

The Impact of Malaria and Gastrointestinal Helminthiasis Co-infection on Anaemia and Severe Malaria among Children in Bugesera District, Rwanda

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Authors' contributions

This work was carried out in collaboration between all authors. Authors UM and BJB were involved in the conception, design, data collection, analysis and interpretation. Authors UN and MT' were involved in data collection and processing. Author MJ was involved in data analysis and interpretation. All authors were involved in the manuscript preparation and approval of the final version submitted.

Original Research Article

ABSTRACT

Aims: Determine the impact of malaria and gastrointestinal helminthiasis co-infection on anaemia and severe malaria among children aged 1 – 15 years.

Study Design: A cross sectional study was carried out.

Place and Duration of Study: The study was carried out in fifteen health centres of Bugesera District – Rwanda, between the months of April and October 2014.

Methodology: A total of 465 children were enrolled. Finger prick blood and stool were collected and examined according to the established standard methods. Data were double entered into EPI info software (Center for Disease Control and prevention, USA) and analysed using STATA Version 12.

Results: The overall prevalence for malaria, helminthiasis and anaemia was 30.8 %, 47.5% and 30.1% respectively. The prevalence of malaria and helminthiasis was highest in the age group of 6 -10 years. The anaemia prevalence was highest in the age group of 1 – 5 years. The prevalence of malaria and helminthiasis co-infection was 61.5% while the associated anaemia prevalence was 38.5%. Severe malaria was dominant in co-infected children (Chi1= 31.5222, $P < 0.000$).

Conclusions: Malaria and helminthiasis co-infection is a better predictor of anaemia than either malaria or helminthiasis. Malaria and helminthiasis co-infection was significantly associated with severe malaria. The impact of malaria and helminthiasis co-infection reported in this study needs further investigation.

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1. INTRODUCTION

Malaria and helminthiasis are common parasitic diseases of the tropics. Malaria is one of the leading causes of mortality especially of children under five years, but helminthiasis is the leading cause of morbidity. Both diseases are major public health concerns in the endemic regions. In addition, different helminthes have different effects when in dual co-infection with *Plasmodium* species [1-3]. Most importantly, these co-infections lead to profound immune conflict because of the different immune control mechanisms with antagonistic mediators [4-7].

One of the main impacts of malaria parasites and helminthic infections is anaemia. Malaria parasites cause anaemia through haemolysis and increased splenic clearance of infected and uninfected red blood cells. On the other hand, intestinal helminthes are significant causes of anaemia as a result of direct blood loss and nutrient depletion [8]. Based on the distinct mechanisms by which malaria parasites and helminthes reduce haemoglobin levels, it can be speculated that their co-infections enhance the risk of anaemia. The different reports from Ethiopia, Kenya, Nigeria, Tanzania and Thailand indicate an additive impact of the co-infections on anaemia [1, 2, 8]. However, the impact of malaria and helminthiasis co-infections in malaria endemic areas like Rwanda is largely under reported. We therefore, investigated the role played by *Plasmodium* species and helminthes co-infections on progression of anaemia and severe malaria among school and pre-school aged children in Bugesera District, Rwanda.

2. METHODOLOGY

2.1 Study Design and Area

A cross sectional study was carried out among school and pre-school aged children from fifteen health centers (Gakurazo, Gashora, Gihinga, Juru, Kamabuye, Mareba, Mayange, Mwogo, Ngeruka, Ntarama, Nyamata, Nyarugenge, Nzangwa, Rilima, Ruhuha) in Bugesera District, Rwanda. The study was carried out between the months of April and October 2014.

2.2 Inclusion Criteria

The study was conducted on children between the age of 1-15 years (pre-school age is 1-5years, school age is 6-15 years) who had signs and symptoms of malaria infections and attending the health centres in Bugesera District, Rwanda. Secondly, those who were diagnosed for *Plasmodium* and helminthes species but had not taken antimalarial and antihelminths within four weeks prior to screening were included. Thirdly, and who consented and assented to the study was included as well.

