Plasmodium and Soil Transmitted Helminth co-infection: Epidemiological interaction and impact among children living in endemic areas of Bagamoyo, Coastal region of Tanzania

INAUGURALDISSERTATION
zur
Erlangung der Würde eines Doktors der Philosophie
vorgelegt der Philosophisch-Naturwissenschaftlichen Fakultät
der Universität Basel

von
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Basel, 2015

Genehmigt von der Philosophisch-Naturwissenschaftlichen Fakultät auf Antrag von
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Basel, den 21. April 2015

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Dekan

Dedication

To the memory of my late parents

To my brothers and sisters

To my compassionate husband Mohammed A.H Mwinyi and our lovely children (Kareem, Imran, Sabrinah, Karisah and Salim)

To Bagamoyo IDEA team, study participants and communities of the study areas

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List of abbreviations

ACT	Artemisinin Combination Therapy
ALU	Artemether-Lumefantrine
ACRP	Adequate Clinical and Parasitological Responses
BDH	Bagamoyo District Hospital
BRTC	Bagamoyo Research and Training Centre
DALYs	Disability Adjusted Life Years
DNA	Deoxyribonucleic Acid
EU	European Union
EPI	Expanded Program on Immunization
EDTA	Ethyl Diamine Tetra acetic Acid
EPG	Egg Per Gram
ELISA	Enzyme Linked Immune Sorbent Assay
FBC	Full Blood Count
FECT	Formalin Ethyl acetate Concentration Technique
GCP	Good Clinical Practice
GPS	Geographical positioning System
Hb	Haemoglobin
HIV	Human Immunodeficiency Virus
HRP	Histidine Rich Proteins
IHI	Ifakara Health Institute
ICH	International Conference on Harmonization
IBD	Inflammatory Bowel Diseases
IL	Interleukin
Ig	Immunoglobulin
ITNs	Insecticide Treated Nets
IRS	Indoor Residual Spraying
LLINs	Long Lasting Insecticidal Nets
LCF	Late Clinical Failure
mRDT	Malaria Rapid Diagnostic Test
MDA	Mass Drug Administration
MUAC	Mid Upper Arm Circumference

MCV	Mean Cell Volume
MCH	Mean Corpuscular Haemoglobin
NTDs	Neglected Tropical Diseases
NTDCP	Neglected Tropical Disease Control Program
NLFEP	National Lymphatic Filariasis Elimination Program
NIMR	National Institute of Medical Research
PCR	Polymerize Chain Reaction
pLDH	*Plasmodium* Lactate Dehydrogenase
RDT	Rapid Diagnostic Test
RCT	Randomized Controlled Trials
Swiss TPH	Swiss Tropical and Public Health institute
STH	Soil transmitted helminth
STHCP	Soil Transmitted Helminth Control Program
SP	Sulphadoxine-pyrimethamine
THMIS	Tanzania HIV/AIDS and Malaria Indicator Survey
UNICEF	United Nations Children's Fund
VHCW	Village Health Care Workers
WHO	World Health Organization
WHZ	Weight for Height Z-score
WAZ	Weight for Age Z-score

Acknowledgement

This PhD thesis was conducted under the umbrella of IDEA project- Tanzania, multicentred, African European global research program funded by the European Union (EU) designed to study the immunological interplay between helminth infections and HIV, tuberculosis (TB), and malaria in partnership with Swiss TPH and Ifakara health institute under the captainship of Prof. Marcel Tanner and Dr. Salim Abdulla respectively. I'm deeply grateful for the PhD opportunity provided, the knowledge, skills and exposures in the field of science as well as social-cultural, collaborations and strong partnership within the research institutes involved. It has been a long journey from the proposal and field work to the fruitful end today. I can only say Asante Sana, for the honored opportunity.

It has been a joyful, motivating, constructive and excellent journey since I met my supervisor who is more than mentor for his continuous unlimited, pleasant and highly stimulating scientific discussions which heighted my enthusiasm to accomplish each step of my work and become what I'm today. Prof. Blaise Genton, Jazakka Allah Kher.

I'm grateful and blessed to meet and getting to know one person who built a strong, young and energetic team which is always moving together to accomplish what is required. Her unique and beautiful lead will never perish in my life. Prof. Claudia Daubenberger, no words can express my deep gratitude in this journey. I believe our journey will continue to grow in many more ways Insha Allah.شكرا جزيلا .

I'm obligated to my young enthusiastic mentor, Dr. Steffi Knopp for her encouragement and endless support throughout this PhD work. She has been more than a leader to our IDEA study team in Bagamoyo within the helminth unit laboratory. Her shared experiences, the knowledge on parasitology and handling of helminth data has been of great importance in achieving what we have today. I'm very proud and feel blessed to find her and be part of my life in many ways. Steffi, nashukuru sana.

Special thanks go to the whole IDEA team in Bagamoyo, Tanzania and Swiss TPH, Switzerland specifically Fabrice, Nicole, Tobi and Julian. My fellow clinicians, colleagues, and the management of Bagamoyo district hospital (BDH) specifically the district immunization and vaccination officer (DIVO) Mr. Farah Mohammed. The work would never be possible without your support in each and every step. Thank you very much for all the support provided.

I'm not a person with many words and don't want to miss out anyone, however I can't finish this without mentioning a few names: Dr. Lweno, Dr. Mtoro, Dr. Jongo, Ummi, Ali Ali, Alisa, Dr. Shekalaghe, Dr. Saumu, Dr. Jennie Jaribu, Dr. Shao, Dr. Daniel, Dr. Khadija, Sammy Khagayi, Idda Romore, Lilian, Jackson, Maxmillian, Solomon, Dorcus, Agnes and Julius Ssempiira. Asanteni sana kwa kuniwezesha kwenye yote.

Additionally, I acknowledge the financial support as granted by the training unit of Ifakara health institute and the Basel city stipend commission for a successful completion of my PhD studies.

My deepest dedication goes to my husband, Mr. Mohammed Mwinyi who has been and is always besides me to support my carrier and our lovely children. Ishkushtish.

Summary

Background: Parasitic infectious agents rarely occur in isolation and multiparasitism is a norm among population living in poor resource settings of developing countries. Soil transmitted helminths (STH) and *Plasmodium falciparum* are among the most common parasitic infections contributing the highest burden of morbidity and mortality in the tropical and sub-tropical countries including Tanzania. Children are the most affected suffering from higher prevalence and heavy parasitic load of both *Plasmodium* and STH. Despite the fact that co-infections occur and cause severe morbidities, less is known and invested on how the two common parasites *Plasmodium* and STH interact. Epidemiological evidence is lacking and intervention efforts are ongoing based on single parasite approach. The progress of intervention program coupled with epidemiological surveys to evaluate the disease burden at the individual level is lacking. New knowledge on interactions and attributed risk is relevant to better understand the disease epidemiology in relation to interventions for policy decision, better management and tailored integrated control measures.

The overall goal and specific objectives: This PhD thesis aimed to explore interactions between *Plasmodium* and STH infections in Bagamoyo, coastal region of Tanzania, an area where both parasitic diseases are prevalent and large scale National control programs to prevent infections are ongoing. The specific objectives pursued were as follows i) to investigate performance of the diagnostic methods used to detect helminth infections among adults and children enrolled within the TB and malaria arms of IDEA project ii) to investigate the relation of STH and *Plasmodium* parasite prevalence rates among children enrolled in a community cross sectional survey of IDEA project, malaria arm iii) to investigate the impact of STH on malaria clinical presentation and treatment outcome among children enrolled in a case control study of IDEA project, malaria arm.

Methods: These studies were conducted as the malaria component of the IDEA project. This is a global research program funded by the European Union (EU) designed to study the immunological interplay between helminth infections and HIV, tuberculosis (TB), and malaria. The studies were conducted in four villages situated in the rural western area about 20 to 60 km from Bagamoyo town, Tanzania. To investigate the sensitivity and performance of the diagnostic methods (objective 1), stool samples were purposely selected for methods comparison from the community survey of adults and children screened within the IDEA arm of TB and malaria respectively. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann and polymerize chain reaction (PCR) methods were analyzed for common STH, hookworm and *S. stercoralis*. To investigate the relation of STH and *Plasmodium* parasite prevalence rates (objective 2), a community cross-sectional survey was conducted among children aged 2 months to 9 years inclusively. This community-based survey was also used to recruit asymptomatic *Plasmodium* parasitemia children (controls) for the case-control study below. The prevalence of *Plasmodium* parasitemia was around 10% within the study area and thus around 1,000 children from the community were screened and enrolled. To investigate the impact of STH on malaria clinical presentation and treatment outcome (objective 3), a case-control study with a semi longitudinal follow-up was conducted. Cases were enrolled in two different groups, cases with severe malaria and cases with uncomplicated malaria. Controls were children with asymptomatic *Plasmodium* parasitemia. The longitudinal short term observational part of the study consisted of the assessment of response to anti-malaria treatment in the three groups according to World

Health Organization (WHO) procedures (day 0, 1, 2, 3, 7, 14, 28, 42). The exposure in this case-control study was taken as presence or absence of an infection with at least one of the helminth species investigated. Included children were assessed at each visit (recruitment and follow up visits) by a qualified, trained study clinician for signs and symptoms of malaria and other common diseases using a structured questionnaire designed for the study.

In community survey and case-control study, stool, urine, adhesive tapes and blood samples were collected and examined using a broad set of quality controlled diagnostic methods for common STH (*Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Enterobius vermicularis*, *and Trichuris trichiura*), schistosoma species and *Wuchereria bancrofti*. Blood slides and malaria rapid tests (mRDTs) were utilized for *Plasmodium* diagnosis. Stool, urine and adhesive tapes were collected at the initial screening (both studies) and during the day 28 follow up visit (case-control study). In order to investigate the impact of STH on malaria clinical presentation and treatment outcome, children diagnosed with STH received a delayed anti-helminthic treatment at the end of study follow up (day 42).

Principle findings: The recruitment of the study was done between June 2011 and November 2012, covering all year seasonal variations. Among the adults and children samples used to investigate the diagnostic performance of the different methods, hookworm (10.0%) and *S. stercoralis* (7.4%) were the most prevalent STH followed by *T. trichiura* (1.9%), *A. lumbricoides* (0.2%) and *S. mansoni* (0.2%). Generally, more than 80% were low intensity infections. Using a direct method comparison as reference FLOTAC had a significantly higher sensitivity (93.8%) than Kato-Katz. This was not the case for PCR. Sensitivity of PCR for *S. stercoralis* diagnosis (17.4%) was significantly lower than that of Baermann method (47.1%). The direct method comparison revealed an equal sensitivity of the PCR and Kato-Katz methods for hookworm diagnosis (73.0%). A significantly negative correlation was found between the PCR cycle thresholds (Ct) values and microscopic egg per gram (EPG) or larvae counts of hookworm and *S. stercoralis* respectively.

Out of 1,033 children included in the cross sectional community survey, 283 (27.4%) were infected with any of the helminth infection. The most prevalent helminth species were *E. vermicularis* (18.0%), hookworm (9.1%) and *S. stercoralis* (6.9%). Other types of helminth isolated were *T. trichiura* (2.5%), *W. bancrofti* (1.4%), *S. haematobium* (0.3%) and *A. lumbricoides* (0.1%). No child was diagnosed with *S. mansoni*. Helminth infection prevalence increased with age, from infants (10.2%), pre-school aged (25.0%) and school aged children (33.5%). *S. stercoralis* was the most common species affecting infants and significantly associated with a higher risk of asymptomatic *Plasmodium* parasitemia [OR=13.0 (95% CI of 1.3 – 127.2)]. Asymptomatic *Plasmodium* parasitemia was associated with mild [OR=1.8 (95% CI of 1.0 – 3.3)] , moderate [OR=5.4 (95% CI of 2.9 – 9.8)] and severe anemia [OR=11.2 (95% CI of 4.2 – 29.9)] in children below two years and those above two years presenting with moderate [OR=3.1 (95% CI of 2.0 – 4.8)] or severe anemia [OR=7.3 (95% CI of 2.0 – 26.0)]. Neither *S. stercoralis*, *E. vermicularis*, hookworm nor any other investigated helminth infection was associated with wasting, underweight, thinness, stunting or anemia in our study population.

Out of 992 children analyzed for dual helminth and *Plasmodium* infection, the prevalence of *Plasmodium* mono-infection was 8.1% (80/992), helminth mono-infection 23.5% (233/992) and co-infection with

Plasmodium and any helminth species 5.0% (50/992). The prevalence rate of *Plasmodium*, specific STH species and co-infections increased significantly with age ($p< 0.001$) with school-aged children mostly affected except for *S. stercoralis* monoinfection and co-infections. There was a trend for STH infections to be associated with *Plasmodium* infection [OR adjusted for age group 1.4 (95% CI 1.0 - 2.1)], which was more marked for *S. stercoralis* [OR= 2.2 (95% CI of 1.1 - 4.3)]. Age and not schooling were risk factors for *Plasmodium* and STH co-infection.

In the case control study, there was a tendency for a protective effect of helminth on the development of clinical malaria [OR=0.6, 95% CI of 0.3 – 1.3] which was more marked for *E. vermicularis* species [OR=0.2, 95% CI of 0.0 – 0.9]. On the contrary, hookworm species tended to be associated with clinical malaria [OR= 3.0, 95% CI of 0.9 – 9.5]. In multiple conditional regression analysis, the overall protective effect was lower for all helminth infection [OR= 0.8, 95% CI of 0.3 – 1.9] but remained significantly protective for *E. vermicularis* species [OR= 0.1, 95% CI of 0.0 – 1.0] and borderline significant for hookworm species [OR= 3.6, 95% CI of 0.9 – 14.3]. Using ordinal logistic regression which better reflects the progression of asymptomatic *Plasmodium* parasitemia to severe malaria, there was a 50% protective effect with overall helminth [OR= 0.5, 95% CI of 0.3 – 0.9]. On the contrary, hookworm species was highly predictive of uncomplicated and severe malaria [OR= 7.8 (95% CI of 1.8 – 33.9) and 49.7 (95% CI of 1.9 – 1298.9) respectively]. Generally, children infected with STH had higher geometric mean time to first clearance of parasitemia.

