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REVIEW

Malaria and soil-transmitted intestinal helminth co-infection and its effect on anemia: a meta-analysis

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This study aimed to synthesize available evidence on the extent of malaria and soil-transmitted intestinal helminth (STH) co-infections in people living in endemic countries and to explore the effect of interactions between malaria and STHs on anemia. We searched relevant studies in electronic databases up to March 2013. Studies comparing malaria and STH co-infected patients with those not co-infected were included and the effect estimates were pooled using a random-effects model. We identified 30 studies for meta-analyses of which 17 were cross-sectional design. The majority of included studies (80%) were carried out in African countries. Among pregnant women, those infected with hookworm were found to have higher association with malaria infection compared with those without (summary OR: 1.36; 95% CI: 1.17–1.59; I^2: 0%). Among non-pregnant adults, the summary OR of the association between anemia and the combined malaria and STH was 2.91 (1.38–6.14). The summary OR of the association between anemia and malaria alone was 1.53 (0.97–2.42), while the association between anemia and STH alone was 0.28 (0.04–1.95). There is no good evidence to support a different effect of malaria and STH on anemia. A subgroup analysis showed a higher risk of malaria infection in the primigravidae (summary OR: 1.61; 95% CI: 1.3–1.99; I^2: 0%). In conclusion, the malaria–STH co-infection was variable with complex outcomes on anemia.

Keywords: Co-infection, Malaria, Helminths, Prevalence, Anemia

Introduction

Mono-infection with parasite species *Plasmodium falciparum* or soil-transmitted helminths (STHs) has been well documented. However, biological phenomena rarely occur in isolation, and this is certainly true for these two parasites.

Numerous studies have reported that two of the most prevalent types of human infection in the developing world, malaria and helminths, overlap extensively in their epidemiological (geographical) distribution and frequently co-infect the same individuals.[1–6] Approximately 30% of the world's population is exposed to malaria and most clinical events attributable to *P. falciparum* occur in the African region.[7] Approximately one third of the world's population is infected with helminths.[3] Thus far, studies on malaria–helminth co-infections provided heterogenous results such as positive association,[8] no significant association[9] and even negative association.[10] Previous reviews have addressed the malaria–helminth co-infections in mice[4] and in humans.[11,12] Since the publication of these reviews there has been a surge in published studies on malaria-helminth co-infections in endemic countries.

Parasitic helminths comprise a highly diverse group of organisms which display different life cycle stages.[13] Human intestinal helminthiasis is most commonly caused by STHs, namely *Ascaris lumbricoides*, *Trichuris trichiura*, and the hookworms *Necator americanus* and *Ancylostoma duodenale*.[14,15] In view of the variation in routes of entry to the host and different clinical outcomes, the interaction of these helminths with malaria parasites is expected to be different from one another. Thus, it is imperative to analyze species-specific infections separately. The present study was carried out to synthesize available evidence on the extent of malaria-intestinal helminth co-infections (pertinent to hookworms, *A. lumbricoides* and *T. trichura*) in people living in endemic countries and to explore the effect of interactions between malaria and STHs on anemia. The protocol of this study is available in PROSPERO (international prospective register of systematic reviews).[16]

Materials and methods

Identification of studies

We searched for studies that assessed malaria and STH co-infection in two electronic databases (MEDLINE and EMBASE), using the following key words: 'malaria', '*Plasmodium falciparum*', '*Plasmodium vivax*' in combination with one of the following STH-related terms: 'soil-transmitted helminths', 'geohelminths', '*Ascaris lumbricoides*', 'round worm', '*Trichuris trichiura*', 'whip worm', 'hookworm', '*Necator americanus*', '*Ancylostoma duodenale*'.

The searches were limited to studies on human participants, published in English language up to 18 March 2013. We also accessed the bibliographic database of the Malaria in Pregnancy library (http://library.mip-consortium.org) and the Cochrane database of systematic reviews (http://www.cochrane.org/). Further, we manually searched the reference sections of the selected studies and relevant review articles for any additional studies that were not found in the initial search.

Criteria for selecting studies

Studies included in the meta-analyses are those which performed randomized controlled trials (RCTs), case-control, cross-sectional, cohort or nested case-control designs; provided prevalence of co-infections; examined anemia as an outcome variable; compared co-infected patients with control groups; provided (or allowed data for computation of an effect estimate) RR, HR or OR and their corresponding 95% CIs. We excluded studies if the study population had taken drug treatment for malaria within the previous two weeks, or if the primary population was co-infected with HIV/AIDS or if the study was done on fewer than 20 participants. We did not include studies on economic evaluation, mathematical modeling or pharmacokinetics.

