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21 **ABSTRACT**

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

24 **Study design:** A cross sectional study was carried out.

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

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

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

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

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

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 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.

METHODOLOGY

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.

67 **Inclusion criteria**

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

73 **Exclusion criteria**

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

76 **Data collection**

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

80 **Blood smear microscopy for malaria parasites**

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

87 **Determination of haemoglobin concentration**

88 Haemoglobin concentration was determined using a portable haemoglobin spectrophotometer,
89 Hemocue Hb 201 analyzer (HemoCue, Angelholm, Sweden) and specially designed microcuvette
90 (Hemocue Hb 201 Microcuvette, Hemocue, Angelholm, Sweden).

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].

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 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 helminthic 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 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 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 table1 below.

Table1: The overall prevalence of malaria, gastrointestinal helminthiasis and anaemia across social –economic and demographic characteristics

	Total examined 465	Malaria (%) 143 (30.8)	Gastrointestinal helminthiasis (%) 221 (47.5)	Anaemia (%) 140 (30.1)
Overall prevalence				
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	43 (51.8)	35 (42.2)

			(45.8)*		
	Second	175	52 (29.7)	83 (47.4)	58 (33.1)
			53		
	Highest	207	(25.6)*	95 (45.9)	47 (22.7)

Note:*(Reference group)

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.

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

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, $P = 0.384$).

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

	Total examine d	Without anaemia (%)		With anaemia (%)	
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)

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

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

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 ($\chi^2 = 31.5222$, $P < 0.000$). This finding is indicated in table 5 below.

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

Co-infection Helminthes-Malaria	Malaria severity		P-value
	Not severe	Severe	
	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, 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 (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 [16].

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.

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COMPETING INTERESTS

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

AUTHORS' CONTRIBUTIONS

‘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 the manuscript preparation and approval of the final version submitted”.

ETHICAL APPROVAL

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

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233 **REFERENCES**

- 234 1. Kinung'hi SM, Magnussen P, Kaatano GM, Kishamawe C, Vennervald BJ (2014). Malaria and
235 Helminth Co-Infections in School and Preschool Children: A Cross-Sectional Study in Magu
236 District, North-Western Tanzania. PLoS ONE 9(1): 680–686.
- 237 2. Mulu Andargachew, Mengistu Legesse, Berhanu Erko, Yeshambel Belyhun, Demise
238 Nugussie, Techalew Shimelis, Afework Kassu, Daniel Elias and Beyene Moges. (2013).
239 Epidemiological and clinical correlates of malaria-helminth co-infections in southern Ethiopia.
240 Malaria J. 12:227.
- 241 3. Alemu Abebe, Yitayal Shiferaw, Aklilu Ambachew, Halima Hamid (2012). Malaria helminth co-
242 infections and their contribution for anaemia in febrile patients attending Azzezo health center,
243 Gondar, Northwest Ethiopia: A cross sectional study. Asian Pacific Journal of Tropical
244 Medicine, Volume 5, Issue 10, 803–809
- 245 4. Ateba-Ngoa Ulysse, Ayola Akim Adegniko, Jeannot F Zinsou, Roland F Kassa Kassa,
246 Hermelijn Smits, Marguerite Massinga-Loembe, Benjamin Mordmüller, Peter G Kremsner and
247 Maria Yazdanbakhsh (2015). Cytokine and chemokine profile of the innate and
248 adaptive immune response of schistosoma haematobium and plasmodium falciparum single
249 and co-infected school-aged children from an endemic area of Lambaréné, Gabon. Malaria
250 Journal, 14:94
- 251 5. Nacher Mathieu (2011). Interactions between worms and malaria: Good worms or bad
252 worms? Malaria Journal, 10:259
- 253 6. Wammes, L.J., Hamid, F., Wiria, A.E., de Gier, B., Sartono, E., Maizels, R.M., Luty, A.J., Fillié,
254 Y., Brice, G.T., Supali, T., Smits, H.H., and Yazdanbakhsh, M (2010). Regulatory T cells in
255 human geohelminth infection suppress immune responses to BCG and Plasmodium
256 falciparum. Eur. J. Immunol. 40: 437–442.

- 257 7. Hartgers, F.C., and Yazdanbakhsh, M (2006). Co-infection of helminths and malaria:
258 modulation of the immune responses to malaria. *Parasite Immunol.* 28: 497–506.
- 259 8. Degarege, A., Animut, A., Legesse, M., and Erko, B (2010). Malaria and helminth co infections
260 in outpatients of Alaba Kulito Health Center, southern Ethiopia: a cross sectional study. *BMC*
261 *Research Notes*, 3:143.
- 262 9. Brooker, S., Clements, A.C.A., Hote, P.J., Hay, S.I., Tatem, A.J., Bundy, D.A., and Snow, R.W
263 (2006). Co-infection with hookworm and malaria is associated with lower haemoglobin levels
264 and is common among African school children. *PLoS Med*, 74:258-271.
- 265 10. Bukusuba, J.W., Hughes, P., Kizza, M., Muhangi, L., Muwanga, M., Whitworth, J.A., and
266 Elliott, A.M (2004). Screening for intestinal helminth infection in a semi urban cohort of
267 pregnant women in Uganda. *Tropical Doctor*, 34: 27–28.
- 268 11. The World Health Organisation Report (1991). *Basic Laboratory Methods in Medical*
269 *Parasitology*. World Health Organization, Geneva.
- 270 12. Katz, N., Chaves, A., and Pellegrino, J (1972). A simple device for quantitative stool
271 thicksmeat technique in *Schistosomiasis mansoni*. *Revista do Instituto de Medicina Tropical*
272 *de Sao Paulo*, 14: 397–400.
- 273 13. Zeukeng, Francis ,Viviane Hélène Matong Tchinda, Jude Daiga Bigoga mail, Clovis Hugues
274 Tiogang Seumen, Edward Shafe Ndzi, Géraldine Abonweh, Valérie Makoge,Amédée
275 Motsebo, Roger Somo Moyou (2014). Co-infections of Malaria and Geohelminthiasis in Two
276 Rural Communities of Nkassomo and Vian in the Mfou Health District, Cameroon. 112(1):8-1
- 277 14. Nkuo-Akenji, Chi Pc, Cho JF, Ndamukong kk, Sumbele I (2006).Malaria and helminth co-
278 infection in children living in a malaria endemic setting of mount Cameroon and predictors of
279 anaemia. *J Parasitology* 92(6):1191-5
- 280 15. Pullan Rachel L., Peter W. Gething,Jennifer L. Smith, Charles S. Mwandawiro, Hugh J. W.
281 Sturrock, Caroline W. Gitonga, Simon I. Hay, Simon Brooker (2011). *Spatial Modelling of Soil-*

282 Transmitted Helminth Infections in Kenya: A Disease Control Planning Tool. DOI:
283 10.1371/journal.pntd.0000958

284 16. Elliott DE and Weinstock JV (2012). Helminth-host immunological interactions: prevention and
285 control of immune-mediated diseases. Ann N Y Acad Sci. Jan; 1247:83-96.