Conclusion: Multiparasitism is common among children, but also in infancy, with school aged children bearing the highest dual burden of both *Plasmodium* and STH infections. The prevalent STH infections were *E. vermicularis*, hookworm and *S. stercoralis*, mostly of light intensity. Multiple diagnostic techniques should be performed including adhesive tapes and Baermann methods when evaluating the burden of helminth in children. Novel technologies with diagnostic assays that can be performed in a high throughput system on large number of population samples to detect all relevant parasite species in low intensity areas would be desirable. The findings of a protective effect of *E. vermicularis* should not deter at this stage deworming programs but rather foster implementation of integrated control program for common parasites to speed up the momentum of moving from morbidity and transmission control to elimination. This is especially true because of the enhancing effect of hookworm on malaria morbidity. Integrated multidisciplinary approach such as distribution of long lasting insecticidal treated bed nets and chemotherapy with dual effect (e.g. ivermectin) associated with health education and improvement of environmental sanitation and hygiene, improved housing and access to safe water should be implemented among school-aged children but also in the under-fives considering the pattern and types of infections within the area. The impact of *E. vermicularis* and *S. stercoralis* on *Plasmodium* parasitemia requires further investigation to better understand its risk, benefits and mechanism involved among children and other risk groups living in different transmission intensity in endemic areas.

Muhtasari

Utangulizi: Vimelea vya maradhi ya parasite kwa wanadamu ni tatizo kubwa katika nchi maskini na nchi zinazoendelea. Ni nadra sana maambukizi haya kuhusisha kimelea cha aina moja, mara nyingi vimelea zaidi ya kimoja husababisha maradhi tofauti kwa muathirika, na hivyo kusababisha maradhi na vifo vingi katika nchi za ukanda wa Tropiki (nchi za joto) ikiwemo Tanzania. Minyoo wanaoambukizwa kwa njia ya udongo (STH) na malaria ya falciparum ni miongoni mwa vimelea vinavyojitokeza kwa wingi na husababisha usugu wa maambukizo wa parasite. Watoto huathirika zaidi na huugua katika kiwango kikubwa na wanabeba parasite wengi wa maradhi yote mawili ya malaria na minyoo. Ijapokuwa muingiliano wa maradhi haya mawili hutokea na husababisha maradhi makali, bado kuna uchache wa uwekezaji na uelewa kati ya maingiliano ya maradhi haya. Kuna ukosefu wa ushuhuda wa kiepidemiologia na bidii za mapambano ya mtazamo wa parasite wa aina moja unaendelea. Mpango wa maendeleo ya bidii na tathmini za kiepidemiologia kutathmini tatizo la maradhi haya kwa mtu mmoja mmoja bado unakosekana. Takwimu ni chache kati ya maingiliano ya jamii za parasite. Maarifa mapya kuhusiana na muingiliano wa vimelea na viashiria vya hatari ni muhimu kwa ajili ya kuelewa athari zitokanazo na vimelea mbalimbali na kushauri njia mahsusi za kudhibiti maambukizo kwa kuhusisha mabadiliko ya sera za afya, njia bora za matibabu na udhibiti wa vimelea kwa kutumia njia jumuishi.

Lengo la ujumla na malengo maalum ya utafiti huu: Lengo kuu la utafiti huu ni kuchunguza muingiliano wa maradhi yatokanayo na vimelea vya *Plasmodium* (malaria) na minyoo katika wilaya ya Bagamoyo, mkoa wa Pwani, nchini Tanzania ambapo mpango wa Taifa wa kudhibiti maradhi haya yatokanayo na vimelea husika unaendeshwa. Malengo mengine ya utafiti huu yalikuwa ni i) Kutathmini ubora na utendaji kazi wa njia mbalimbali za kimaabara zilizotumika kuchunguza uwepo wa minyoo ya aina mbalimbali katika mwili wa binadamu kutumia takwimu zilizokusanywa kwenye mradi wa IDEA ya kifua kikuu na malaria kwa watu wazima na watoto ii) Kuchunguza mahusiano ya vimelea vya *Plasmodium* na minyoo ya aina mbalimbali kwa kupitia taarifa zilizokusanywa kwenye utafiti jamii wa afya za watoto uliofanyika kutumia takwimu za mradi wa IDEA malaria iii) Kuchunguza athari za minyoo kwa watoto wanaougua malaria, maendeleo yao kiafya baada ya kuanza matibabu ya malaria kwa kulinganisha kundi la waliogua na la uhakiki kutumia takwimu za mradi wa IDEA malaria.

Methodolojia: Takwimu zilizokusanywa katika mradi ulokwishafanyika wa IDEA zilitumika. Huu ni mpango wa dunia wa utafiti uliodhaminiwa na jumuiya ya Ulaya, uliokuwa na lengo la kuchunguza mahusiano ya kinga ya mwili inayosababishwa kati ya maingiliano baina ya minyoo na ukimwi, kifua kikuu na malaria. Utafiti wa kazi hii ulihusisha vijiji vilivyopo ukanda wa magharibi umbali wa kati ya kilomita 20 na 60 kutoka Bagamoyo mjini. Vitongoji vilivyohusika ni Magomeni, Kiwangwa, Mkange na Msata. Kutathmini ubora na utendaji kazi wa njia mbalimbali za kimaabara zilizotumika kuchunguza uwepo wa minyoo ya aina mbalimbali (lengo la kwanza), ugunduzi yakinifu wa njia tofauti (Kato-Katz, FLOTAC, Baermann and polymerize chain reaction (PCR)) ulitumika kuchambua kwa makusudi kutoka kwenye sampuli zilizo chaguliwa za baadhi ya minyoo (hookworm and *S. stercoralis*) kwa watu wazima na watoto waliochujwa kutoka kwenye mradi wa IDEA kundi la kifua kikuu na malaria kwa ufuatanisho. Ili kuchunguza mahusiano ya vimelea vya *Plasmodium* na minyoo ya aina mbalimbali (lengo la pili), taarifa za watoto umri wa miezi 2 hadi miaka 9 kutoka kwenye jamii zilizotumika. Taarifa hizi pia za watoto kutoka kwenye jamii zilitumika kuingiza watoto wenye vimelea vya malaria ndani ya damu yao pasipo na dalili za malaria (kundi la uhakiki)

kwenye uchunguzi wa Kesi na uhakiki hapa chini. Kiwango cha maambukizi vya vimelea vya malaria katika damu ilikuwa karibu asilimia 10% ndani ya eneo la utafiti, kwa hiyo karibu watoto 1000 kutoka katika jamii walichunguzwa na kuingizwa katika utafiti. Mlinganisho wa kundi lililougua na la uhakiki (Kesi na uhakiki) lenye ufuatiliaji mfupi lilitumika kuchunguza athari za minyoo kwa watoto waliougua malaria na matokeo yao walipopewa matibabu (lengo la tatu). Kesi zilipatikana kupitia makundi mawili, kundi lenye malaria kali na kundi lenye malaria ya kawaida. Kundi la uhakiki walikuwa wenye vimelea vya malaria bila ya uwepo wa dalili za malaria. Mfuatilio wa muda mfupi wa utafiti huu ulikuwa una lengo la kuangalia athari za minyoo dhidi ya matibabu ya malaria katika makundi haya matatu kwa mfuatilio ulifuata utaratibu wa ufuatiliaji wa shirika la Afya la Dunia, (WHO) siku ya 0, 1, 2, 3, 7, 14, 28 na 42. Kitenganishi kati ya kundi waliougua na kundi la uhakiki ni kuwepo au kutokuwepo kwa maradhi ya mwingiliano mojawapo ya minyoo. Watoto walioshiriki walichunguzwa na daktari wa utafiti aliekobea katika kila ziara (pale wakati wa kujiunga na wakati wa kufuatiliwa kwao), kwa kuangalia dalili za maradhi ya malaria na maradhi mengine ya kawaida kwa kutumia dondoo zilizotengenezwa kwa ajili ya utafiti huu.

Kwenye chunguzi zote mbili, za jamii na kesi na uhakiki, choo kikubwa, kidogo na sampuli za damu zilichukuliwa na kuchunguzwa kwa kutumia vifaa vingi vya kudhibiti ubora wa kuchunguza minyoo wa ardhini na ya kwenye damu wanaojitokeza mara kwa mara *Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Enterobius vermicularis*, *Trichuris trichiura*, Schistosomiasis, na *Wuchereria bancrofti*. Vipimo vya malaria vilifanyika kwa kutumia darubini ya maabara na vipimo vya haraka kwa kutumia damu (malaria rapid tests). Sampuli za minyoo (mkojo na choo kikubwa) vilipimwa katika siku ya kwanza ya uchunguzi wa jamii na kesi na uhakiki na siku ya 28 baada ya kuanza ushiriki kwenye uchunguzi wa kesi na uhakiki. Ili kuweza kuelewa athari ya minyoo katika kinga ya watoto wenye maambukizi ya vimelea vya malaria, matibabu ya minyoo kwa watoto husika yalicheleweshwa na kufanyika siku ya mwisho ya utafiti ambayo ni siku ya 42 ya ufuatiliaji.

Matokeo: Utafiti wa IDEA ulifanyika kati ya mwezi wa Juni 2011 na mwezi wa Novemba 2012 na ulihusisha majira yote ya mwaka. Majibu kutokana na kundi la watu wa wazima na watoto walioshiriki katika kutathmini ubora na utendaji wa njia mbalimbali za kimaabara yalionyesha hookworm (10.0%) na *S. stercoralis* (7.4%) kuwa wengi wakifuata *T. trichiura* (1.9%), *A. lumbricoides* (0.2%) na *Schistosoma mansoni* (0.2%). Kwa ujumla zaidi ya asilimia themanini (80%) ya sampuli zilizopimwa ilikuwa na kiwango kidogo cha minyoo. Kwa kulinganisha ubora wa njia tofauti, FLOTAC na sio PCR ilionekana ina ubora zaidi wa kugundua minyoo (93.8%) ilipolinganishwa na Kato-Katz. Ubora wa kipimo cha PCR kugundua minyoo ya *S. stercoralis* (17.4%) ulikuwa ni wa chini kulinganisha na kipimo cha Baermann (47.1%). Kwa upande mwingine ubora wa kipimo cha PCR ulikuwa sawa na ule wa kipimo cha Kato-Katz katika kugundua minyoo aina ya Hookworm (73%). Kimsingi uhusiano hasi uligundulika kati ya majibu ya PCR cycle threshold (Ct) na uiano wa mayai ya minyoo ya hookworm au kuhesabu viluilui vya *S. stercoralis* kwa kutumia darubini.

Katika jumla ya watoto 1,033 walioshiriki katika tafiti jamii husika, 283 (27.4%) waligundulika kuwa na minyoo. Minyoo iliyogundulika kwa wingi na asilimia kwenye mabano ni *E. vermicularis* (18.0%), hookworm (9.1%) na *S. stercoralis* (6.9%). Aina nyingine za minyoo iliyogunduliwa ni *T. trichiura* (2.5%), *W. bancrofti* (1.4%), *S. haematobium* (0.3%) na *A. lumbricoides* (0.1%). Hakuna mtoto aliyegundulika na minyoo aina ya *S. mansoni*. Mgawanyo wa watoto katika makundi matatu yatokanayo na umri ulionyesha ya kuwa maambukizi kwa watoto chini ya umri wa miaka miwili yalikuwa asilimia 10.2 (10.2%), kufuatiwa

na umri kabla ya kujiunga na shule asilimia 25 (25%) na umri waendao shule asilimia 33.5 (33.5%). Minyoo iliyogunduliwa kwa wingi katika watoto chini ya umri wa miaka miwili ni "*S. stercoralis* na kwa kiasi kikubwa iliambatana na uwepo wa vimelea vya malaria bila ya kuwepo kwa dalili za ugonjwa huo [OR=13.0 (95% CI ya 1.3 - 127.2)]. Uwepo wa vimelea vya malaria bila ya dalili viliambatana na upungufu mdogo wa damu [OR=1.8 (95% CI ya 1.0 - 3.3)], upungufu wa wastani wa damu [OR=5.4 (95% CI ya 2.9 - 9.8) na upungufu mkubwa wa damu [OR=11.2 (95% CI ya 4.2 - 29.9)] kwa watoto chini ya miaka miwili. Kwa watoto wenye umri zaidi ya miaka miwili, vimelea vya malaria bila uwepo wa dalili viliambatana na upungufu wa wastani wa damu [OR=3.1 (95% CI ya 2.0 - 4.8)] na upungufu mkubwa wa damu [OR=7.3 (95% CI ya 2.0 - 26.0)]. Maambukizi ya minyoo hayakuwa na mahusiano ya moja kwa moja na matatizo yatokanayo na lishe hafifu na kuathiri maendeleo ya ukuaji wa watoto walioshiriki.