In the included studies, the diagnosis of malaria was confirmed by microscopic examination of blood smears or by rapid-onsite diagnostic test (a positive immunochromatographic test). STHs were confirmed by microscopic examination of stool specimens using the Kato-Katz method for quantitative determination of helminth ova. The intensity of helminth infection was described according to WHO criteria based on eggs per gram of feces (epg).[17] Anemia was defined according to WHO criteria based on the age related hemoglobin(Hb) cut off levels.[18] Packed cell volume (PCV) values $<31\%$ were considered as anemia, which was further classified as mild (PCV 21–30%), moderate (PCV 15–20%) or severe (PCV $<15\%$).[18,19]

Data extraction and quality assessment

We assumed that the RR from cohort studies approximates the OR from case-control studies[20] and HR from cohort studies. All searches were conducted by two authors who independently read the papers and extracted data on study country, study design, sample size, outcome measures, adjusted ORs or RRs with 95% CI, adjustment factors and methods of confirmation of malaria infection and STHs. If articles contained information on the same or overlapping study population, we included the study with the most complete information. The methodological quality of the studies was judged using the Newcastle-Ottawa Scale (NOS), which determines study quality on the basis of selection, comparability and outcomes.[21]

Data synthesis and analysis

Studies reporting estimates adjusted for at least a potential confounding factor of age or gender (or both) in their analyses were preferentially included in the meta-analysis. However, unadjusted ORs were extracted if adjusted ORs were not provided. If RR or HR were not provided, raw data were extracted to calculate ORs. We entered the ratio measures of the (adjusted) effect as a log OR and the standard error (SE) of the log OR using generic inverse-variance weighting method[22] and then included this summary estimate in the meta-analysis by the fixed-effects or random-effects models according to the heterogeneity test. We assessed heterogeneity of effect estimates within each group of studies using I^2 test which describes the percentage of total variation across studies that is due to heterogeneity rather than chance. A calculated value of $I^2 > 50\%$ indicated substantial heterogeneity.[22]

To study the effect of malaria and STH infection on anemia, we calculated the interaction, using the methods described by Altman and Bland[23] to address the size of association between anemia and malaria alone, the size of association between anemia and STH infection alone and the size of association between anemia and combined malaria and STH infection.

We stratified the analysis by species of STH, type of malaria (uncomplicated malaria, cerebral malaria, severe noncerebral malaria), target study population (pregnant women, preschool children, school children, adult) or type of study design, whenever available.

For robustness of results, we performed a sensitivity analysis on the studies carried out in unstable malaria transmission areas. To investigate the potential publication bias we visually examined the funnel plots. Data entry and analysis were carried out with RevMan version 5.2 (The Nordic Cochrane Centre, Copenhagen, Denmark). The methods and findings of the present review have been reported based on the PRISMA[24] guidelines.

Results

Figure 1 presents the flow of the study search. An initial search yielded 368 citations. Of these, a total of 44 full-text articles were reviewed, of which only 30 were included in the meta-analysis.[8–10,25–51] Studies were excluded if data on the non-coinfected group were not available,[52–57] data on the co-infected group were not available,[58] the majority were HIV-infected participants or tested positive for HIV,[59,60] samples were duplicated,[61,62] data were insufficient to compute effect estimate as only abstracts were available[63,64] and the study was on treatment efficacy.[65]

Table 1 gives the characteristics of the included studies. Of 30 studies, 17 were cross-sectional studies, five were case-control studies, two were nested case-control in RCT studies, one was a RCT and five were cohort studies. Twenty-four studies (80%) were performed in African countries and six (20%) were performed in Thailand. Among the 30 studies, 19 (63%) made adjustments for various factors; 16 (53%) did matching based on age and 9 (30%) did matching on both age and gender. We were not able to assess the quality of studies using NOS because the validity (of the criteria used) was unknown in most studies, and some studies included invalid individual items.[66] This was

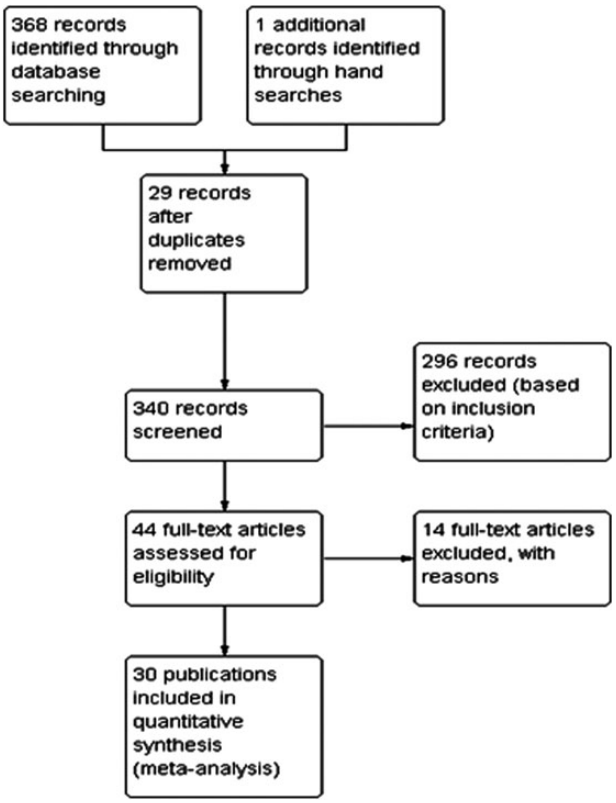

Figure 1. Flowchart of the study selection process

especially true for the cross-sectional studies, which accounted for the greatest proportion of the studies included.

Of the 30 studies, 12 assessed all three species of STH (*A. lumbricoides*, *T. trichiura* and hookworm)[8–10,26,28,32,37–41,44] one assessed two species (*A. lumbricoides* and hookworm)[48] and two assessed two species (*A. lumbricoides* and *T. trichiura*).[29,33] Seven studies[34,42,43,45,47,49,51] assessed hookworm only, while four studies[25,27,31,50] assessed *A. lumbricoides* only. Three studies described STH without speciation.[35,36,46]

Twelve studies[8,26,28,32,33,35,41,43,46,49–51] assessed the impact of co-infection on anemia, using various measurements. Five studies[26,28,32,33,49] quantitatively reported anemia with the mean Hb level in various age groups and pregnant women. One study reported anemia with the Hb cut-off level.[8] Out of eight studies[8,28,32,41,43,46,50,51] which reported presence or absence of anemia, one study[32] assessed anemia using the mean PCV.

