



1 **The impact of malaria and helminthiasis co-**
2 **infection on anemia and severe malaria among**
3 **children in Bugesera district, Rwanda**

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20 **ABSTRACT**

21 **Aims:** ~~To~~ determine the impact of malaria and soil transmitted helminthiasis co-infection
22 on anemia and severe malaria among children aged 1 – 15 years.

23 **Study design:** A cross sectional study was carried.

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

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

30 **Results:** The overall prevalence for malaria, helminthiasis and anemia was 30.8 %,
31 47.5% and 30.1-% respectively. The prevalence of malaria and helminthiasis was
32 highest in the age group of 6 -10 years. The anemia prevalence was highest in the age
33 group of 1 – 5 years. The prevalence of malaria and helminthiasis co-infection was
34 61.5% while the associated anemia prevalence was 38.5%. Severe malaria was
35 dominant in co-infected children ($\chi^2 = 31.5222$, $P < 0.000$).

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

40 **Keywords:** Malaria, helminthiasis, coinfection, anemia, severe-malaria, children,

41 Busegera-district, Rwanda

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, 2, 3]. Most importantly, these co-infections lead to profound immune conflict because of the different immune control mechanisms with antagonistic mediators [4, 5, 6, 7].

One of the main impacts of malaria parasite and helminthic infections is anemia. Malaria causes anemia, through hemolysis and increased splenic clearance of infected and uninfected red blood cells. On the other hand, intestinal helminthes are significant causes of anemia as a result of direct blood loss and nutrient depletion [8]. Based on the distinct mechanisms by which malaria parasites and helminthes reduce hemoglobin levels, it can be speculated that their co-infections enhance the risk of anemia. The different reports from Ethiopia, Kenya, Nigeria, Tanzania and Thailand indicate an additive impact of the co-infections on anemia [1, 2, 8, 9]. 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 anemia and severe malaria among school and pre-school aged children in Bugesera District, Rwanda.

METHODOLOGY

Study design and area

This was a cross sectional study 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.

66 **Inclusion criteria**

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

72 **Exclusion criteria**

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

75 **Data collection**

76 All participants at the health centers with malaria symptoms were finger pricked and the blood sample
77 for malaria diagnosis, hemoglobin determination was taken. Stool for helminthes diagnosis was
78 obtained as well.

79 **Blood smear microscopy for malaria parasites**

80 Thick and thin smears were prepared on a single slide for each patient from capillary blood by finger
81 pricking using a sterile lancet. Each blood smear was stained with May Grunuwald Giemsa and
82 examined under the oil immersion microscope objective. Blood smears (thick) were reported negative
83 after examination of 300 high-power fields. The type of *Plasmodium* spp and parasitemia were
84 determined according the WHO standard procedure [10].

85 **Determination of hemoglobin concentration**

86 Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer,
87 Hemocue Hb 201 analyzer (HemoCue, Angelholm, Sweden) and specially designed microcuvette
88 (Hemocue Hb 201 Microcuvette, Hemocue, Angelholm, Sweden) [10].

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 [11, 12, 13].

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. Helminthic infection intensities were calculated as geometric mean of eggs per gram of feces and malaria parasites per microliter of blood. The Chi-square test was used to compare proportions and to test for association between malaria, anemia and helminthic infections' prevalence.

In the multivariate analysis, presence or absence of infection or anemia was compared among schools, age groups, sexes and other infections using logistic regression analysis fitted as a generalized linear model with a logit 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 $P < 0.05$.

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 anemia was, 30.8 %, 47.5% and 30.1% respectively. Malaria and anemia 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, anemia and helminthiasis prevalence were equally distributed. Anemia 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 table1 below.

Table1: The overall prevalence of malaria, intestinal parasites and anemia across social –economic and demographic characteristics.

	Total examined	Malaria infection (%)	Parasitic infection (%)	Anemia (%)
Overall prevalence	465	143 (30.8)	221 (47.5)	140 (30.1)
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)

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126 Out of the 465 children included in the analysis, 45 (9.68%, CI: 7.15 – 12.73) were infected with at
 127 least one of the helminthes *Ascaris lumbricoides*, *Trichuris trichiura*, and ~~Hook-worm~~. Thirty nine
 128 children (86.67%) were heavily infected with ~~*Ascaris lumbricoides*~~. Whereas 4 children (8.89%) had
 129 light ~~*Trichuris trichiura*~~ infection, only 2 children (4.4%) were infected with the hookworm. The results
 130 are summarized in table 2 below.



131 **Table 2: Species of helminthes with the respective prevalence**

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

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

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144 **Table 3: The anemia status among children co-infected and non-co-infected**

	Total examine d	Without anemia (%)		With anemia (%)	
Overall prevalence	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)

145 Multivariate logistic regression analysis was also performed to identify predictors of anemia. Variables
 146 included in the analysis were Malaria status, Helminthiasis status and Malaria-helminthiasis co-
 147 infection. The results are indicated in table 4.

148 **Table 4: Multivariate logistic regression analysis showing important predictors of**
 149 **anemia 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

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

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

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

Note: * (Reference group)**

Discussion

In this study, the malaria prevalence is lower than reported elsewhere [1, 14]. However, our findings compare well with the earlier report from Cameroon [15]. 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 *Ascaris lumbricoides* (86.7%). Therefore, our finding suggests that the co-infection of *P. falciparum* and *A. lumbricoides* is a better predictor of anemia than *P. falciparum* and hookworm.

Our results differ from a similar study conducted in Cameroon [14], 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 (Chi1 = 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 [17].

CONCLUSION

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

Malaria – helminthiasis coinfection was major predictor of anemia compared to malaria and helminthiasis alone. The prevalence of anemia 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 anemia 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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