Miongoni mwa watoto 992 waliochunguzwa kwa maabukizi ya minyoo na vimelea vya *Plasmodium*, maambukizi ya *Plasmodium* kwa ujumla yalikua asilimia 13 (130/992). Asilimia 8.1 (80/992) ya washiriki walikua na maambukizi ya *Plasmodium* pekee, maambukizi ya minyoo ya aina moja pekee yalikuwa asilimia 23.5 (233/992) na asilimia tano (50/992) ya washiriki walikuwa na maambukizi ya pamoja ya minyoo na vimelea vya *Plasmodium*. Maambukizi ya vimelea vya *Plasmodium* na minyoo yalionekana kuongezeka kadri ya umri wa washiriki ulivyoongezeka na waathirika wakubwa walikuwa ni watoto wenye umri wa kwenda shule isipokuwa kwa minyoo aina ya *S. stercoralis* ambayo iliathiri zaidi watoto wenye umri chini ya miaka miwili. Kulikua na mahusiano ya maambukizi ya pamoja ya minyoo na vimelea vya *Plasmodium* [OR=1.4 (95%CI ya 1.0 - 2.1)] na hii ilionekana dhahiri kwa minyoo aina ya *S. stercoralis* [OR=2.2 (95%CI ya 1.1 - 4.3)]. Umri wa mtoto na kutokwenda shule vilikuwa ndio viashiria vya hatari ya maambukizi ya pamoja (shirikishi) ya vimelea vya *Plasmodium* na minyoo ya aina mbalimbali.

Utafiti wa kesi na uhakiki, ulionyesha minyoo kwa ujumla kuwa na kinga na kuzuia kupata dalili za ugonjwa wa malaria [OR=0.2 (95% CI ya 0.3 – 1.3)] ambayo ilionekana zaidi kwa minyoo ya aina ya *E. vermicularis* [OR=0.2 (95% CI ya 0.0 – 0.9)]. Kwa upande mwingine, minyoo aina ya hookworm ilionekana kuchochea ugonjwa wa malaria [OR=3.0 (95% CI ya 0.9 – 14.3)]. Kwa njia ya uchambuzi iitwayo ordinal logistic regression ambayo inaonyesha vizuri mtiririko kutoka kuwa na vimelea vya malaria bila dalili mpaka kuwa na malaria kali, kwa ujumla minyoo ilionyesha kinga thidi ya kupata dalili za malaria kwa asilimia hamsini [OR=0.5 (95% CI ya 0.3 – 0.9)]. Kinyume chake kwa minyoo ya aina ya hookworm ambayo ilikua inachochea ugonjwa wa malaria, [OR=7.8 (95% CI ya 1.8 – 33.9)] kwa malaria isiyo kali na [OR=49.7 (95% CI ya 1.9 – 1298.9)] kwa malaria kali]. Wastani wa muda kwa masaa kigeometria wa kuondoa vimelea vya malaria baada ya matitbabu ulikua mkubwa kwa watoto wenye maabukizi ya aina zote za minyoo inayoambukizwa kwa udongo.

Hitimisho: Maambukizi ya zaidi ya kimelea kimoja ni kawaida kwa watoto kuanzia kwenye umri wa chini na watoto wa umri wa kwenda shule wanaathirika zaidi na vimelea vyote viwili, vya *Plasmodium* na minyoo inayoambukizwa kwa udongo. Minyoo iliyoonekana kwa kiwango kikubwa ni ya aina ya *E. vermicularis*, hookworm na *S. stercoralis* ambayo ilikua kwa kiwango kidogo. Njia mbalimabali za kiamaabara ikiwemo adhesive tapes na Baermann inabidi zitumike wakati tunachunguza tatizo la minyoo kwa watoto. Ubunifu wa njia ya kimaabara ambayo inaweza kufanywa kwa wingi na kwa mara moja kwenye jamii kugundua vimelea mbalimbali vya magonjwa kwenye maeneo yenye maambukizi machache inahitajika. Matokea yanayoonyesha kinga kwa minyoo ya aina *E. vermicularis* na uchochezi kwa minyoo

ya hookworm yasisitishe matumizi ya dawa za minyoo badala yake yatumike kupanga mbinu za pamoja katika mpango mzima wa kuzuia maradhi mpaka uenezaji na kutokomeza kabisa vimelea hivi. Wito wa mfumo wa pamoja wa usambazaji wa vyandarua vyenye viwatilifu vya muda mrefu na dawa zenye athari mbili kama ivermectin ikisindikizwa na elimu ya afya ya pamoja kuboresha usafi wa mazingira, uboreshaji wa makazi na upatikanaji wa maji salama ukizingatia muundo na aina ya maambukizi katika maeoneo ili kuzuia maambukizi yote ya minyoo inayosababishwa na udongo na vimelea vya *Plasmodium* kwa watoto wote walio na umri wa kwenda shule na wale chini ya miaka mitano unahitajika. Athari za minyoo ya *E. vermicularis* na *S. stercoralis* kwa vimelea vya *Plasmodium* inahitaji tafiti nyengine zaidi ili ieleweke vizuri visababishi, faida na utaratibu husika kwa watoto na makundi hatari wanaoishi maeneo kwenye viwango tofauti vya maambukizi katika maeneo yenye magonjwa husika.

Zusammenfassung

Hintergrund: Vertreter infektiöser oder parasitärer Organismen treten selten in Isolation zueinander auf. Gerade in ressourcenarmen Verhältnissen von Entwicklungsländern ist das Auftreten von Multiparasitismus in der Bevölkerung Standart. Durch Bodenkontakt übertragene parasitische Würmer (Helminthen) und *Plasmodium falciparum* stellen dabei die meistverbreiteten parasitären Infektionen dar und tragen einen Grossteil zur Mortalität und Morbidität in tropischen und sub-tropischen Ländern wie Tansania bei. Von solchen Parasiten am stärksten betroffen sind Kinder, welche im Vergleich zu Erwachsenen einer höheren Parasiten-Prävalenz ausgesetzt sind und unter einer stärkeren Infektionsintensität leiden. Obwohl dabei auftretende Koinfektionen häufig und mit einem verstärkten Krankheitsbild assoziiert sind, ist relativ wenig über die genaue Interaktion zwischen Helminthen und *P. falciparum* bekannt. Detaillierte epidemiologische Daten fehlen weitgehend und Interventionen beschränken sich in der Regel auf nur einen Parasiten. Es mangelt generell an der Weiterentwicklung eines effizienten Interventions-Programms sowie epidemiologischer Studien welche zu einem Verständnis dieser Koinfektion auf individueller Basis führen würden. Neue Erkenntnisse zur genauen Interaktion zwischen Helminthen und *P. falciparum* und die dadurch assoziierten Risiken sind notwendig für die Implementierung von Interventions-Strategien, besserem Management sowie einheitlicher und massgeschneiderter Massnahmen zur besseren Kontrolle dieser Infektionskrankheiten.

Ziel dieser Arbeit: Im Rahmen dieses PhD Projekts sollten die zwischen einander auftretenden Interaktionen bei Infektion mit *Plasmodium* und Helminthen erforscht werden. Dies in Bagamoyo, im Küstengebiet Tansanias, wo beide parasitären Krankheiten verbreitet sind und grossflächig nationale Kontrollprogramme zur Prävention dieser Infektionen etabliert sind. Die genauen Zielsetzungen dieser Arbeit wurden wie folgt definiert: i) die Erforschung der Effizienz verschiedener diagnostischer Methoden zum Nachweis von Helminthen in Erwachsenen und Kindern, welche im Rahmen des IDEA-Projekts für Tuberkulose (TB) und Malaria Kohorten rekrutiert wurden ii) die Untersuchung der Beziehung zwischen Helminthen und *Plasmodium* Prävalenzraten in Kindern welche im Rahmen einer IDEA-Malaria Gemeinschafts Querschnittsstudie rekrutiert wurden iii) die Erforschung des Einflusses von Helminthen auf die klinische Präsentation, sowie den Behandlungsausgang von Malaria in Kindern welche im Rahmen von IDEA-Malaria in einer Fall-Kontrollstudie rekrutiert wurden.

Methoden: Die Studien wurden als Bestandteil der Malaria Komponente im Rahmen des IDEA Projekts ausgeführt. Es handelt sich dabei um ein globales Forschungsprogramm, finanziert von der Europäischen Union (EU) und mit dem Ziel, die immunologischen Wechselwirkungen welche bei Infektion mit Helminthen und HIV, TB oder Malaria auftreten, zu erforschen. Durchgeführt wurden die Studien im Westen Tansanias, 20 bis 60 km entfernt von der grösseren Ortschaft Bagamoyo, in vier kleinen Siedlungen in ländlichem Gebiet. Zur Untersuchung der Sensitivität und Effizienz diagnostischer Methoden (Zielsetzung 1), wurden gezielt Stuhlproben zum Methodenvergleich verwendet, welche im Rahmen der IDEA-TB und IDEA-Malaria Gemeinschaftserhebungen von Erwachsenen bzw. Kindern gesammelt wurden. Die diagnostische Genauigkeit von Kato-Katz, FLOTAC, Baermann und Polymerase Kettenreaktion (PCR) wurden mittels Diagnose bekannter Helminthen, Hakenwürmer und *S. stercoralis* untersucht. Um den Zusammenhang der Prävalenzraten von Helminthen und *Plasmodium* zu erforschen (Zielsetzung 2), wurde eine Querschnittstudie mit Kindern zwischen 2 Monate bis 9 Jahre Alter

durchgeführt. Diese Gemeinde-basierte Studie diente gleichzeitig zur Rekrutierung von Kindern mit asymptomatischer *Plasmodium* Parasitämie (Kontrollen) für die parallel durchgeführte Fall-Kontrollstudie. Die Prävalenz von *Plasmodium* Parasitämie lag im Untersuchungsgebiet bei ca. 10%, dementsprechend wurden ca. 1,000 Kinder untersucht und rekrutiert. Um die Auswirkung von Helminthen Infektionen auf die klinische Manifestation und Behandlungsausgang von Malaria zu untersuchen (Zielsetzung 3), wurde eine Fall-Kontrollstudie mit semi-longitudinalen Nachfolgeuntersuchungen (follow-ups) ausgeführt. Die Studienteilnehmer wurden in zwei verschiedene Gruppen aufgeteilt: Fälle mit schwerer Malariaverlaufsform und Fälle mit unkomplizierter Malaria. Als Kontrolle dienten Kinder mit asymptomatischer *Plasmodium* Parasitämie.

Der longitudinal observative Teil der Studie bestand darin, den drei Gruppen über einen kurzen Zeitraum zu folgen, um mögliche Reaktionen gegenüber der Malaria Behandlung zu dokumentieren. Diese Nachuntersuchungen fanden nach dem Schema der Weltgesundheitsbehörde (WHO) statt (Tag 0, 1, 2, 3, 7, 14, 28, 42). Exposition war in dieser Fall-Kontrollstudie definiert als Präsenz oder Absenz einer Infektion mit einer beliebigen Helminthen-Art. Die rekrutierten Kinder wurden bei jedem Besuch (Rekrutierung und Nachfolgeuntersuchungen) von einem qualifizierten und speziell ausgebildetem Studienarzt für Zeichen und Symptome von Malaria und weiterer häufiger Krankheiten untersucht. Dies wurde unterstützt mittels eines extra für die Studie angefertigten Fragebogens.

In Querschnittsstudie und Fall-Kontrollstudie wurden Stuhlproben, Urin, Klebestreifen und Blutproben mit einem breiten Set etablierter diagnostischer Methoden auf die häufigsten Helminthen-Arten (*Ascaris lumbricoides*, Hakenwürmer, *Strongyloides stercoralis, Enterobius vermicularis* und *Trichuris trichiura*) sowie *Schistosoma* und *Wuchereria bancrofti* analysiert. Blut Ausstriche und Malaria Schnelltests (mRDTs) dienten zur Diagnose von *Plasmodium falciparum*. Stuhlproben, Urin und Klebestreifen wurden während dem ersten Screening (beide Studien), sowie 28 Tage später während der Nachuntersuchung (Fall-Kontrollstudie) gesammelt. Um den direkten Einfluss von Helminthen auf die klinische Malaria Manifestation und Behandlungsausgang untersuchen zu können, wurden Helminthen-positive Kinder erst nach Studienende (Tag 42) behandelt.

Resultate: Die Rekrutierung der Studienteilnehmer fand zwischen Juni 2011 und November 2012 statt, wodurch allfälligen saisonalen Variationen Rechnung getragen wurde. In den Proben, welche von Erwachsenen und Kindern gesammelt wurden und zur Qualitätskontrolle der verschiedenen Methoden verwendet wurden, waren Hakenwümer (10.0%) und *S. stercoralis* (7.4%) am stärksten verbreitet, gefolgt von *T. trichiura* (1.9%), *A. lumbricoides* (0.2%) und *S. mansoni* (0.2%). Bei mehr als 80% dieser Fälle handelte es sich um leichte Infektionen. Im direkten Methodenvergleich hatte FLOTAC insgesamt eine signifikant höhere Sensitivität (93.8%) als Kato-Katz. Dies war nicht der Fall für PCR. Die Sensitivität von PCR für die Diagnose von *S. stercoralis* (17.4%) war deutlich tiefer als diejenige der Baermann Methode (47.1%). Der direkte Methodenvergleich zeigte eine gleiche Sensitivität von PCR und Kato-Katz Methode für die Diagnose von Hakenwürmern (73.0%). Eine signifikante negative Korrelation wurde gefunden zwischen den PCR Zyklus Schwellenwerten (Ct) und den mikroskopisch bestimmten Eier pro Gramm (EPG) Werten von Hakenwürmern oder den gezählten Larven von *S. stercoralis*.