Co-infection with uncomplicated malaria

Table 2 provides the number of included studies and the summary results of the co-infection in various age groups as well as in pregnant women.

The available data showed that there was high variability in the prevalence of malaria-STH co-infection from 4.2%[44] to 48.3%.[26] Of interest, the highest prevalence was found among pregnant women in Nigeria, whilst the lowest was among the school-age group in the same country.

Among non-pregnant women, there were comparable proportions of malaria infections among those co-infected with *A. lumbricoides*, hookworms, *T. trichura*, any helminth and those without. Among preschool children, there was a comparable proportion of malaria infections between those co-infected with hookworms and those without. Only one study assessed the co-infection with *T. trichiuria*,[8] providing a significant positive association (OR 2.77; 95% CI: 1.15–6.65). Among school children, there were comparable proportions of malaria infections between those co-infected with *A. lumbricoides*, hookworms or *T.trichiura* and those without. But the pooled analysis of three studies[8,9,30] showed a significant positive association between malaria and co-infection with those co-infected with STH (summary OR 1.71; 95% CI: 1.03–2.82; I^2: 72%). Of note, there was substantial (statistical) heterogeneity among studies.

Among pregnant women, the pooled analysis of seven studies[9,34,37–39,40,45] showed a significant positive association between malaria and hookworms (summary OR 1.36; 95% CI: 1.17–1.59; I^2: 0%). However, there was no significant association between malaria and *A. lumbricoides*, *T. trichiura* or any other STH (Figure 2).

Co-infection with severe malaria

Among non-pregnant adults, the pooled analysis of two studies[25,31] showed no significant differences in the proportion of severe malaria between those co-infected with *A. lumbricoides* and those non co-infected (summary OR 1.76; 95% CI: 0.05–64.93; I^2: 89%). Of note, 95% CI is rather wide and there was substantial (statistical) heterogeneity among studies. The pooled analysis of two studies[35,36] documented a significant negative association between anemia and co-infection with any STH (summary OR 0.36; 95% CI: 0.22–0.58, I^2: 0%).

Effect of co-infection on anemia

Among non-pregnant adults, three studies[8,41,43] qualitatively assessed anemia (i.e., presence/absence). The summary OR of the association between anemia and the combined malaria and STH infection was 2.91 (1.38–6.14) (Figure 3). The summary OR of the association between anemia and malaria alone was 1.53 (0.97–2.42), while the association between anemia and STH infection alone was 0.28 (0.04–1.95). There is no good evidence to support the different effects[23] of malaria and STH co-infection on anemia (Supplementary Table 1).

Two studies on non-pregnant adults[8,29] assessed anemia using Hb cut-off level and reported a significantly higher risk of anemia in those co-infected with any STH compared with those not co-infected (summary OR: 2.2; 95% CI: 1.9–4.45; I^2: 40%). Two studies (n=624)[28,29] assessed the effect of co-infection on anemia and reported a significantly lower mean Hb level in the co-infected group compared with those not co-infected (mean difference (MD) 0.98; 95% CI:−0.59 to −1.37; I^2: 0%). Overall, among non-pregnant adults, a higher risk of anemia was found in the co-infected group, regardless of hematological outcome.

Among school-aged children, the pooled analysis of three studies (n=476)[32,33,49] showed that there was comparable mean Hb levels in the two groups (MD: 0.47; 95% CI:−0.1 to 1.03). The pooled estimates of two studies[43,51] gave a higher risk of anemia in those co-infected with *A. lumbricoides* (summary OR: 2.72; 95% CI: 1.11–6.7; I^2: 0%). Due to a paucity of data, we could not summarize the effect of malaria–STH