2.3 Exclusion Criteria

The study excluded children with concomitant infections that warrant admission in the Hospital and the Health Centres in the study area.

2.4 Data Collection

All participants at the health centres with malaria symptoms were finger pricked and the blood sample for malaria diagnosis, haemoglobin determination was taken. Stool for helminthes diagnosis was obtained as well.

2.5 Blood Smear Microscopy for Malaria Parasites

Thick and thin smears were prepared on a single slide for each patient from capillary blood by finger pricking using a sterile lancet. Each blood smear was stained with May Grunwald Giemsa and examined under the oil immersion microscope objective. Blood smears (thick) were reported negative after examination of 300 high-power fields. The type of *Plasmodium* spp and parasitemia were determined according the WHO standard procedure [11]. Only *Plasmodium falciparum* was identified in all the samples.

2.6 Determination of Haemoglobin Concentration

Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer, Hemocue Hb 201 analyzer (HemoCue, Angelholm, Sweden) and specially

designed microcuvette (Hemocue Hb 201 Microcuvette, Hemocue, Angelholm, Sweden).

2.7 Kato-Katz Technique for Helminth Eggs

Stool containers were provided to the participant for specimen collection. Intestinal helminths were detected microscopically by wet preparation and by Kato–Katz method [10,11,12].

2.8 Data Analysis

Data was double entered into EPI info software (Center for Disease Control and prevention, USA) and analyzed using STATA Version 12 (STATA Corp., Texas, USA) and R software for Graphing using Geographical Information System (GIS) in the study area.

Parasite counts were normalized by log transformation, averaged and at that time back transformed to the original scale. Helminth infection intensities were calculated as geometric mean of eggs per gram of faeces and malaria parasites per microliter of blood. The Chi-square test was used to compare proportions and to test for association between malaria, anaemia and helminth infections' prevalence.

In the multivariate analysis, presence or absence of infection or anaemia was compared among schools, age groups, sexes and other infections using logistic regression analysis fitted as a generalized linear model with a logic link function and adjusting for possible clustering among siblings. All predictors were initially tested for significance separately and then equally in a multi-variable model. Box plots and bar chart were drawn using R version 3.3.1. The rest of the graphs were drawn using STATA software. Tests were deliberated statistically significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

The study enrolled 465 pre-school and school age children (1 – 15 years). The mean age was 7.1 years while the median was 6 years. Pre-school children were 166 (37.5%). Overall 200

(43%) of the participants never attended school. According to the wealth index, 207 (44.5%) of the study participants fell into the highest quartile while 83 (17.9%) were in the lowest quartile. Most of the children, 99% were from a rural but suburb set up.

Out of 465 children enrolled, the overall prevalence for malaria, intestinal parasites and anaemia was, 30.8 %, 47.5% and 30.1% respectively. Malaria and anaemia prevalence were spread evenly across age groups, while intestinal parasites were more prevalent among children in the (6-10) age group (55.4%, $P = 0.0142$) compared to other age groups. With respect to sex, the malaria, anaemia and helminthiasis prevalence were equally distributed. Anaemia was more prevalent among children attending nursery schools (60%, $P = 0.0322$). On the other hand malaria was more prevalent among children from the lowest wealth index (45.8%, $P = 0.0225$). The results are summarized in Table 1 below.

Out of the 465 children included in the analysis, 45 (9.68%, CI: 7.15 – 12.73) were infected with at least one of the helminthes *Ascaris lumbricoides*, *Trichuris trichiura*, and *Ancylostoma duodenale/Necator americanus*. Thirty nine children (86.67%) were heavily infected with *A. lumbricoides*. Whereas 4 children (8.89%) had light *T. trichiura* infection, only 2 children (4.4%) were infected with *A. duodenale/N. americanus*. The results are summarised in Table 2 below.