Von den insgesamt 1,033 Kindern, welche in die Querschnittsstudie eingeschlossen wurden, waren 283 (27.4%) mit Helminthen infiziert. Die am häufigsten auftretenden Helminthen-Arten waren *E. vermicularis* (18.0%), Hakenwürmer (9.1%) und *S. stercoralis* (6.9%). Weitere vorhandene Helminthen waren *T. trichiura* (2.5%), *W. bancrofti* (1.4%), *S. haematobium* (0.3%) und *A. lumbricoides* (0.1%). Bei keinem der Kinder wurde eine Infektion mit *S. mansoni* diagnostiziert. Die Helminthen Prävalenz stieg mit zunehmendem Alter von Säuglingen (10.2%) zu Kinder im Vorschul- (25.0%) und im Schulalter (33.5%). *S. stercoralis* wurde in Säuglingen am häufigsten gefunden und war deutlich assoziiert mit einem erhöhten Risiko von asymptomatischer *Plasmodium* Parasitämie [OR=13.0 (95% CI 1.3 – 127.2)]. Asymptomatische *Plasmodium* Parasitämie war assoziiert mit milder [OR=1.8 (95% CI 1.0 – 3.3)] , moderater [OR=5.4 (95% CI 2.9 – 9.8)] und schwerer Anämie [OR=11.2 (95% CI 4.2 – 29.9)] in Kindern unter zwei Jahren und in den Älteren mit moderater [OR=3.1 (95% CI 2.0 – 4.8)] oder schwerer Anämie [OR=7.3 (95% CI von 2.0 – 26.0)]. Weder *S. stercoralis*, *E. vermicularis*, Hakenwürmer oder einer der übrigen untersuchten Helminthen-Arten waren assoziiert mit Schwund (Atrophie), Untergewicht, Magerkeit, Unterentwickeltheit oder Anämie in der untersuchten Studienpopulation.

Innerhalb der 992 auf Doppelinfektion mit Helminthen und *Plasmodium* untersuchten Kinder, betrug die Prävalenz für *Plasmodium*-Monoinfektion 8.1% (80/992), Helminthen-Monoinfektion 23,5% (233/992) und Koinfektion mit *Plasmodium* und einer beliebigen Helminthen-Art 5.0% (50/992). Die Prävalenzrate für *Plasmodium*, spezifischer Helminthen-Arten und Koinfektionen stiegt signifikant mit fortschreitendem Alter ($p< 0.001$). Dabei am meisten betroffen sind Kinder im Schulalter, mit Ausnahme für *S. stercoralis*-Monoinfektion und Koinfektionen. Infektion mit Helminthen war generell assoziiert mit *Plasmodium*-Infektion [OR angepasst für Altergruppe 1.4 (95% CI 1.0 – 2.1)], was ausgeprägter bei *S. stercoralis* zur Geltung kam [OR= 2.2 (95% CI 1.1 – 4.3)]. Alter und nicht Einschulung war der Risikofaktor für *Plasmodium* und Helminthen Koinfektion.

In der Fall-Kontrollstudie zeigte sich die Tendenz, dass Infektion mit Helminthen einen schützenden Effekt auf die Entwicklung von klinischer Malaria hat [OR=0.6, 95% CI 0.3 – 1.3]. Dies war stärker ausgeprägt für *E. vermicularis* [OR=0.2, 95% CI 0.0 – 0.9]. Im Gegenteil dazu schienen Hakenwümer mit klinischer Malaria assoziiert zu sein [OR=3.0, 95% CI 0.9 – 9.5]. Eine multiple konditionale Regressionsanalyse indizierte einen tieferen allgemeinen Schutzeffekt für sämtliche Helminthen Infektionen [OR=0.8, 95% CI 0.3 – 1.9], bestätigte jedoch eine signifikanten Schutzeffekt bei Infektion mit *E. vermicularis* [OR=0.1 95% CI 0.0 – 0.1] und grenzwertige Signifikanz für Hakenwürmer [OR=3.6 95% CI 0.9 – 14.3]. Statistische Analyse mit ordinaler logistischer Regression, welche die Progression von asymptomatischer zu schwerer Malaria besser reflektiert, zeigte einen 50% schützenden Effekt über alle Helminthen betrachtet [OR=0.5 CI 95% 0.3 – 0.9]. Im Gegenteil dazu waren Hakenwürmer ausschlaggebend für unkomplizierte [OR=7.8 95% CI 1.8 – 33.9] und schwere Malaria [OR=49.7 95% CI 1.9 – 1298.9]. Generell hatten Kinder mit Helminthen-Infektion eine höhere geometrische Mittelwertszeit bis zu einer ersten Beseitigung der *Plasmodium* Parasitämie.

Schlussfolgerung: Multiparasitismus ist verbreitet im Kinds- aber auch Säuglingsalter, während Kinder im Schulalter die höchste Zweifachbelastung durch Infektion mit *Plasmodium* und Helminthen aufweisen. Die am häufigsten auftretenden Helminthen waren *E. vermicularis*, Hakenwürmer und *S. stercoralis*, die meisten davon in leichter Infektionsintensität. Will man die Helminthen-Belastung in Kindern verlässlich

evaluieren, sollten mehrere diagnostische Methoden inkl. Baermann und adhesiver Klebestreifen durchgeführt werden. Erstrebenswert wären neue Technologien und diagnostische Testverfahren welche in hohem Durchsatz verwendet werden können, die gleichzeitige Analyse von einer Vielzahl Proben ermöglichen und sämtliche relevante Parasiten inkl. leichter Infektionen detektieren. Die Erkenntnis des schützenden Effekts von *E. vermicularis* sollte kein Argument für die Unterlassung stattfindender Entwurmungsprogramme sein. Vielmehr ist es ein Grund zur verstärkten Weiterentwicklung einheitlicher Parasiten-Kontrollprogramme, mit dem Ziel möglichst rasch von Morbiditäts- und Transmissionskontrolle Richtung Eliminierung fortzuschreiten. Ein solcher Schritt wäre von grosser Bedeutung, bedenkt man den unterstützenden Effekt von Hakenwürmer auf die durch Malaria verursachte Morbidität. Ein einheitlicher, multidisziplinärer Ansatz, wie beispielsweise die lang anhaltende Verteilung von Insektizid-behandelten Moskitonetzen und medikamentöser Behandlung mit Zweifachwirkung (z.B. Ivermectin) in Kombination mit gesundheitlicher Aufklärung und Ausbildung der Bevölkerung, sowie der Verbesserung von sanitären Einrichtungen und Hygienestandarte, verbessertem Wohnungsbau und Zugang zu sauberem Trinkwasser sollte etabliert werden. Kinder im Schulalter, sowie die unter Fünfjährigen müssen in einem solchen Programm integriert sein, bedenkt man die Arten und Vorkommen der hier beschriebenen Infektionen. Der Einfluss von *E. vermicularis* und *S. stercoralis* auf *Plasmodium* Parasitämie muss weiter erforscht werden, will man genauere Aussagen bezüglich assoziierter Risiken, allfälliger Vorteile und Mechanismen machen, welche Kinder und andere Risikogruppen in Transmissionsgebieten betreffen.

Chapter 1: INTRODUCTION

1.0 Multiparasitism

Multiparasitism also known as polyparasitism is concurrent infestation in a single host individual with two or more parasite species (Steinmann et al., 2010). The parasite species are divided into two subgroups namely macroparasites and microparasites (Anderson and May, 1979). Macroparasites includes parasitic helminth (nematodes and trematodes) and arthropods, both falling onto the field of parasitology. These parasites have no direct reproduction within their host, have long duration (generation time occupying an appreciable fraction of the host life span), have weak and short immunity and reinfection is common. On the contary, microparasites species are small with high reproduction rate within the host and short generation time producing short duration infection but ending up with the host acquiring long term immunity. Microparasites include protozoa (in the field of parasitology) and rickettisial, bacteria, fungi and viruses falling within the scope of microbiology (Anderson and May, 1979). This PhD thesis addresses the interaction of the most prevalent parasitic diseases, *Plasmodium* (protozoa) and soil transmitted helminth (STH), (intestinal nematodes) among children.

Multiparasitism is a norm among children living in poor resource settings of developing countries (Petney and Andrews, 1998, Steinmann et al., 2010, Mazigo and Ambrose-Mazigo, 2012, Elliott and Yazdanbakhsh, 2012). The agent, host and environment interact in a complex way, the balance and interactions are different for different infections. Variety of environmental and host related factors can influence the structure and dynamics of the parasite communities which make up these multiple infections (Petney and Andrews, 1998, Rothman and Greenland, 2005). These conditions include poverty, environmental contamination, water bodies, lack of effective preventive measures (Booth, 2006) and the immunity of the host. In addition, overlap of *Plasmodium* infection and other pathogens depends on the conditions that favor multiple parasitic species survival and transmission such as exposure related risk, within host interactions between co-infecting species and shared geographical distribution of the parasites. (Booth, 2006, Brooker et al., 2007, Brooker et al., 2012)

1.1 Geographical distribution of malaria and soil transmitted helminth (STH)

1.1.1 Geographical distribution of malaria

Malaria is mainly transmitted in the tropical and subtropical regions. The geographical distribution of malaria mainly depends on climatic factors such as temperature, humidity and rainfall. Other factors which can influence its spatial distribution include environmental conditions, socioeconomic status, population movement, control measures and drug resistance. All these factors affect the survival and multiplication of mosquito population, specifically *Anopheles* species where malaria parasite can complete its life cycle. Figure 1 summarizes the life cycle of malaria parasite.

Temperature is particularly critical (Craig et al., 1999, Caminade et al., 2014) and in many malaria endemic countries transmission does not occur in all parts. Parasite development ceases at 16^0C, transmission become stable at a temperature above 22^0C and thermal death of the mosquitoes occurs at around 40-42^0C (Craig et al., 1999). Transmission will not occur at very high altitudes 2500 meters above the sea level as duration necessary to complete the sporogonic cycle increases and thus unlikely for the mosquitoes to survive long enough to transmit malaria parasites.

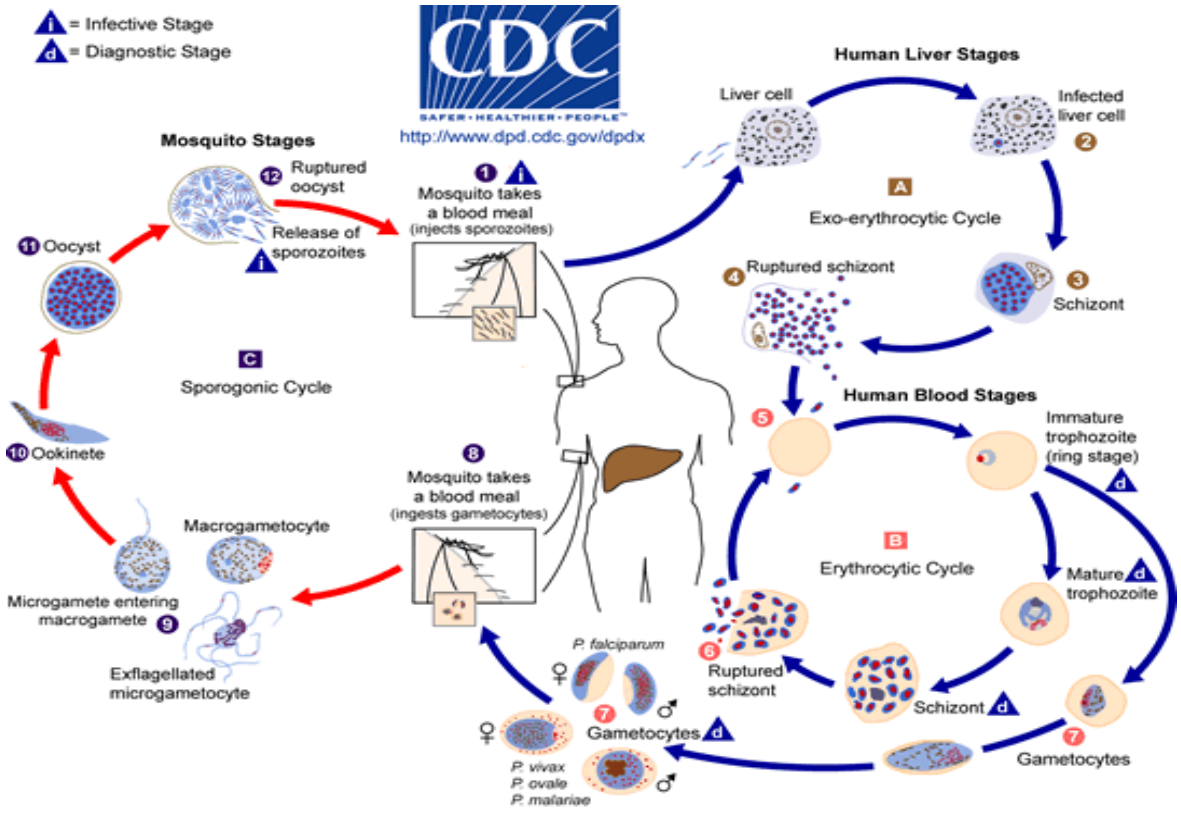


Figure 1. The life cycle of malaria parasite

(Source: http//www.dpd.cdc.gov/dpdx): accessed on 08.02.2015

The malaria parasite life cycle involves two hosts. During a blood meal, a malaria-infected female *Anopheles* mosquito inoculates sporozoites into the human host ❶. Sporozoites infect liver cells ❷and mature into schizonts ❸, which rupture and release merozoites ❹. (Of note, in *P. vivax* and *P. ovale* a dormant stage [hypnozoites] can persist in the liver and cause relapses by invading the bloodstream weeks, or even years later.) After this initial replication in the liver (exo-erythrocytic schizogony A), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony B). Merozoites infect red blood cells ❺. The ring stage trophozoites mature into schizonts, which rupture releasing merozoites ❻. Some parasites differentiate into sexual erythrocytic stages (gametocytes) ❼. Blood stage parasites are responsible for the clinical manifestations of the disease.

