study has several additional limitations. First, we are unable to determine if the positive filarial serology indicates current or past infection. Because we do not have measures of current infection, we are unable to determine if our results correlate with infection or exposure and are limited to describing the associations with filarial sero-reactivity. However, one advantage of using serology is that we are able to observe cumulative exposure, rather than assessing exposure at a single point in time. Secondly, this well-established serologic assay specifically uses Brugia malayi antigens, based on the substantial antigenic cross-reactivity among all filarial species [8–10, 39], and as a result this assesses filarial exposure without assignment to an exact species. However, the age-specific profile aligns much more closely with W. bancrofti than with O. volvulus infection [48–51] as does the geospatial data. Although we now have filarial species-specific recombinants for W. bancrofti [52–54] and O. volvulus [55], insufficient serum was available to perform these specific assays. Nonetheless, the results still suggest filarial antibodies are an important biomarker of increased risk for severe malaria and further suggest that efforts to reduce exposure to the vectors associated with W. bancrofti or O. volvulus infection may also have a substantial impact on reducing severe malaria.

Supporting information
S1 Table. Samples per participant, by timepoint used in the analysis. Samples were the remaining sera from the Mother-Offspring Malaria Study (MOMS) Project conducted from 2002 to 2006 in Muheza, Tanzania. (TIF)
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References

1. Kung’u JK, Goodman D, Haji HJ, Ramsan M, Wright VJ, Bickle QD, et al. Early helminth infections are inversely related to anemia, malnutrition, and malaria and are not associated with inflammation in 6- to 23-month-old Zanzibari children. Am J Trop Med Hyg. 2009; 81(6):1062–70. Epub 2009/12/10. https://doi.org/10.4269/ajtmh.2009.09-0091 PMID: 19996438.
2. Crompton DW, Nesheim MC. Nutritional impact of intestinal helminthiasis during the human life cycle. Annu Rev Nutr. 2002; 22:35–59. Epub 2002/06/11. https://doi.org/10.1146/annurev.nutr.22.120501.134539 PMID: 12055337.
3. World Health Organization. Key Malaria Facts, 2012. Available from: http://www.rbm.who.int/keyfacts.html.
4. World Health Organization. World Malaria Report 2017. Geneva, Switzerland: World Health Organization, November 29, 2017, Licence: CC BY-NC-SA 3.0 IGO.
5. Trampuz A, Jereb M, Muzlovic I, Prabhu RM. Clinical review: Severe malaria. Crit Care. 2003; 7(4):315–23. https://doi.org/10.1186/cc183 PMID: 12930555; PubMed Central PMCID: PMCPMC270697.
6. Anstey NM, Russell B, Yeo TW, Price RN. The pathophysiology of vivax malaria. Trends in parasitol- ogy. 2009; 25(5):220–7. Epub 2009/04/08. https://doi.org/10.1016/j.pt.2009.02.003 PMID: 19349210.
7. Streit T, Lafontant JG. Eliminating lymphatic filariasis: a view from the field. Ann N Y Acad Sci. 2008; 1136:53–63. https://doi.org/10.1196/annals.1425.036 PMID: 18579875.
8. Simonsen PE, Pedersen EM, Rwegoshora RT, Malecela MN, Derua YA, Magesa SM. Lymphatic filaria- sis control in Tanzania: effect of repeated mass drug administration with ivermectin and albendazole on infection and transmission. PLoS Negl Trop Dis. 2010; 4(6):e696. https://doi.org/10.1371/journal.pntd.0000696 PMID: 20532226; PubMed Central PMCID: PMCPMC2879369.
9. Meyrowitsch DW, Simonsen PE, Makunde WH. Bancroftian filariasis: analysis of infection and disease in five endemic communities of north-eastern Tanzania. Ann Trop Med Parasitol. 1995; 89(6):653–63. PMID: 8745940.

10. Simonsen PE, Meyrowitsch DW, Jaoko WG, Malecela MN, Mukoko D, Pedersen EM, et al. Bancroftian filariasis infection, disease, and specific antibody response patterns in a high and a low endemicity community in East Africa. Am J Trop Med Hyg. 2002; 66(5):550–9. PMID: 12201589.

11. Malecela M.N., Lazarus W, Mwingira U, Mwakitalu E, Makene C, Kabali C, et al. Eliminating LF: a progress report from Tanzania. Journal of Lymphoedema. 2009; 4(1):10–2.