Table 1. Characteristics of included studies

Reference	Country	Study design	Sample size	Target population	Prevalence of co-infection, %	Mean age in years (±SD) or (range)	STH species[a]	Malaria transmission	Dominant malaria species/malaria status	Adjusted factors[b]	Remarks
Degarege, 2012[8]	South Ethiopia	CS	1065	U5, SC & adult	19.4	18.6 (1–82)	*AL*, HW, *TT*	Unstable, seasonal	*Pv*/UM	1, 2, 7	
Shapiro, 2005[9]	Uganda	CC	856	All ages	54.8	NA	*AL*, HW, *TT*	Low, unstable	Asymptomatic	1, 2, 4, 14	Active surveillance
van Eijk, 2009[10]	Kenya	CS	390	PG	37.8	25 (21–31)[c]	*AL*, HW, *TT*	Holo, perennial (low-moderate)	Asymptomatic	18, 19, 20, 21	<20 yr: 15.8%
Nacher, 2000[25]	Thailand	CC	182 cases 355 controls	NA	NA	NA	*AL*	Low, seasonal	UM, CM	1, 3, 6, 9, 10	532 cases: 40 control
Egwunyenga, 2001[26]	Nigeria	CS	816	PG	48.3	NA	*AL*, HW, *TT*	Perennial	Asymptomatic	NA	
Nacher, 2001[27]	Thailand	CS	80	NA	NA	24[c]	*AL*	Low, seasonal	UM	1, 3, 10, 11	Contemporaneous *Pf* & *Pv*
Nacher, 2001[28]	Thailand	CS	307	NA	NA	25 (15–62)	*AL*, HW, *TT*	Low, seasonal	UM (*Pf* only)	1, 2, 5, 9, 12, 13	20% SS & 10% *Opistorchis Viverini* 120: gametocytes +ve
Nacher, 2001[29]	Thailand	CO	291	NA	NA	NA	*Al*, *TT*	Low, seasonal	UM, CM	NA	16% SS
Spiegel, 2003[30]	Madagascar	CO	80	U5 & SC	NA	1–14	*AL*, HW, *TT*	Low, unstable	Asymptomatic	NA	29/80 cases were U5
Le Hesran, 2004[31]	Senegal	CC	64 cases 64 controls	SC	NA	6.6 (±3)	*AL*	Seasonal	SCM	2, 22	*Strongyloides stercoralis* as an adjustment factor
Nkuo-Akenji, 2006[32]	Cameroon	CS	425	U5 & SC	29.9	(0.75–14)	*AL*, HW, *TT*	Hyperendemic, perennial	Asymptomatic	NA	PCV level for anemia 228/228: SC
Achidi, 2008[33]	Cameroon	CS	263	SC	13.9 (29/209)	7.56 (1.82)	*AL*, *TT*	Meso-hyperendemic	*Pf*/asymptomatic	NA	Hb only
Hillier, 2008[34]	Uganda	CS	2507	PG	7.5	23.7 (14–47)	HW	Stable	Asymptomatic	1, 2, 4, 14, 17	
Nacher, 2008[35]	Thailand	CC	67 cases 217 controls	NA	NA	NA	Any	Low, seasonal	CM	1, 5, 6, 9	
Degarege, 2009[36]	South Ethiopia	CO	1802	U5 & SC	37.8 (*AL*) 24.7 (HW) 8.3 (*TT*)	NA	*AL*, HW, *TT*	Unstable, seasonal	*Pv*/(79.9%), UM, SCM	NA	Post-treatment
Woodburn, 2009[37]	Uganda	CO	2507[d]	PG	NA	23[e] (14–47)	*AL*, HW, *TT*	High	*Pf*	1, 3, 22, 23	11.9% HIV +ve 9% taken anthelmintics also assessed *Strongyloides stercoralis*
Yatichi, 2009[38]	Ghana	CS	785	PG	16.6 (all) 45.0 (PG)	26.8 (15–48)	*AL*, HW, *TT*	High & perennial	*Pf*	NA	also assessed *Strongyloides stercoralis*

Continued

Table 1. *Continued*

Reference	Country	Study design	Sample size	Target population	Prevalence of co-infection, %	Mean age in years (±SD) or (range)	STH species[a]	Malaria transmission	Dominant malaria species/malaria status	Adjusted factors[b]	Remarks
Adegnika, 2010[39]	Gabon	CO	388	PG	15.0	24.4 (6.6)	*AL*, HW, *TT*	High	Asymptomatic	1, 24	
Boel, 2010[40]	Thailand	CS	829	PG	19.0	25 (6) in 1996 27 (7) in 2007	*AL*, HW, *TT*	Low, seasonal	Symptomatic	NA	No de-worming policy for PG
Degareg, 2010[41]	South Ethiopia	CS	1082	U5, SC & adult	39.6	>1	*AL*, HW, *TT*	Unstable, seasonal	*Pv*/UM	1, 2	
Pullan, 2010[42]	Uganda	CS	1770	U5, SC & adult	15.5	(0–80)	HW	stable	Asymptomatic	1, 2, 14	
Humphries, 2011[43]	Ghana	CS	258	Any age	10.0 (adult) 51.0 (children)	(1–80); 51.2%: ≤15	HW	High & perennial	Asymptomatic	1, 2	
Ojurongbe, 2011[44]	Nigeria	CS	117	SC	4.3	9.9 (3.1)	*AL*, HW, *TT*	Perennial	Asymptomatic	NA	
Thigpen, 2011[45]	Malawi	RCT (nested CC)	848[b]	PG	21.4	20.1±2.9	HW	Perennial	Asymptomatic, UM	NA	14.2% HIV +ve
Alemu, 2012[46]	Ethiopia	CS	384	Any age	5.1	23.8 (range: 1–80)	Any	Unstable, seasonal	*Pv*/UM	NA	Sample size calculated; 73% :>15 yrs old
Brooker, 2011[47]	East Africa (Kenya, Uganda, Ethiopia)	CS	28 050	SC	0.9 (Kenya) 9.3 (Uganda, 2006) 11.1 (Uganda, 2009)	IQR: 7–13	HW	Unstable (Ethiopia) low-moderate (Kenya) high (Uganda)	Asymptomatic	14	
Nano, 2012[48]	Columbia	CC	68 cases 1769 controls	SC & adult[f]	31.6 (*AL*) 17.4 (HW)	27; (6–59)	*AL*, HW	High	Asymptomatic	1, 2	
Righetti, 2012[49]	Cote d'ivoire	CS	732	3 groups (infant, SC & adult women)	1.1 (infant) 27.9 (SC) 4.9 (adult)	0.5–25	HW	Perennial	Asymptomatic	1, 2, 12, 15, 16	
Abanyie, 2013[50]	Nigeria	RCT (nested CC)	690	U5	42.9	35.5 (12.9), month	*AL*	Perennial	UM	1, 4	Nigeria
Bustinduy, 2013[51]	Kenya	CS	2013	SC	4.7	(5–18)	HW	Holo, perennial	Asymptomatic	1, 8	*Schistosoma haematobium*-endemic area