The gametocytes, male (microgametocytes) and female (macrogametocytes), are ingested by an *Anopheles* mosquito during a blood meal ❽. The parasites' multiplication in the mosquito is known as the sporogonic cycle C. While in the mosquito's stomach, the microgametes penetrate the macrogametes generating zygotes ❾. The zygotes in turn become motile and elongated (ookinetes) ❿ which invade the midgut wall of the mosquito where they develop into oocysts ⓫. The oocysts grow, rupture, and release sporozoites ⓬, which make their way to the mosquito's salivary glands. Inoculation of the sporozoites ❶into a new human host perpetuates the malaria life cycle.

Rainfall tend to regulates temperature and saturation deficit, important factors for mosquito survival (Craig et al., 1999). The highest transmission occurs in Africa south of Sahara and parts of Oceania in Papua New Guinea. In Western Europe and United states, economic development and public health measures have succeeded in eliminating malaria. However, most of these areas have *Anopheles* mosquitoes and reintroduction of the disease is a constant risk. The global distribution and estimated malaria cases, 2012 are shown in Figure 2.

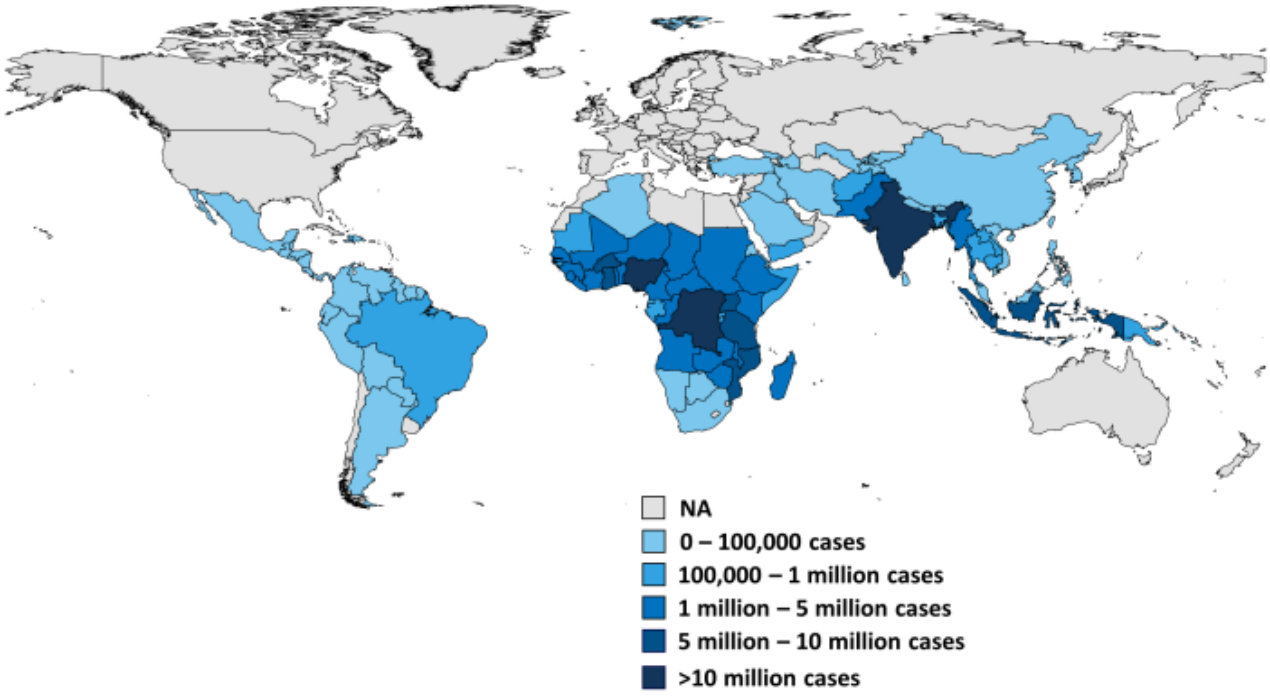


Figure 2. Global distribution of estimated malaria cases in 2012

Source: World malaria report (WHO, 2013)

In Tanzania, regional heterogeneity of malaria transmission is noted despite the efforts of ongoing malaria interventions (Oesterholt et al., 2006). Comparing the Tanzania HIV and malaria indicator report of 2008 and that of 2011/2012, there is a tendency of clustering of malaria prevalence in certain regions. Malaria prevalence is still high around the lake and coastal zones compared to the central and highland regions (Tanzania HIV/AIDS and Malaria Indicator Survey, 2012). The overall prevalence of malaria in coastal region is estimated to be 10%. Figure 3 shows the distribution of malaria prevalence in children by region, Tanzania as estimated using malaria rapid diagnostic tests (mRDTs).

Subnational heterogeneity of malaria prevalence in children, Tanzania

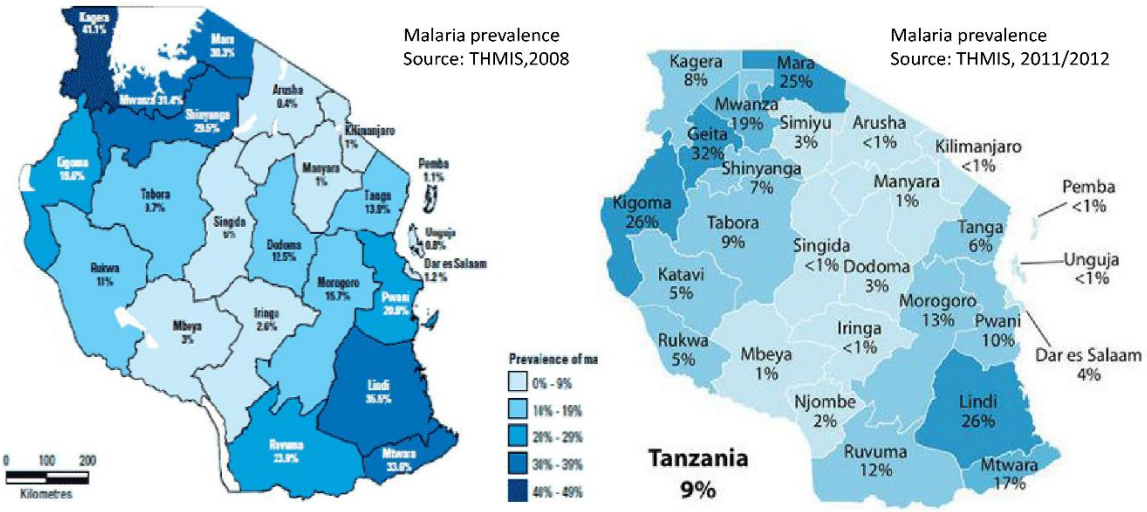


Figure 3. Malaria prevalence in children by region as reported in 2008 and 2012, Tanzania

Source: Tanzania HIV/AIDS and Malaria Indicator Survey (THMIS 2008 and 2011/2012), National Bureau of Statistics.

1.1.2 Geographical distribution of soil transmitted helminth (STH)

Soil transmitted helminth infections (STH) are among the most common infection globally, widely distributed in tropical and subtropical areas with greatest numbers occurring in Sub-Saharan Africa, East Asia, South America, China and India (de Silva et al., 2003, Pullan et al., 2014). WHO estimates that more than 4 million children in European region need preventive chemotherapy (WHO, 2015). Countries in central Asia, Caucasus and the Balkan are considered to have the highest burden of STH. The countries mostly affected include Azerbaijan, Kyrgyzstan and Tajikistan. The Rostropovich-Vishnevskaya Foundation in close collaboration with the WHO/Europe is continuing to make assessments of country-level problems and needs related to STH in Armenia, Georgia, Kazakhstan, the Republic of Moldova and Romania.

Transmission occurs through eggs and larvae thriving in contaminated soil with faeces containing helminth eggs. Figure 4 shows the schematic life cycle of STH. Climate is an important factor for survival and transmission coupled with inadequate sanitation and hygiene in resource poor settings (Brooker and Michael, 2000, Brooker et al., 2006, Bethony et al., 2006). Adequate moisture and warm temperature are essential for the larvae development and survival in the soil (Brooker et al., 2006). High prevalence and intensity of hookworm is found in coastal regions and areas of extreme high temperatures of 37–40^{0}C such as Cameroon, chad and Mali (Hotez and Kamath, 2009). Maturation and infective cycle have been noted to continue at a temperature of 20-26^{0}C (for *A. lumbricoides* and *T. trichiura*) and development ceases below 5 and above 38^{0}C (Knight, 1982, Brooker et al., 2006). *A. lumbricoides* are more resistant to extreme temperature than *T. trichiura* eggs (Knight, 1982, Bundy and Cooper, 1989).

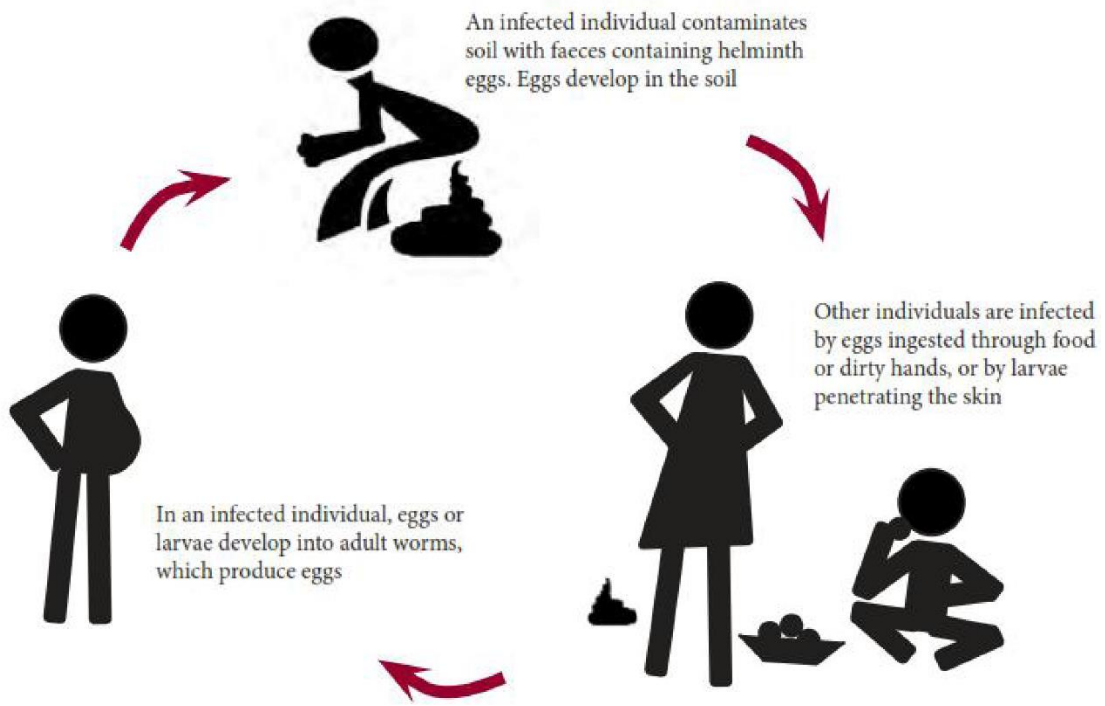


Figure 4. Schematic life cycle of soil transmitted helminth

Source: WHO, 2011

Unlike other intestinal parasites no intermediate host is required, humans are the definitive host and generally the parasite do not multiply within the host (Bethony et al., 2006). The adult worms inhabit the intestinal tract although there are variations in the modes of transmission and infection. The main species that infect human includes the roundworms (*Ascaris lumbricoides*), hookworm (*Ancylostoma duodenale* and *Necator Americanus*), and whipworm (*Trichuris trichiura*). In addition to the four major STH species, there are two intestinal nematodes infecting humans, the threadworm (*Strongyloides stercoralis*) and the pinworm (*Enterobius vermicularis*) that are mostly neglected in prevalence report and global burden of disease estimates due to their rather unpleasant and cumbersome diagnostic techniques involved. Infection with *A. lumbricoides*, *T. trichiura* and *E. vermicularis* are transmitted by swallowing mature eggs from contaminated food and fingers. In case of hookworm and *S. stercoralis*, infections are transmitted via active larvae penetration through the skin. The ingested mature eggs of *T. trichiura* and *E. vermicularis* hatch into larvae and directly reach the large intestines. *E. vermicularis* larvae migrate to the anus to deposit their eggs on the perianal skin (Cook, 1994). Autoinfection with *E. vermicularis* is possible since eggs can become infective within hours (Knight, 1982). Autoinfection tendency also occurs with *S. stercoralis* as the larvae already hatch in the intestinal lumen where infective stages can develop causing chronic infection /carrier (Concha et al., 2005). The larvae of *A. lumbricoides*, hookworm and *S. stercoralis* enter the circulatory system and are transported via the lungs, trachea and swallowed again through the oesophagus (Bethony et al., 2006, Schär et al., 2013b). Figure 5 shows the global distribution of STH species (hookworm, *A. lumbricoides* and *T. trichiura*).