12. van den Berg H, Kelly-Hope LA, Lindsay SW. Malaria and lymphatic filariasis: the case for integrated vector management. Lancet Infect Dis. 2013; 13(1):89–94. https://doi.org/10.1016/S1473-3099(12)70148-2 PMID: 23084831.

13. World Health Organization. Geographical distribution of arthropod-borne diseases and their principal vectors. World Health Organization [Internet]. 1989.

14. Ellman R, Maxwell C, Finch R, Shayo D. Malaria and anaemia at different altitudes in the Muheza district of Tanzania: Childhood morbidity in relation to level of exposure to infection. Annals of Tropical Medicine and Parasitology. 1998; 92(7):741–53. PubMed PMID: WOS:000076972800001. PMID: 9924532

15. Manguin S, Bangs MJ, Pothikasikorn J, Charoenviriyaphap T. Review on global co-transmission of human Plasmodium species and Wuchereria bancrofti by Anopheles mosquitoes. Infect Genet Evol. 2010; 10(2):159–77. https://doi.org/10.1016/j.meegid.2009.11.014 PMID: 19941975.

16. Mboera LEG, Senkoro KP, Rumisha SF, Mayala BK, Shayo EH, Mlozi MRS. Plasmodium falciparum and lymphatic infestations among schoolchildren in relation to agro-ecosystems in Mvomero District, Tanzania. Acta Tropica. 2011; 120(1–2):95–102. https://doi.org/10.1016/j.actatropica.2011.06.007 PubMed PMID: WOS:000295304900013. PMID: 21741929.

17. Burkot TR, Molineaux L, Graves PM, Paru R, Battistutta D, Dagoro H, et al. The prevalence of naturally acquired multiple infections of Wuchereria bancrofti and human malarias in anophelines. Parasitology. 1990;100 Pt 3:369–75. PMID: 2194153.

18. Simonsen PE, Derua YA, Kisinza WN, Magasa SM, Malecela MN, Pedersen EM. Lymphatic filariasis control in Tanzania: effect of six rounds of mass drug administration with ivermectin and albendazole on infection and transmission. BMC Infectious Diseases. 2013; 13(1):335. https://doi.org/10.1186/1471-2334-13-335 PMID: 23870103.

19. Nacher M, Singhasivanon P, Yimsamran S, Manibunyo W, Thanyavich N, Wuthisen R, et al. Intestinal helminth infections are associated with increased incidence of Plasmodium falciparum malaria in Thailand. J Parasitol. 2002; 88(1):55–8. https://doi.org/10.1645/0022-3395(2002)088[0055:IMHIAAW]2.CO;2 PMID: 12053980.

20. Murray J, Murray A, Murray M, Murray C. The biological suppression of malaria: an ecological and nutritional interrelationship of a host and two parasites. Am J Clin Nutr. 1978; 31(8):1363–6. PMID: 354372.

21. Lyke KE, Dicko A, Dabo A, Sangare L, Kone A, Coulibaly D, et al. Association of Schistosoma haematobium infection with protection against acute Plasmodium falciparum malaria in Malian children. Am J Trop Med Hyg. 2005; 73(6):1124–30. Epub 2005/12/16. doi: 73/6/1124 [pii]. PMID: 16354824; PubMed Central PMCID: PMC2738948.

22. Nacher M. Worms and malaria: blind men feeling the elephant? Parasitology. 2008; 135(7):861–8. https://doi.org/10.1017/S0031182008000358 PMID: 18377695.

23. Spiegel A, Tall A, Raphenon G, Trape JF, Druilhe P. Increased frequency of malaria attacks in subjects co-infected by intestinal worms and Plasmodium falciparum malaria. Trans R Soc Trop Med Hyg. 2003; 97(2):198–9. PMID: 14584377.

24. Brandao E, Bonfim C, Cabral D, Lima JL, Aguiar-Santos AM, Maciel A, et al. Mapping of Wuchereria bancrofti infection in children and adolescents in an endemic area of Brazil. Acta Trop. 2011; 120(1–2):151–4. https://doi.org/10.1016/j.actatropica.2011.06.004 PMID: 21726520.

25. Michael E, Bundy DA, Grenfell BT. Re-assessing the global prevalence and distribution of lymphatic filariasis. Parasitology. 1996; 112 (Pt 4):409–28. PMID: 8935952.