The prevalence of malaria and helminthiasis co-infection and anaemia was 61.5% and 38.5 % respectively. The results summarized in Table 3 below also indicate that there is a statistically significant relationship between malaria and anaemia status (Chi1= 25.2684, $P < 0.000$). However, these results suggest that there is no statistical significant relationship between helminthiasis and anaemia status. (Chi1= 0.7593, $P = 0.384$).

Multivariate logistic regression analysis was also performed to identify predictors of anaemia. The results are indicated in Table 4 below.

Table 1. The overall prevalence of malaria, gastrointestinal helminthiasis and anaemia across social –economic and demographic characteristics

Overall prevalence	Total examined 465	Malaria (% 143 (30.8)	Gastrointestinal helminthiasis (%) 221 (47.5)	Anaemia (%) 140 (30.1)
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Age					
children(1-5 Yrs)	166	40 (24.1)	63 (38)*		56 (33.7)
children(6-10 Yrs)	193	73 (37.8)	107 (55.4)		61 (31.6)
children(11-15 Yrs)	106	30 (28.3)	51 (48.1)		23 (21.7)
Sex					
Male	210	62 (29.5)	92 (43.8)		62 (29.5)
Female	255	81 (31.8)	129 (50.6)		78 (30.6)
Level of education					
None	200	56 (28)	79 (39.5)*		73 (36.5)
Nursery	45	18 (40)	27 (60)*		14 (31.1)
Primary	214	69 (32.2)	112 (52.3)		53 (24.8)
Secondary	6	0 (0)	3 (50)		0 (0)
With medical assurance					
No	15	1 (6.7)	8 (53.3)		5 (33.3)
Yes	450	142 (31.6)	213 (47.3)		135(30)
Wealth index					
Lowest	83	38 (45.8)*	43 (51.8)		35 (42.2)
Second	175	52 (29.7)	83 (47.4)		58 (33.1)
Highest	207	53 (25.6)*	95 (45.9)		47 (22.7)

Note:*(Reference group)

Table 2. Species of gastrointestinal nematodes with the respective prevalence

	Species of gastrointestinal nematodes		
	<i>Ascaris lumbricoides</i>	<i>Trichuris trichiura</i>	<i>Ancylostoma duodenale/ Necator americanus</i>
Prevalence	86.67	8.89	4.4
Geometric mean parasite count	22.81(17.97 27.65)	20.31 (7.4 33.23)	
Maximum density	67.53	28.14	4.9
No. infected	39	4	2

Table 3. The anaemia status among children co-infected and non-co-infected with malaria and helminthiasis

Overall prevalence	Total examined	Without anaemia (%)		With anaemia (%)	
	465	325	(69.9)	140	(30.1)
Malaria					
Negative	322	248	(77)	74	(23)
Positive	143	77	(53.8)	66	(46.2)
Helminthiasis					
Negative	420	291	(69.3)	129	(30.7)
Positive	45	34	(75.6)	11	(24.4)
Co-infection	13	8	(61.5)	5	(38.46)

Table 4. Multivariate logistic regression analysis showing important predictors of anaemia with adjusted odds ratios confidence interval and p-values

		Adjusted OR	CI	P-value
Malaria	Negative	1		
Malaria	Positive	2.87	(1.89 - 4.37)	<0.000
Helminthiasis	Negative	1		
	Positive	0.73	(.36 - 1.4)	0.385
Malaria-Helminthes co-infection	No	1		
	Yes	3.1	(2.278 - 4.83)	<0.000

Table 5. Malaria and helminthiasis co-infection and severity of malaria

Co-infection Helminthes-Malaria	Malaria severity		P-value
	Not severe	Severe	
No co-infected	138(100%)	5 (100%)	***
co-infected	129(93.5%)	1 (20%)	
	9(6.5%)	4 (80%)	<0.000

Note: *** (Reference group)

In our study, there were 13 malaria-helminthes co-infection cases. Out of these, 5 were found to have severe malaria. Comparing severity of malaria with malaria-helminthes co-infection status, the chi-square test shows a strong relationship (Chi²= 31.5222, $P < 0.000$). This finding is indicated in Table 5 below.