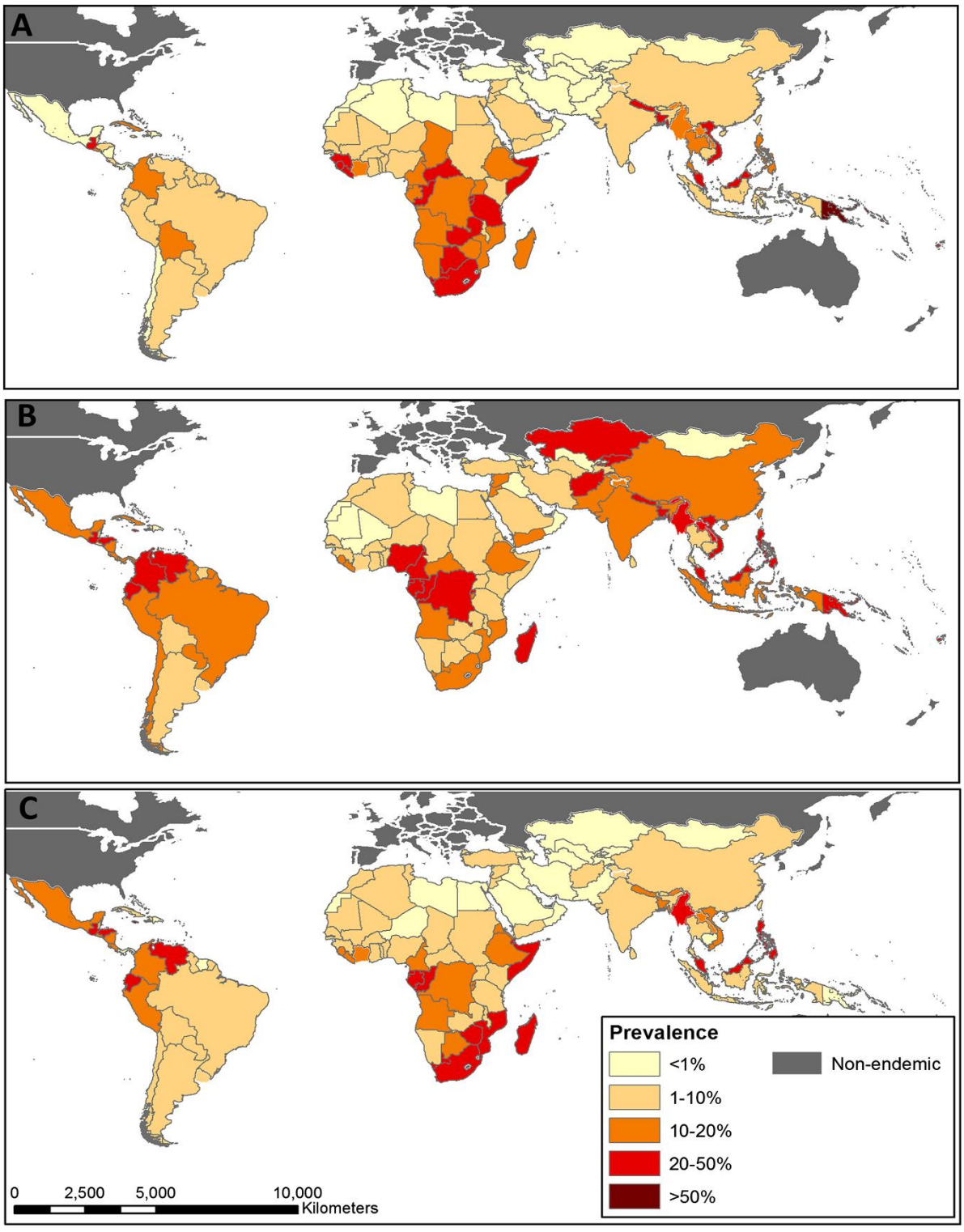


Figure 5. Distribution of STH infection species prevalence in 2010

Source: Pullan et al, 2014. (A) Hookworm, (B) *Ascaris lumbricoides*, (C) *Trichuris trichiura*.

1.2 Burden of parasitic diseases

Parasitic diseases are illnesses caused by infestation (infection) with parasites such as protozoa, helminth or arthropods. Parasites thrive in warm and moist environment and thus common in sub-Saharan Africa, Southeastern Asia, India, Central and South America. Most of the parasitic diseases are categorized among the neglected tropical diseases (NTDs) with helminth infection contributing approximately to 85% of the NTDs, primarily occurring in impoverished communities (Hotez and Kamath, 2009). Often causing chronic infections with perceived little damage with the exception of acute infection like malaria which may lead to high mortality early in life. The diseases mostly cause morbidities rather than mortality. The morbidities include childhood growth, children's mental and cognitive development in terms of school performance and school absenteeism, work productivity and even disfigurement and stigmatization (Hotez et al., 2014). Current health metrics used to estimate the burden of parasitic diseases are criticized to underestimate the actual burden particularly for helminth infections which cause subtle morbidities (Hotez et al., 2014, King, 2015). Main priorities for better disease burden assessment were listed as i) more accurate case counting (active and previous infection) ii) use of patient based measurements of disease impact on disability status (overall impact, nutritional status, physical performance, cognitive level, scholastic achievements, employment and economic impact) iii) use of randomized trials with long term follow up to define the benefits of intervention (King, 2015).

1.2.1 Burden of malaria and existing interventions

Malaria is a life threatening disease caused by parasites of the genus *Plasmodium* associated with high morbidity and mortality. The four main species of *Plasmodium* that cause human malaria are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. The *P. falciparum* is the most predominant and dangerous species associated with high morbidity and mortality in the tropics (WHO, 2013). Globally in 2010, the burden of malaria was estimated to be more than 80 million disability adjusted life years (DALYs) (Murray et al., 2012). Between 2000 and 2012, there has been a dramatic decrease in the malaria specific mortality following the scaling up of malaria control strategies. Estimates of malaria mortality have shown a reduction of 45% in all age groups worldwide and 51% in children under-five years (WHO, 2013). Of the total deaths averted, 90% are estimated to be in children from Sub Saharan Africa where the burden of malaria is huge, contributing to 20% decline in all cause child mortality (WHO, 2013).

Malaria control scale-up has progressed on both mainland Tanzania and Zanzibar through the National malaria control program. This has significantly contributed to the reduction of infant and child mortality in the country (WHO, 2013). The different interventions included scaling up of insecticide treated nets (ITNs) for the most vulnerable groups since 2004 and the delivery of free Long Lasting Insecticidal Nets (LLINs) to children under five years of age since 2009 followed by universal coverage in 2011 (Bonner et al., 2011, Koenker et al., 2013, Renggli et al., 2013), a change in the malaria treatment policy from sulfadoxine-pyrimethamine (SP) to artemether-lumefantrine (ALu) in December 2006, introduction of indoor residual spraying (IRS) in epidemic prone areas and most recently the introduction of malaria Rapid Diagnostic Tests (mRDTs) which are being phased in all regions since early 2009 (Masanja et al., 2012). Based both on country reports and those published in peer-reviewed journals, there is consistent temporal evidence for a beneficial impact on infection, morbidity and mortality as malaria program coverage increased in the country (Wang et al., 2006, Geissbühler et al., 2009, Khatib et al., 2012, Tanzania

HIV/AIDS and Malaria Indicator Survey, 2012, RBM, 2012).There is indication from bednet studies that the magnitude of the overall mortality reduction due to the malaria intervention was actually greater than expected from reduction due to malaria mortality alone (Schellenberg et al., 2001). In Tanzania, the under-five mortality rate declined by 41% from 137 deaths per 1,000 live births in 1992-1996 to 81 deaths per 1,000 live births in 2006-2010. Over the same period, the infant mortality rate declined by 42%, from 88 to 51 deaths per 1,000 live births (Tanzania HIV/AIDS and Malaria Indicator Survey, 2012). Malaria dropped in the last ten years from 44% to 22% of *P. falciparum* parasitaemia associated with fever in a systematic review from Africa (D'Acremont et al., 2010), from 18% to 9% as diagnosed by mRDTs at the country level (Tanzania HIV/AIDS and Malaria Indicator Survey, 2012) and from above 40% to less than 5% in districts such as Rufiji, Kilombero/Ulanga and Korogwe (Mmbando et al., 2010).

1.2.1.1 Diagnosis of *Plasmodium* infection

Early detection and prompt management of *Plasmodium* disease is crucial especially in children due to associated high morbidity and mortality. Presumptive diagnosis based on signs and symptoms is not uncommon in malaria endemic areas (D'Acremont et al., 2009). Parasite based diagnosis is important considering the development of antimalarial resistance and the use of expensive artemisinin combination therapy (ACT) in treatment of cases. In recent years, mRDTs, which target specific *Plasmodium* antigens mainly histidine rich proteins (HRP), *Plasmodium* lactate dehydrogenase (pLDH) and aldolase enzyme in blood of infected humans, have been introduced as essential part for case management. The sensitivity and specificity of mRDTs have been shown to be high in field studies (Moody, 2002, Stauffer et al., 2009, Batwala et al., 2010). The main disadvantage of mRTDs includes inability to quantify parasitemia (Mueller et al., 2007). The ability of pLDH based RDTs to detect gametocytes and generation of positive results due residual HRP antigen even after parasite clearance requires further assessment in clinical settings (Bell et al., 2005). The lowest detection limit for mRDTs is estimated to be 50 – 100 parasites/µl. Performance of mRDT varies depending on the level of parasitemia as does with microscopy (Ochola et al., 2006, Abba et al., 2011).

The results of microscopy reading depend on the quality of smear preparation, staining techniques and competence of the microscopist for correct interpretation. Similar to mRDTs, the light microscopy method attains a field limit of detection of 50 – 100 parasites/µl, subjective to the quality of smear and microscopist competence (Moody, 2002, Wongsrichanalai et al., 2007).

The polymerase chain reaction (PCR) has proven to be a sensitive method for the diagnosis of all the four human malaria species (*P. falciparum*, *P. ovale*, *P. malariae*, *and P. vivax*). The detection limit of <5 parasites/µl and the identification to the species level makes it the most accurate technique. However, the technique is not yet ready to be used for routine acute malaria identification because of the time and cost involved and technical experience required (Moody, 2002, Kamau et al., 2011, Mosha et al., 2013).

1.2.2 Burden of soil transmitted helminth (STH) and existing interventions

Recent estimates suggest that globally around 1.45 billion people are infected with at least one intestinal helminth species and a global burden of 5.2 million disability adjusted life years (DALYs) is attributed to these infections (Pullan et al., 2014). Out of the total global burden (DALYs), hookworm contributes up to 439 million people equivalent to 3.2 DALYs, 819 million people (1.3 DALYs) for *A. lumbricoides* and 465

million people (0.6 DALYs) for *T. trichiura* infection (Pullan et al., 2014). Overall prevalence of any STH in all endemic countries has dropped from 38.6% from 1990 to 25.7% in 2010 representing a reduction of 140 million infected individuals indicating a substantial public health gain for the past 20 years (Pullan et al., 2014).

The general prevalence of STH in Tanzania is estimated between 57 – 85% (Bundy et al., 2000). Local reports suggests that all regions have some level of infection which may go up to 100% in certain ecological settings (Ministry of Health and Social Welfare, 2009). Generally, high prevalence of STH have been documented in the island of Pemba, Zanzibar and parts of Northeastern Tanzania along the coast and lake zones (Brooker et al., 2009). Hookworm is the most widely distributed species while *A. lumbricoides* and *T. trichiura* have a much more restricted distribution (Brooker et al., 2009), the prevalence being higher in the islands than mainland, Tanzania with spatial variations (Knopp et al., 2008a). The burden of STH infections in Tanzania is mostly distributed along the lake zones and coastal areas although absence of surveys in other regions could explain the distribution. Figure 6 shows the distribution of STH in Tanzania.

Morbidity and rate of transmission are directly related to the number of worms (infection intensity) in the host (Knight, 1982). Infection clustering is common, where a relatively large proportion of the total infective potential of a population is derived from a small number of hosts (Knight, 1982) bringing non-linear relationship between prevalence and infection intensity (Bethony et al., 2006, Pullan et al., 2014) although prevalence remain to be the key indicator for the initial selection of the control measures. Children (school aged) are at a higher risk of infection, reinfection and thus morbidities associated with STH (WHO, 2011b).

Global strategies to control STH include large scale preventive chemotherapy intervention of the endemic population with a broad spectrum antihelminthic drug, a cheap and effective means of reducing the burden and its related morbidities (WHO, 2011b, Pullan et al., 2014). World Health Organization (WHO) recommend a conduct of pretreatment survey to understand the prevalence and intensity of the area plus a comprehensive control program approach which includes deworming, provision of adequate sanitation, health and hygiene education (WHO, 2011b). The frequency of periodic treatment is recommended based on the helminth prevalence of the area. Prevalence of any STH of above or equal to 50% (twice a year deworming), between ≥20 to ≤50% (once a year deworming) is recommended. In both categories all school aged children, enrolled and not enrolled, pre-schoolers, women of child bearing age and adults at high risk occupation have to receive preventive chemotherapy. No large scale preventive chemotherapy is recommended when prevalence is less than 20% (WHO, 2011b).