26. Metenou S, Dembele B, Konate S, Dolo H, Coulibaly SY, Coulibaly YI, et al. Patent Filarial Infection Modulates Malaria-Specific Type 1 Cytokine Responses in an IL-10-Dependent Manner in a Filaria/ Malaria-Coinfected Population. Journal of Immunology. 2009; 183(2):916–24. https://doi.org/10.4049/jimmunol.0800257 PubMed PMID: WOS:000267812600017. PMID: 19561105.

27. Hartgers FC, Obeng BB, Kruize YC, Dijkhuize A, McCall M, Sauerwein RW, et al. Responses to malarial antigens are altered in helminth-infected children. J Infect Dis. 2009; 199(10):1528–35. Epub 2009/04/28. https://doi.org/10.1086/598667 PMID: 19392626.
39. Makunde WH, Kamugi M, Massaga JJ, Makunde RW, Sauvel ZK, Akida J, et al. Treatment of co-

38. Metenou S, Dembele B, Konate S, Dolo H, Coulibaly YI, Diallo AA, et al. Filarial infection suppresses malaria-specific multifunctional Th1 and Th17 responses in malaria and filarial coinfections. J Immunol. 2011; 186(8):4725–33. https://doi.org/10.4049/jimmunol.1003778 PMID: 21411732; PubMed Central PMCID: PMCPMC3407819.

37. Nash SD, Prevots DR, Kabyemela E, Khasa Y-L, Fried M, et al. Maternal malaria and gravidity interaction predict severe malaria during infancy. PLoS One. 2013; 8(10):e77214. https://doi.org/10.1371/journal.pone.0077214 PMID: 24130857; PubMed Central PMCID: PMCPMC3795067.

36. Mutabingwa TK, Bolla MC, Li JL, Domingo GJ, Li X, Fried M, et al. Maternal malaria and gravidity interaction to modify infant susceptibility to malaria. PLoS Med. 2005; 2(12):e407. https://doi.org/10.1371/journal.pmed.0020407 PMID: 16259531; PubMed Central PMCID: PMCPMC1277932.

35. Fouda GG, Leke RFG, Long C, Drulhe P, Zhou A, Taylor DW, et al. Co-infection with Plasmodium falciparum antigens: Clinical and Vaccine Immunology. 2006; 13:1307–13. https://doi.org/10.1128/CVI.00183-06 PMID: 17035513

34. Lwambo NJS, Siza JE, Brooker S, Bundy DAP, Guyatt H. Patterns of concurrent hookworm infection and schistosomiasis in schoolchildren in Tanzania. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000; 94(S1–S90). PMID: 11103309

33. Beales PF, Brabin B, Dorman E, Gilles HM, Loutain L, Marsh K, et al. Severe falciparum malaria. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2000; 94(S1–S90). PMID: 11103309

32. Sokhna C, Le Hesran JY, Mbaye PA, Akiana J, Camara P, Diop M, et al. Increase of malaria attacks among children presenting concomitant infection by Schistosoma mansoni in Senegal. Malar J. 2004; 3(1):43. https://doi.org/10.1186/1475-2875-3-43 PMID: 15544703; PubMed Central PMCID: PMCPMC3538224.

31. Van de Perre P. Transfer of antibody via mother’s milk. Vaccine. 2003; 21(24):3374–6. PMID: 12850343.

30. McCarthy JS, Zhong M, Gopinath R, Ottesen EA, Williams SA, Nutman TB. Evaluation of a polymerase chain reaction-based assay for diagnosis of Wuchereria bancrofti infection. J Infect Dis. 1996; 173(6):1510–4. https://doi.org/10.1093/infdis/173.6.1510 PMID: 8649232.

29. Mandala WL, Msefula CL, Gondwe EN, Drayson MT, Molyneux ME, MacLennan CA. Cytokine Profiles in Malawian Children Presenting with Uncomplicated Malaria, Severe Malaria Anemia, and Cerebral Malaria. Clin Vaccine Immunol. 2017; 24(4). https://doi.org/10.1128/CVI.00533-16 PMID: 28122790; PubMed Central PMCID: PMCPMC5382826.