4. DISCUSSION

In this study, the malaria prevalence is lower than reported elsewhere [1, 13]. However, our findings compare well with the earlier report from Cameroon [14]. In their report [15], the prevalence of malaria, helminthiasis and anemia in 425 children was; 64%, 38% and 31% respectively. Even, though, they reported higher malaria prevalence than in our study, the prevalence of anemia was not significantly different in both studies. This is could be due to the lower prevalence of helminthiasis in their study than in ours. Furthermore, in their study the prevalence of the co-infection was 25% which is significantly lower than our findings (62%). This discrepancy could be due to the fact that the impact of malaria and helminthiasis co-infection on anemia is influenced by the species of the helminthes involved in the co-infection. In a similar study [1], the prevalence of the co-infection of *P. falciparum* with hookworm was 60%. However, the prevalence of anemia (34%) was attributed to malaria. This is because the co-infection was not significant on the multivariate logistic regression analysis of (OR= 1.320, $P = 0.064$). Their finding contradicts the result of our study. It is necessarily so, because, in our study the most prevalent helminth was *A. lumbricoides* (86.7%). Therefore, our finding suggests that the co-infection of *P. falciparum* and *A. lumbricoides* is a better predictor of anaemia than *P. falciparum* and *A. duodenale/N. americanus*.

Our results differ from a similar study conducted in Cameroon [13], where the prevalence of the co-infection was lower, 22% but with higher prevalence of anemia, 42%. This is perhaps

because the malaria prevalence was higher than double (77%) of our study.

Interestingly, our study reports for the first time that malaria and helminthiasis co-infection is a better predictor of anemia (OR = 3.1, $P = <0.000$) than malaria and helminthiasis alone (OR = 2.9, $P = <0.000$ and OR = 0.7, $P = 0.385$ respectively). Helminthiasis is a chronic disease which potentially predisposes and enhances the establishment of *Plasmodium* and the subsequent pathogenesis. Secondly, we report the prevalence of helminthiasis of 50% and 0 % for malaria and anaemia among the secondary school children in the age group 11 -15 years. This could be due to the age associated activities and the social economic status.

The findings of this study show a significant relationship between malaria – helminthiasis co-infection and severe malaria (Chi² = 31.522, $P = <0.000$). This is in agreement with other studies elsewhere [2, 8]. This is mainly due to the inherent antagonism of the immune system and other immune regulatory mechanisms. It is now established that chronic helminthiasis skews the immune system into a Th2 response mediated by IL-4, which is antagonistic with IFN- γ , the mediator of a Th1 immune response for controlling *P. falciparum* infection and hence malaria. Of late, it has also been reported that helminthic infections activate T regulatory cells that suppress Th1 immune responses [16].

5. CONCLUSION

The prevalence for both malaria and helminthiasis was higher in the age group of (6 – 9) years than in the age group of (1 – 5) years. There was no malaria and anaemia among the secondary school children age group (11 – 15) years while helminthiasis was 50%. On the contrary, the anaemia prevalence was higher in the age group (1 – 5) years than the age group (10 – 14) years. The most prevalent helminth in Busegera district of Rwanda was *A. lumbricoides* (86.7%), followed by *T. trichiura* (8.9%) and hook worm (4.4%).

Malaria – helminthiasis coinfection was major predictor of anaemia compared to malaria and helminthiasis alone. The prevalence of anaemia due to helminthiasis was lower than in participants who were negative for both diseases and the co-infection. This study provides the first report on the impact of malaria – helminthiasis co-infection on anaemia in Rwanda. In addition, we provide the first report on the malaria – helminthiasis co-infection as a significant predictor of severe malaria in the East African region. The impact of malaria - helminthiasis co-infection reported in this study calls for further investigations.

ETHICAL APPROVAL

The protocol was implemented as approved by the National Health Research Committee, and Rwanda National Ethics Committee.

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COMPETING INTERESTS

The authors declare that they have no competing interests whatsoever in relation to this manuscript.

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