AL: *Ascaris lumbricoides*; CC: case-control study; CM: cerebral malaria; CO: cohort study; CS: cross-sectional study; HW: hookworms; NA: not available/not provided/not mentioned/not specified; *Pf*: *Plasmodium falciparum*; PG: pregnant mother; *Pv*: *Plasmodium vivax*; RCT: randomized control trial; RDT: rapid-onsite diagnostic test for malaria; SC: school age; SCM: severe and complicated malaria; STH: soil-transmitted helminths; *TT*: *Trichuris trichiuria*; UM: uncomplicated malaria; U5:under 5 years old.
[a]species focused in the present study median; [b]1: age; 2: gender; 3: ethnicity; 4: socioeconomic status; 5: BMI; 6: mean corpuscular volume; 7: nutrition; 8: other infections; 9: duration of symptoms/fever; 10: treatment status; 11: clinical status; 12: parasitaemia; 13: splenomegaly; 14: residential location/location cluster/spatial factors; 15: stunting; 16: inflammation; 17: HIV status; 18: marital status; 19: water treatment; 20: reported soil eating, 21: other geohelminth; 22: education; 23: place of birth, 24: parity; [c]median and IQR; [d]<15% of patients with HIV included; [e]median; [f]14.3% (35/256) under 15 years.

Table 2. Association of malaria infections and helminthes stratified by species of helminth

Description	Target group	Number of included studies	Reference	Summary OR (95% CI)	I^2 test (%)
Co-infection with Ascaris	Adults (nonpregnant)	5	8, 9, 27, 44, 48	1.21 (0.64–2.29)	85
	Pregnant women	5	10, 34, 38–40	1.13 (0.58–2.18)	88
	School children	2	8, 44	1.32 (0.39–4.43)	93
	Pre-school children	2	42, 49	3.13 (0.81–12.1)	73
Co-infection with hookworms	Adults (nonpregnant)	7	8, 9, 42–44, 48, 49	0.81 (0.43–1.52)	82
	Pregnant women	7	10, 34, 37–40, 45	1.36 (1.17–1.59)	0
	School children	6	8, 42–44, 47, 49	1.48 (0.85–2.57)	76
	Pre-school children	2	42, 49	3.13 (0.81–12.1)	73
Co-infection with Trichuris	Adults (nonpregnant)	3	8, 9, 44	1.16 (0.73–1.86)	0
	Pregnant women	5	10, 34, 37–39	1.15 (0.92–1.45)	0
	School children	3	8, 9, 44	1.58 (0.87–2.88)	21
	Pre-school children	1	8	2.77 (1.15–6.65)	NA
Co-infection with any STH	Adults (nonpregnant)	5	8, 9, 28, 36, 44	1.22 (0.84–1.77)	72
	Pregnant women	3	38, 40, 45	2.5 (0.88–7.09)	83
	School children	3	8, 9, 30	1.71 (1.03–2.82)	72

NA: not applicable; STH: soil-transmitted helminth.

co-infection on anemia in pregnant women. One study on pregnant women (n=816)[26] gave a significantly lower mean Hb level in the co-infected group (MD: −1.1; 95% CI: 0.19–2.39). Another study (n=111)[29] showed a significantly lower mean Hb level in those co-infected with severe malaria and STHs compared with those non co-infected with STH (MD −1.1; 95% CI: −0.06 to 0.19). Funnel plots show no obvious publication bias (not shown).

Subgroup analysis

In the summary estimate of four studies on pregnant women,[10,37–39] the risk of co-infection with malaria and STHs was found to be higher in the primigravidae than that in the multigravidae (summary OR: 1.61; 95% CI: 1.3–1.99; I^2: 0%). Inadequate data precluded us from performing subgroup analysis with the gradient of parasitemia.

Sensitivity analysis

Among non-pregnant adults, the significant positive effect of co-infection with any STH on anemia remained even after removal of a study carried out in an unstable malaria transmission area[46] (summary OR: 1.99; 95% CI: 1.35–2.94; I^2: 14%). Of interest, the 95% CI showed a narrow width and the observed value of heterogeneity dropped from initial 79% to 14%; the latter indicated that heterogeneity might no longer be an important issue. However, the strength of association has become smaller (i.e., from summary OR 2.91 to 1.99), indicating malaria in areas of stable transmission has a pronounced effect on anemia.

Discussion

The present analysis on available data reveals that malaria–STH co-infection gives complex outcomes with regard to the occurrence of anemia. This occurrence may reflect the complex interaction between STHs and *Plasmodium* parasites and the variability of the consequences of the host's immunological responses.

It is reasonable to assume that the immune system of co-infected individuals would differ from that of non co-infected individuals. Co-infecting parasites may interact either positively or negatively in the receptive hosts through a range of mechanisms including resource competition, immune-mediated interactions and direct interference.[5] The significant positive association between malaria and any STH infection in school children in the present analysis could be explained in a socioeconomic context. The high risk of geohelminth infection (i.e. STH in this case) in children has been associated with their particularly poor hygiene (at home) and recreational or other activities that regularly bring them close to areas contaminated with human feces.[15,33]

Based on available data, we found the positive association between malaria and hookworm was pronounced in pregnant mothers; a stream of bioimmunological mechanisms appeared to be involved in such interactions. Not all of these interactions are mutually exclusive and some are worthy of special mention.