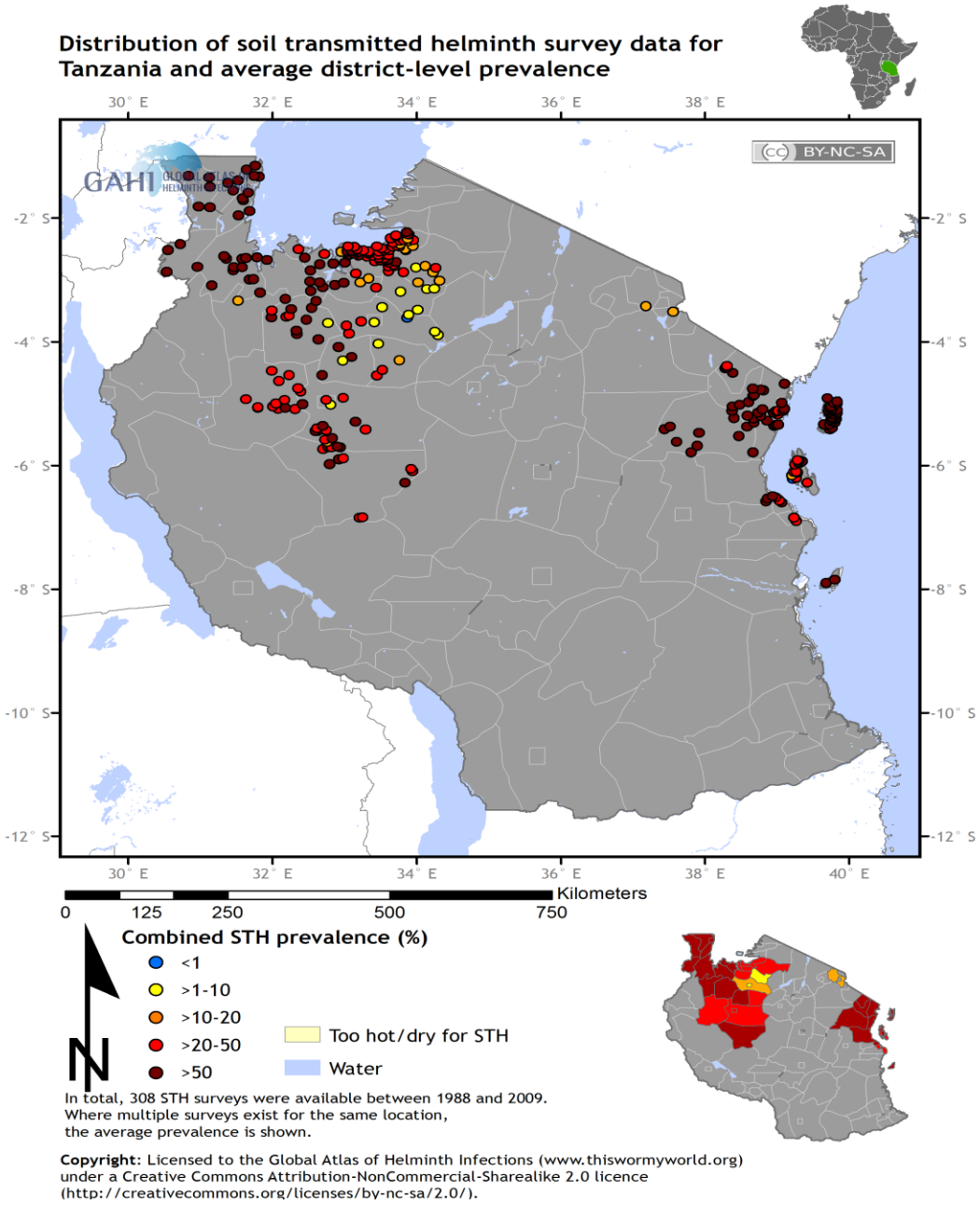


Figure 6. Distribution of soil transmitted helminth (STH) in Tanzania

Source: www.thiswormyworld.org, accessed on 08.02.2015

In Tanzania, a holistic approach to combine all tropical diseases including soil transmitted helminth which was under soil transmitted helminth control program (STHCP) was decided and a neglected tropical disease control program (NTDCP) was developed (Ministry of Health and Social Welfare, 2009). The Bagamoyo district, within the coastal regions of Tanzania was included in the second phase of the program for implementation in 2010. The program was initiated through the United Nations Children's Fund (UNICEF) in 2001. A single dose of antihelminthic therapy with either albendazole or mebendazole is administered as part of mass drug administration (MDA) campaign to children under-five years twice a year every June and December. The targeted interval between the two doses is four months. A combination therapy of ivermectin and albendazole is provided among school aged children as part of the National lymphatic filariasis elimination program (NLFEP) once a year. The choice of antihelminthic drugs used depends on the availability of the drug at the district and donor funding. In 2011, the cumulative coverage of antihelminthic MDA using mebendazole integrated with vitamin A campaign in Bagamoyo was 80.4% (personal communication from Bagamoyo district hospital report). Coverage variations between villages was documented ranging as low as 39.3% in Masuguru, 61.7% in Kiwangwa to more than 100% in areas such as Mkange, Matipwili, Makurunge and Msata with the targed coverage of 80%. The approach is aimed at removing the adult worm from the intestinal tract. Evaluation of infection intensity is the recommended measure of success for a large scale deworming program as per WHO guidelines (WHO, 2011b). Infection intensity is categorized into three thresholds (high, moderate and low) according to STH species and number of eggs detected in one gram of stool (EPG). The thresholds are not only indicator for worm burden but also reflects the degree of environmental contamination with eggs and hence transmission (Montresor et al., 1998). The classification of STH species infection intensity is summarized in Table 1. Effectiveness evaluation is the main drawback with the campaign in most endemic areas including Bagamoyo.

Table 1. Classes of infection intensity for soil transmitted helminth species

	Infection intensity		
STH species	**Light**	**Moderate**	**Heavy**
A. lumbricoides	1 - 4,999	5,000 – 49,999	>50,0000
T. trichiura	1 - 999	1,000 – 9,999	>10,000
Hookworm	1 -1,999	2000 – 3,999	>4,000
S. stercoralis	Not applicable	Not applicable	Not applicable

Source: WHO, 2011

1.2.2.1 Diagnosis of Soil transmitted helminth (STH) infections

Accurate diagnosis is important in estimation of disease burden, monitoring interventions and drug efficacy. Data on the prevalence of STH are greatly influenced by the diagnostic methods and sampling effort (Speich et al., 2014, Sayasone et al., 2015). The sensitivity of parasitological methods are related to infection intensity (Knopp et al., 2008b). Microscopic examination of Kato-Katz thick smears for eggs and larvae of intestinal nematodes in stool samples is widely used (Katz et al., 1972). The Kato- Katz technique is recommended by WHO for community based epidemiological surveys (Montresor et al., 1998). The advantages of Kato-Katz include its performance in places where microscopy is available, cheap and

reusable kits, easy to perform the test and rapidly available results. Only tiny amount of stool is examined (41.7 mg), this negatively impacts on the test sensitivity especially when infection intensity is low (Knopp et al., 2009). Moreover, Kato-Katz method can't detect *S. stercoralis* larvae. Formalin ethyl acetate concentration technique (FECT) which is routinely used for intestinal protozoa but also applicable to STH has a low sensitivity to *S. stercoralis* (Steinmann et al., 2007, Utzinger et al., 2010). The Baermann (Garcia, 2006) or Koga agar plate (Koga et al., 1991) technique are utilized to detect the larvae of *S. stercoralis* in stool. These technique are rarely utilized in routine screening for STH and thus *S. stercoralis* is among the neglected STH (Olsen et al., 2009). Evidence has shown lower sensitivity of a triplicate Kato-Katz as compared to a single FLOTAC method (Knopp et al., 2009). FLOTAC is a new technique with acceptable sensitivity in low infection intensity areas which allows the quantification of eggs and larvae in up to 1 gram of stool (Utzinger et al., 2008).

Routine diagnosis of stool can identify 5 -15% of *E. vermicularis* eggs/adult worm (Cook, 1994). The *E. vermicularis* adult worm tends to hatch its eggs around the perianal skin at night causing itching and discomfort. Microscopic examination of a cellophane adhesive tape applied early in the morning before any cleaning gives a higher yield of positive results. Perianal scraping or swabs from the perianal skin and/or beneath fingernails can be examined directly on clean glass slide with a drop of oil under low power magnification of light microscopy (Cook, 1994).

Serological tests such as enzyme linked immune sorbent assay (ELISA), can also be used for the diagnosis of STH including *S. stercoralis*. These are invasive (requiring blood) and cross reactive antibodies between STH and filarial worms can impact the result specificity (van Doorn et al., 2007).

Polymerase chain reaction (PCR) for detection of the helminth Deoxyribonucleic Acid (DNA) in stool sample have been shown to be specific and sensitive for detecting STH infection (Phuphisut et al., 2014). Its use in routine practice is limited considering the cost associated with the technique.

1.3 Possible modes of interactions between malaria and soil transmitted helminth (STH)

Children living in endemic countries are constantly exposed to STH infections as soon as they start to crawl. Reinfection with the same or different STH species is common leading to co-infection of helminth species. *Plasmodium* species being a common parasitic disease among children living in the tropical and subtropical countries including Tanzania, its coexistence with STH is not uncommon. Recently, a number of conflicting results have been published and other scientific work ongoing to explore the interactions between *Plasmodium* and STH (Nacher, 2011, Adegnika and Kremsner, 2012). This is not a new field in such as for many years scientists have been considering that worms are somehow good for our health, which of course questions the public health benefit, and hence relevance of the deworming (Bundy et al., 2000, Nacher, 2002, Nacher, 2011, Wammes et al., 2014). Experiences from industrialized developed countries shows an increment of inflammatory diseases and allergies (atopy and asthma) as per "hygiene hypothesis theory" (Strachan, 1989). Success stories from case reports and clinical trials on using a porcine whipworm (*Trichuris suis*), hookworm (*Necator americanus*) and *E. vermicularis* for treatment of inflammatory bowel diseases (IBD) have been documented (Elliott and Weinstock, 2012) and many other clinical trials are in the pipeline (Wammes et al., 2014). Here we summarize the possible mechanisms of interaction between STH and *Plasmodium,* either directly or mediated through the host.

1.3.1 Direct interaction and resource competition

STH are macroparasites mostly occupying the human intestinal lumen (Jackson et al., 2009). Generally, the outcome of pathogenesis to the host varies depending on the co-infected species as outlined by Petney & Andrew (Petney and Andrews, 1998), and summarized in Table 2. The pathological damage caused by one parasite could influence the susceptibility of the host to a different species. Competition between parasite species can either limit population size or can cause changes in the anatomical site of the infection in the host (change in the occupied niche) (Petney and Andrews, 1998). This is a characteristic of most of the parasite within the host, for example hookworm, *A. lumbricoides* and *S. stercoralis* with visceral migration as compared to *E. vermicularis* and *T. trichiura* which are mostly localized in the lumen of gastrointestinal tract. Different mechanism of interaction occurs most likely at different stages of the life cycles (Churcher et al., 2006, Yakob et al., 2013). The rate and multiplicity of species observed as disease progression process for example with *Plasmodium* species could be influenced by other co-infecting species. Factors triggering the progression of *Plasmodium* infection from the liver stage to a blood stage or from asymptomatic *Plasmodium* infection to a clinical disease state are still vague (Stanisic et al., 2013). Complex mechanisms are involved in structuring the interactions which could be synergistic, antagonistic or neutral depending on external and internal factors shaping the co-infection (Petney and Andrews, 1998). Both interference and exploitation competition can occur depending on the co-infecting species virulence, abundance, over-dispersion within the host and sequence in which the host acquired the infections (Fakae et al., 1994, May and Nowak, 1995, Petney and Andrews, 1998).

Table 2. The possible outcomes of the two species parasite infection on the pathogenicity to the host

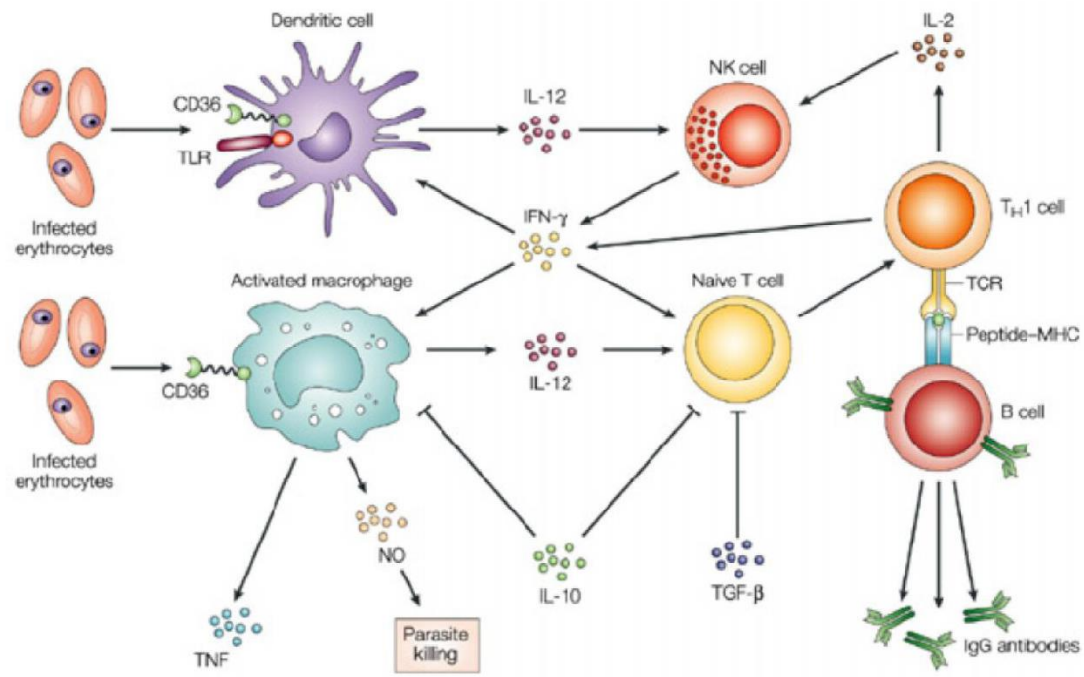
Influence of parasite 1 on parasite 2	Influence of parasite 2 on parasite 1	Relationship to pathogenesis
+	+	Each species increases pathogenicity of the others
+	0	One specie increase pathogenicity, the other its usual pathogenicity
+	-	One specie increase pathogenicity, the other reduced pathogenicity
0	0	Neither species affect the others
0	-	One specie shows its usual pathogenicity, the other reduced pathogenicity
-	-	Both species show reduced pathogenicity

+ indicated increased pathogenicity; 0 indicates no change; - indicates reduced pathogenicity

Source: Petney and Andrews, 1998

1.3.2 Immune mechanisms

Different immunological mechanisms induced by helminth infection have been highlighted as potentially protective against *Plasmodium* infection or increasing the risk. Infection with helminth has a profound effect on the immune system resulting in polarisation towards T helper 2 (Th2) responses, characterized by high levels of cytokines such as interleukin-4 (IL-4), IL-5, IL-13 and high serum levels of immunoglobulin E (IgE) (Maizels and Yazdanbakhsh, 2003, Jackson et al., 2009). Despite these strong Th2 responses, adult helminth often survives in the human host, sometimes for decades. The mechanisms include the induction of regulatory T (Treg) cells and modulation of cells of the innate immune system (Wammes et al., 2014), such as alternatively activated macrophages (AAMϕ), myeloid depressed suppressor cells (MDSC)(Gabrilovich and Nagaraj, 2009), regulatory dendritic cells (DCreg) and regulatory natural killer cells (NKreg)(Jewett et al., 2013), which results in an anti-inflammatory environment, characterized by increased levels of IL-10 and transforming growth factor-β (TGF-β). The excretory and secretory products of helminth causing immune-regulatory activities have been documented (McSorley et al., 2013). This regulatory network prevents the elimination of the helminth and at the same time protects the host against pathology that would otherwise result from excessive inflammation. The hypo-responsiveness also termed as "modified Th2 immune response" is not only directed towards helminth antigens, but appears to extend to third party antigens. These includes *Plasmodium* infection, allergies and inflammatory conditions such as autoimmune diseases and IBD (Jackson et al., 2009, Nacher, 2011, Elliott and Weinstock, 2012). The immune correlates of protection for the *Plasmodium* are not well characterized. Cytophilic antibodies immunoglobulin-G1 (IgG1) and IgG3 are probably the major effectors of *Plasmodium* parasite clearance during the blood stage (Cohen et al., 1961). The role of CD4 T-cells against blood-stage malaria has been investigated in rodent models and indicates that Th1 cells are involved during the acute phase through the production of pro-inflammatory cytokines such as IFN-γ and TNF-α while Th2 cells are important for the clearance of the parasite, involving active cooperation of innate immunity including monocytes/macrophages, dendritic cells, natural killer (NK) cells, natural killer T (NKT) cells and γδT cells (Bouharoun-Tayoun et al., 1990, Achtman et al., 2005, Stanisic et al., 2013). Figure 7 summarizes A. the immune response to malaria infection and B. the immune response to helminth infection.