28. Stothard JR, Sousa-Figuereido JC, Betson M, Adriko M, Ainaitwe M, Rowell C, et al. Schistosoma mansoni Infections in Young Children: When Are Schistosome Antigens in Urine, Eggs in Stool and Schistosomiasis in Schoolchildren in Tanzania, Transactions of the Royal Society of Tropical Medicine and Hygiene. 1999; 93(5):497–502. https://doi.org/10.1016/S0035-9203(99)90349-8. PMID: 10696404

27. Tshikuka JG, Scott ME, Gray-Donald K, Kalumba ON. Multiple infection with Plasmodium and helminths in communities of low and relatively high socio-economic status. Ann Trop Med Parasitol. 1996; 90(3):277–93. PMID: 8758142.
45. Briand V, Watier L, JY LEH, Garcia A, Cot M. Coinfection with Plasmodium falciparum and schistosoma haematobium: protective effect of schistosomiasis on malaria in senegalese children? Am J Trop Med Hyg. 2005; 72(6):702–7. PMID: 15964953.

46. Shapiro AE, Tukahebwa EM, Kasten J, Clarke SE, Magnusen P, Olsen A, et al. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. Trans R Soc Trop Med Hyg. 2005; 99(1):18–24. https://doi.org/10.1016/j.trstmh.2004.02.006 PMID: 15550257.

47. Diallo TO, Remoue F, Schacht AM, Charrier N, Dompnier JP, Pillet S, et al. Schistosomiasis co-infection in humans influences inflammatory markers in uncomplicated Plasmodium falciparum malaria. Parasite Immunol. 2004; 26(8–9):365–9. https://doi.org/10.1111/j.0141-9838.2004.00719.x PMID: 15679634.

48. Jaoko WG, Michael E, Meyrowitsch DW, Estambale BB, Malecela MN, Simonsen PE. Immunopathology of Wuchereria bancrofti infection: parasite transmission intensity, filaria-specific antibodies, and host immunity in two East African communities. Infect Immun. 2007; 75(12):5651–62. https://doi.org/10.1128/IAI.00970-07 PMID: 17908811; PubMed Central PMCID: PMCPMC2168322.

49. Beuria MK, Bal M, Dash AP, Das MK. Age-related prevalence of antibodies to infective larvae of Wuchereria bancrofti in normal individuals from a filaria-endemic region. Journal of Biosciences. 1995; 20(2):167–74. https://doi.org/10.1007/bf02703266

50. Filipe JA, Boussinesq M, Renz A, Collins RC, Vivas-Martinez S, Grillet ME, et al. Human infection patterns and heterogeneous exposure in river blindness. Proc Natl Acad Sci U S A. 2005; 102(42):15265–70. https://doi.org/10.1073/pnas.0502591102 PMID: 16217028; PubMed Central PMCID: PMCPMC1257694.

51. Faulkner H, Gardon J, Kamgno J, Enyong P, Boussinesq M, Bradley JE. Antibody responses in onchocerciasis as a function of age and infection intensity. Parasite Immunology. 2001; 23(9):509–16. https://doi.org/10.1046/j.1365-3024.2001.00408.x PubMed PMID: WOS:000171022000006. PMID: 11589780

52. Steel C, Golden A, Kubolcik J, LaRue N, de Los Santos T, Domingo GJ, et al. Rapid Wuchereria bancrofti-specific antigen Wb123-based IgG4 immunoassays as tools for surveillance following mass drug administration programs on lymphatic filariasis. Clin Vaccine Immunol. 2013; 20(8):1155–61. https://doi.org/10.1128/CVI.00252-13 PMID: 23740923; PubMed Central PMCID: PMC3754496.

53. Kubolcik J, Fink DL, Nutman TB. Identification of Wb 123 as an early and specific marker of Wuchereria bancrofti infection. PLoS Negl Trop Dis. 2012; 6(12):e1930. https://doi.org/10.1371/journal.pntd.0001930 PMID: 23236529; PubMed Central PMCID: PMCPMC3516582.

54. Steel C, Kubolcik J, Ottesen EA, Nutman TB. Antibody to the filarial antigen Wb123 reflects reduced transmission and decreased exposure in children born following single mass drug administration (MDA). PLoS Negl Trop Dis. 2012; 6(12):e1940. https://doi.org/10.1371/journal.pntd.0001940 PMID: 23236533; PubMed Central PMCID: PMCPMC3516579.

55. Lobos E, Weiss N, Karam M, Taylor HR, Ottesen EA, Nutman TB. An immunogenic Onchocerca volvulus antigen: a specific and early marker of infection. Science. 1991; 251(5001):1603–5. https://doi.org/10.1126/science.2011741 PMID: 2011741.