A synergistic effect of co-infection with hookworm

T-helper type 2 or T-helper type 1

The resolution of *Plasmodium* infection requires a coordinated succession from a T-helper type 1 (Th1) to a T-helper type 2 (Th2) type response. Anything that upsets the timing or balance of this process can lead to chronic or severe infection.[47] The immunoregulatory effects of helminths, and/or which allow for their own long-term

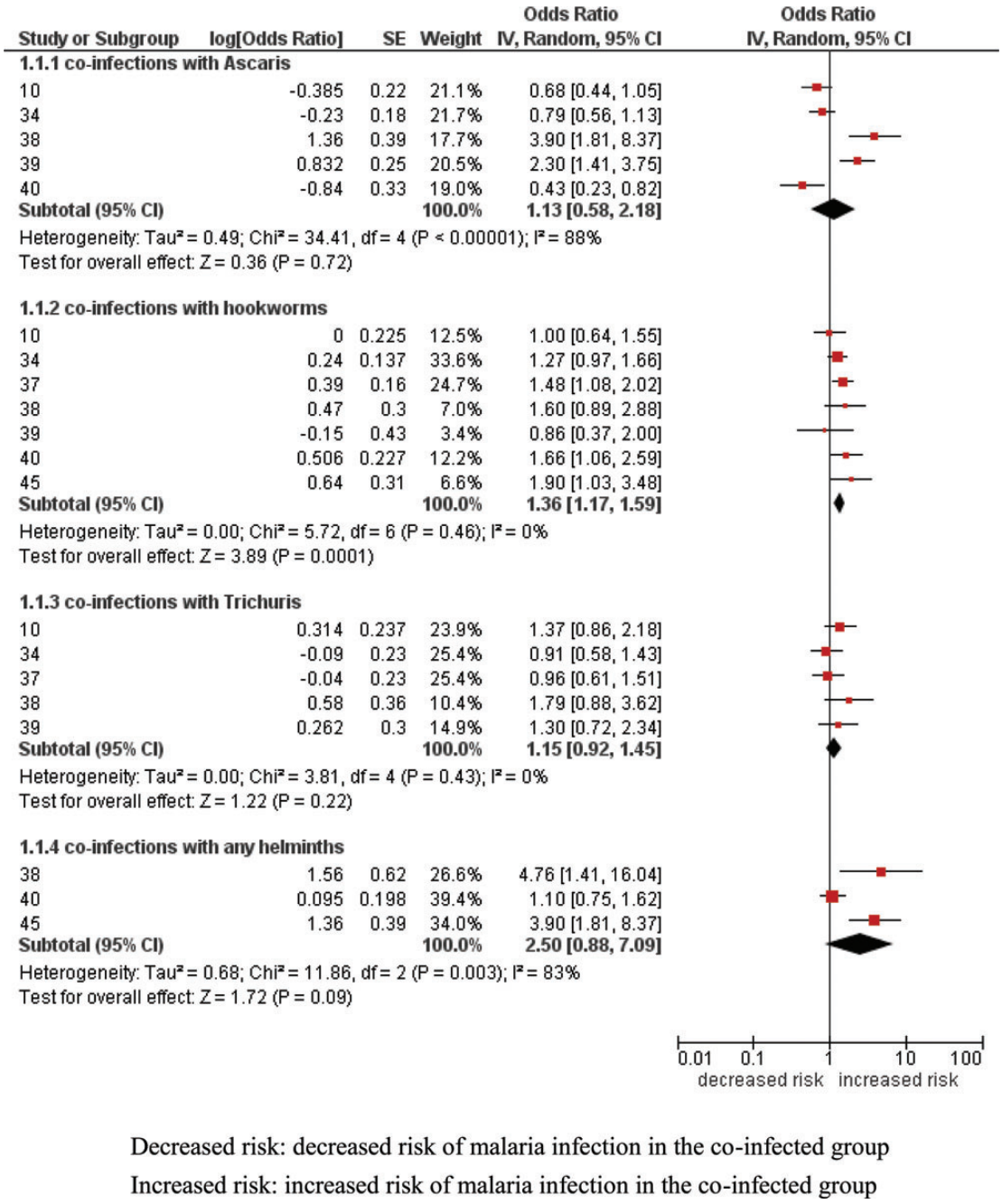

Figure 2. Relationship between malaria and helminth co-infections in pregnant women

survival in the host[34] could give a 'spill-over' effect that impairs the host immune response required to protect against or eliminate malaria parasites.[34,63,67]

Helminth infections are often more chronic and long-lived than infections caused by *P. falciparum*. The observed positive association between helminths (hookworms in this case) and asymptomatic *P. falciparum* parasitaemia may imply a relationship between an increased likelihood of *P. falciparum* infection and a reduced likelihood of clearing *P. falciparum* and/or to a reduced likelihood of developing symptoms and seeking medication.[34] Of note, the epidemiology of hookworm varies greatly between geographical locations. This unequal distribution might be partly attributed to a positive association of co-infection with hookworm as most of the primary studies were conducted in areas where hookworm infection was more prevalent. A study in Thailand also reported that the incidence of hookworm was 57%, while those of *Ascaris* and *Trichuris* were 6% and 15%, respectively.[35] When considering the association between hookworm and malaria, an unmatched bias could not be ruled out as higher prevalence of hookworms compared with that of malaria could be due to the relatively longer

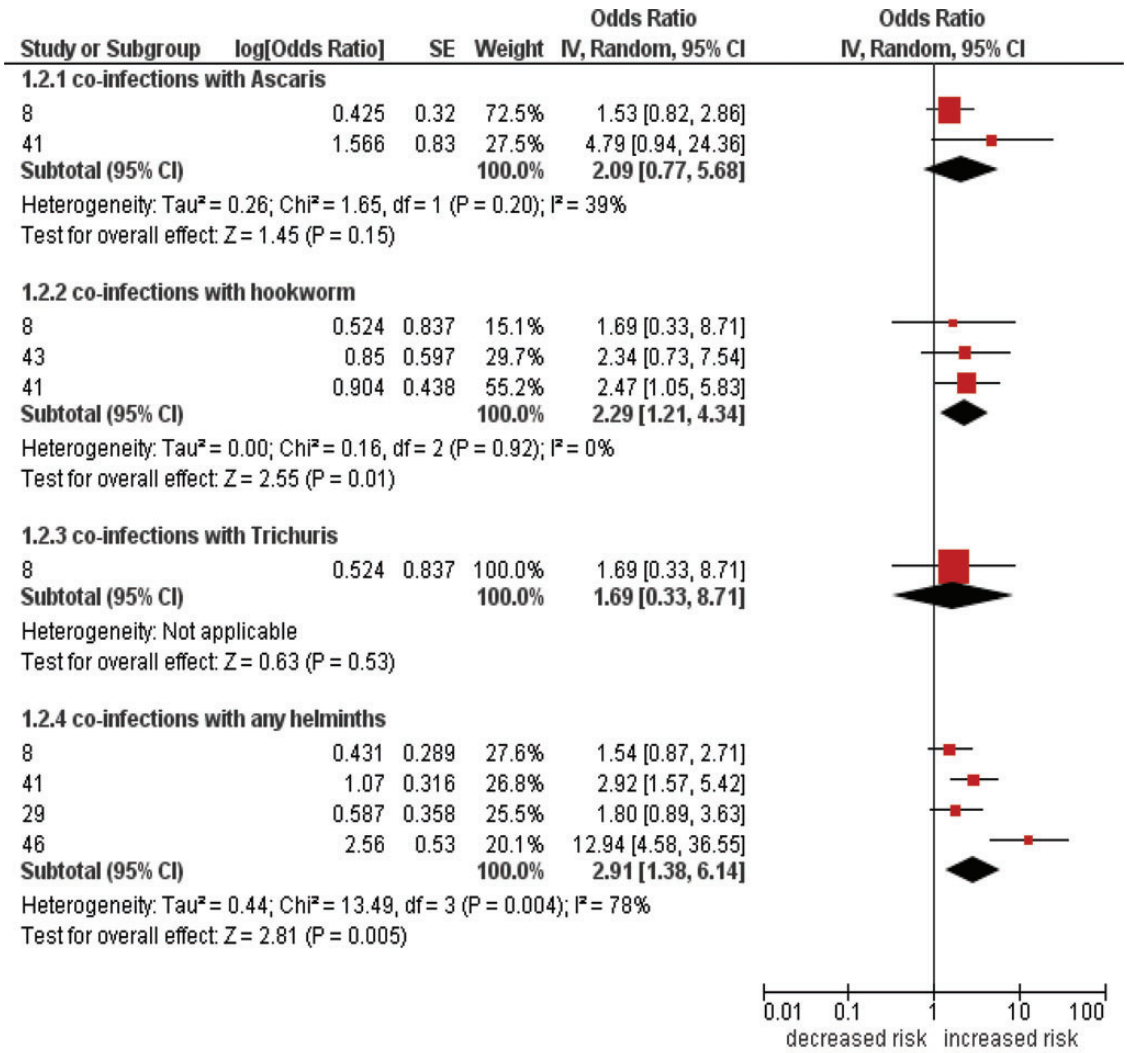

Figure 3. Effect of malaria–helminth co-infection on anemia in non-pregnant adults

life span of hookworms. We found a higher association of malaria and STH co-infection in the primigravidae. This could be explained by immunobiological mechanism. Pregnancy increases the risk of malarial infection in relation to the shifts in immunity from Th1 to Th2 responses. During normal pregnancy, the woman's immunity favors more Th2 cellular responses in the placenta. This is a favorable response as the proinflammatory Th1 cytokines have deleterious effects on pregnancy.[45,68] It is well known that the majority of helminths and their antigens are able to stimulate a Th2 host response.[45] This could have rendered the positive association between these two parasites more likely.

Immune hyporesponsiveness

Common functions of dendritic cells (DCs) during the different stages of parasitic infection include antigen-processing and T-lymphocyte activation, followed by changes of their surface markers, migration pattern and cytokine production.[69] It is not unreasonable to assume that helminth induced immune hyporesponsiveness could contribute towards increased susceptibility to other infections prevalent in the region, notably malaria. Hartgers and co-workers[63] reported that the immune hyporesponsiveness induced during chronic helminth infection affects responses not only to helminth antigens but also to bystander antigens.

Is there protective effect of co-infection with *Ascaris*?

Our study could not find a negative association between *Ascaris* infection (in any age group and in women) and uncomplicated malaria, regardless of pregnancy status. There are three probable explanations as to why we could not find this negative association of *Ascaris* infection in the current review. First, the different

helminth infections have different immunological consequences. Interferon gamma (IFN-γ) production in response to parasite antigens is depressed in hookworm infections, but not in *A. lumbricoides* infection.[9,67] Perhaps, among the populations reported in these studies, the IFN-γ production might have not yet reached the threshold limit of Th2 activation. Second, some low grade or moderate infection with *A. lumbricoides* that could have contributed to this association may have been overlooked. Third, we are not sure whether the primary studies had adequate power to detect differences in the two groups as most of them did not provide information on sample size calculation.

In a RCT, it was found that treatment of *Ascaris* in adults, was associated with a two-fold increase in malaria parasitaemia, suggesting a protective effect of the *ascaris* co-infection.[45] However, a concern is that this effect could also be due to intrinsic immunomodulatory properties of an anti-helminthic drug, levamisole.[65]

Anemia in co-infected patients

Findings of the present study suggest that malaria-helminth co-infection has an impact on anemia. The postulated mechanisms by which *Plasmodium* infection causes anemia include an increased destruction of red blood cells (RBCs) through hemolysis and increased splenic clearance of infected and uninfected RBCs;[46] and cytokine-induced dyserythropoiesis.[42,46] Hookworms contribute to anemia by direct intestinal blood loss,[4246] nutritional theft and impairment of appetite due to immunological factors.[46] Over a prolonged period, even small hookworm loads may cause blood loss sufficient to deplete the body's iron stores.[70] Taken collectively, co-infection with *Plasmodium* species and hookworm is likely to increase the severity of anemia because of the distinct mechanisms by which each parasite causes anemia.[3,71] The present analysis showed that the level of Hb in co-infected populations was significantly lower compared with non co-infected populations. This suggests that the anemia observed in co-infected populations was not likely to be a direct result of dietary deficiency alone.

The majority of helminths and their antigens are able to stimulate a Th2 host response.[45] IL-4 (i.e., Th2 cytokine) could increase iron uptake and storage in activated macrophages. The resultant diversion of iron from developing erythroblasts[72] could also contribute to anemia. In this way, Th2 response modulates both splenic and macrophage functions and thus exacerbates anemia in those cases already infected with malaria.[45]

Is the co-infection due to the effect of polyhelminths?

Although we have attempted to provide evidence on the relationship between malaria and particular species of STH, many patients in primary studies might have co-infections with more than one intestinal helminth. Hence, interspecies association in hosts is a concern. However, a recent review, assessing data from South America has highlighted the lack of evidence to show that the three helminthic infections are heavily inter-dependent.[73]

Study limitations

We acknowledge limitations in the present study. The confirmation of malaria or STH in the primary studies may have introduced some bias due to a variety of reasons: the limited sensitivity of microscopy for malaria (i.e., false negatives); misclassification of low to moderate intensity intestinal helminth infections as negatives; temporal variation in excretion of epg in hours or days and the effect on the sensitivity of single sampling; variations in helminth infection intensities; and variations in the definition of malaria.[43] Published studies have documented that obtaining only a single fecal sample for *A. lumbricoides* and *T. trichiuria* can lead to underestimation of infection rates by up to 50%.[54,74] Sensitivity for low infection rates increased from 20 to 54% when three fecal samples were examined on separate days.[74,75]

Although confounding factors were addressed in many of the primary studies identified for the current analysis, it is likely that there still may be unmeasured confounding factors which might have induced bias to this meta-analysis. There could have been a temporal mismatch between the data, which came from several different time points, ranging from 2003 to 2013. In those areas, we were not able to take into account the possible effect of any health intervention (related to malaria, STH infection or both). Furthermore, ecological bias is a concern as the data are collected from disparate populations

Public health implications

The summary estimates suggest that there is malaria-STH co-infection pertinent to hookworm, albeit with some limitations in the individual studies. We would suggest that combined intervention would be particularly relevant for vulnerable populations who are at the highest risk for anemia, such as children and pregnant women. The potential additive benefit of combining interventions targeted at anemia is highlighted by a study in Sri Lanka which showed that mebendazole given in combination with iron-folate can improve hemoglobin and iron status more than iron-folate alone.[4] However, relevant interventions should be tailored to the epidemiological conditions so that resources are optimally utilized.[47] If malaria does have a positive association with implicated helminth infections, acquisition of immunity against *P. falciparum* could alter the course of disease in these co-infected patients.[67]

Conclusions

The findings of the present study suggest that there are variabilities in the prevalence of malaria–STH co-infection as well as in the impact of the co-infection on anemia, which is dependent on the age, immune status of the host and the common social or environmental factors. Well-designed prospective studies with adequate samples are recommended to substantiate these points.

Supplementary data are available at Transactions Online (http://trstmh.oxfordjournals.org/).

Authors' contributions: JWM, MT conceived the study; MAW, MT, CN designed the study protocol; CN, VNW carried out the literature review; CN entered data, analyzed and cross-checked by VNW and SFW; MT, MAW, JWM, VNW, SR, CN interpreted these data; CN wrote the manuscript with the help of MAW and VNW; JWM, MT, SFW, SR critically revised the manuscript for intellectual content. All authors read and approved the final manuscript. MAW, VNW and CN are guarantors of the paper.

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