A: Immune response to malaria infection

B: Immune response to helminth infection

Source: Montaner et al, 2014

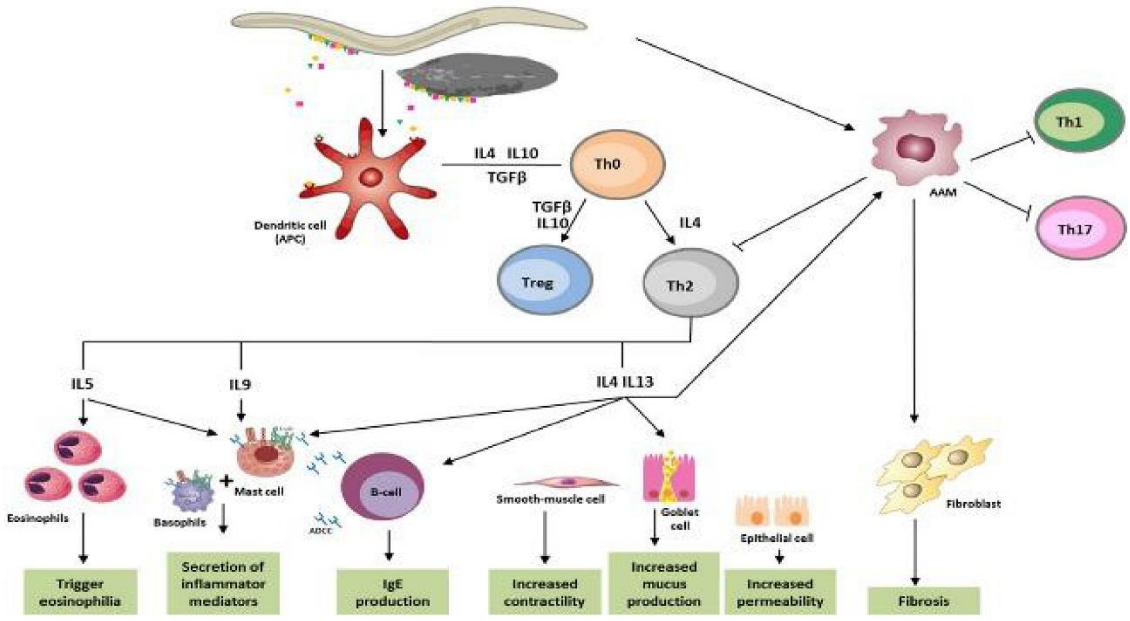


Figure 7. A. The immune response to malaria infection. 7. B. The immune response to helminth infection.

The Th2/Treg response is presumed to modify the malaria immunity in two main hypotheses which support either the protective or the enhancing role of helminths for severe malaria. First, the helminth could protect through an increase in IgE complexes that activate high affinity Fc receptors FceRII (CD23) and the anti-inflammatory IL-10 which activate the nitric oxide synthase releasing the nitric oxide leading to reduced sequestration of parasited red blood cells (Nacher, 2002). Additionally, T cells with regulatory function (Treg) in helminth co-infected children may lead to suppression of Th1 and pro-inflammatory responses which are key for *Plasmodium* parasite clearance within the host (Hartgers et al., 2009). Secondly, the helminths are presumed to decrease cytophilic IgG1 and IgG3 and increase non-cytophilic IgG2, IgG4 and IgM antibodies. This alters antibody dependent cellular inhibition (ADCI) leading to increase in incidence and severity of malaria (Druilhe et al., 2005, Roussilhon et al., 2010).

Chapter 2: RATIONALE AND RESEARCH QUESTION

Plasmodium and STH helminth co-infections are common considering the wide geographical overlap in occurrence, chronicity nature of the infections, environmental factors and poor socioeconomic status which facilitate continuous exposure and reinfection of the host. The pathogenic process of co-infection can complicate the diagnosis and prognosis of specific diseases. Children are the most affected leading to severe morbidities and mortality specifically in developing countries where management of cases is challenging. Sensitive diagnostic methods are central in identifying and accurately assess the disease burden attributable to individual infections. The composition of parasite community making up co-infection is not random and plays part in evolution of both host and parasites. Despite the fact that co-infections occur and cause severe morbidities, less is known and invested on how the two common parasites *Plasmodium* and STH interact. Epidemiological evidence is lacking and intervention efforts are ongoing based on single parasite approach. Data are insufficient on between species parasite interaction. New knowledge on interactions and attributed risk is relevant to better understand the disease epidemiology in relation to interventions for policy decision, better management and tailored integrated control measures.

Similar situation is observed in Tanzania, research information on co-infections is lacking and basic epidemiology of common parasitic co-infections in different transmission zones is urgently required. The progress of intervention program coupled with epidemiological surveys to evaluate the disease burden at the individual level is lacking. More data is required for the programs to be tailored in the right way particularly when moving from morbidity to transmission and elimination control in the momentum of disease eradication. Less is known on how *Plasmodium* interact with the ancient species of STH, what are the risks and benefits and how best to tackle them.

This PhD research was conducted to explore interaction between *Plasmodium* and STH infections in Bagamoyo district, coastal region of Tanzania.

The research questions were as follows;

I. How sensitive are the diagnostic methods used in identifying the STH species in Bagamoyo district, coastal region of Tanzania?

II. Does (do) STH helminth co-infection(s) influence susceptibility to *Plasmodium* infection?

III. Does (do) STH helminth co-infection(s) influence clinical presentation and severity of *Plasmodium* infection?

Chapter 3: GOAL AND OBJECTIVES

3.0 Goals

3.1 General objective

To explore the interaction of *Plasmodium* and STH helminth infection among children living in endemic areas of Bagamoyo district, coastal region of Tanzania.

3.2 Specific objectives

I. To investigate the sensitivity and performance of the diagnostic methods to detect helminth infections used in a rural area within Bagamoyo district, in the context of National helminth control program.

II. To investigate the relation of STH and *Plasmodium* parasite prevalence rates using data from community cross sectional survey of children living in Bagamoyo district.

III. To investigate the impact of STH on clinical presentation and treatment outcome of malaria using a case control study among children in Bagamoyo district, coastal region of Tanzania.

CHAPTER 4: METHODOLOGY

4.1 Study area

The study was conducted in Bagamoyo district, coastal region of Tanzania under the Ifakara health institute (IHI) research platform. Four main villages situated in rural western area of Bagamoyo district, about 20 to 60 km from Bagamoyo town were purposely selected considering the transmission of malaria and helminth. The villages included were Magomeni, Kiwangwa, Mkange and Msata. Ifakara health institute (IHI) through its Bagamoyo research and training centre (BRTC) work in close collaboration with the Bagamoyo district hospital (BDH) officials to ensure quality health care delivery using its research platforms. This includes administrative, laboratory, data management facilities and clinical support at the main Bagamoyo district hospital and sentinel dispensaries within the study area.

The IHI study area (Figure 8) covers about 1160 square kilometres. The Eastern border of the study area is formed by the Indian Ocean, with the Ruvu River forming part of the western and northern borders. The area extends for approximately 7km on either side of a road running westwards for 62 kilometres. To the south is an uninhabited forest reserve. According to the 2012 Tanzania National Census, the population of the Bagamoyo District was 311,740 which can be reached by dirt road throughout the year; all the villages of the study area are within one hour drive.

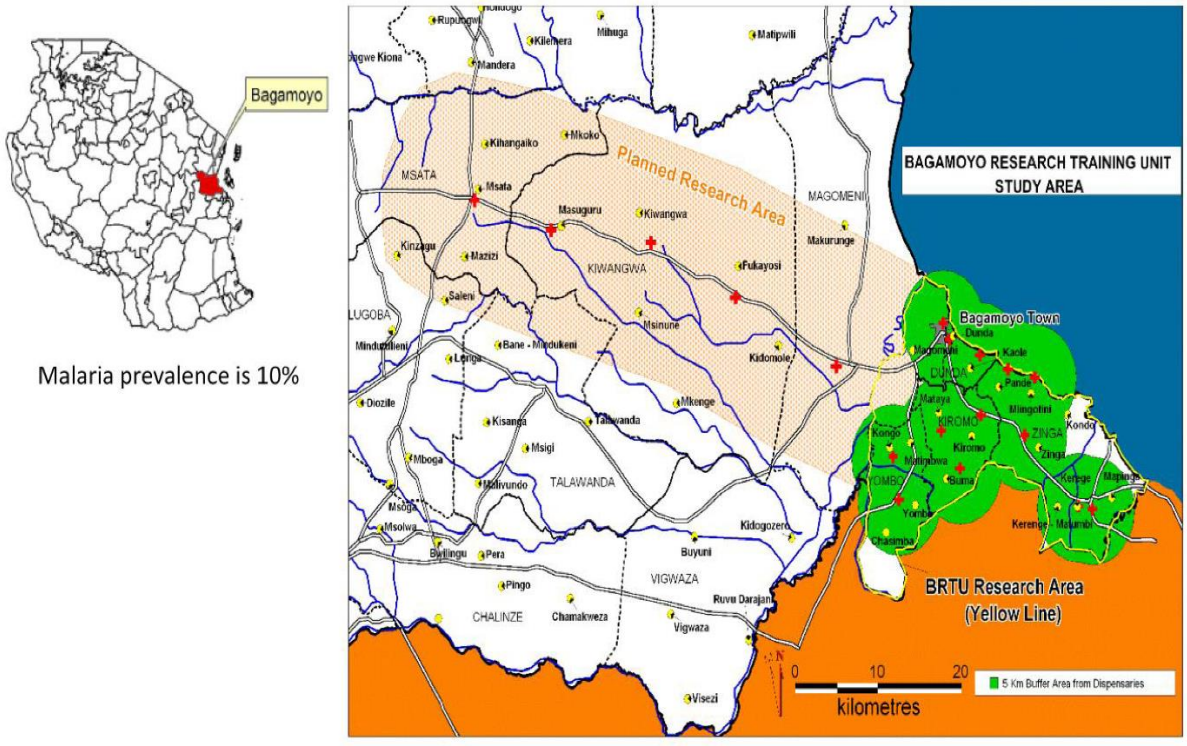


Figure 8. The Bagamoyo research and training centre (BRTC) study area within the platform of Ifakara health institute (IHI)

The main rainy season is from March to May, with a second period from November to December, although occasional rain occurs at all times of the year. The average rainfall is 1200 to 2100 mm per year. There is year round grassland vegetation or subsistence agriculture throughout the study area. According to meteorological statistics, the average temperature for the region is about 28^0C.
The recruitment of the study was done between June 2011 and November 2012, covering all year seasonal variations.

4.2 Study population and design

Diverse ethnic groups including the Zaramo, Kwere, Doe and Zigua inhabit the study area and the majority of them are either subsistence farmers who cultivate rice, maize and cassava, or fish from the sea or the Ruvu River and its tributaries. Agriculture employs 76% of the population. Kiswahili, the national language, is widely spoken in the area. The literacy rate is moderate in both men and women (about 57%) (National Bureau of Statistics (NBS) [Tanzania] and ICF Macro, 2010).
Studies were conducted as the malaria component of the IDEA project, a global research program designed to study the immunological interplay between helminth infections and the neglected tropical diseases namely HIV, TB, and Malaria (Willyard, 2009). A number of research institutions from different countries in Europe and Africa are participating in IDEA project. The present study concerns the IHI and Swiss tropical and public health institute (Swiss TPH), part of IDEA, Tanzania (IDEA-Tz).
To investigate the sensitivity and performance of the diagnostic methods (objective 1), stool samples were selected for methods comparison from the community survey of adults and children screened within the IDEA arm of TB and malaria respectively. Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann and polymerize chain reaction (PCR) methods were analyzed for common STH, hookworm and *S. stercoralis*.

To investigate the relation of STH and *Plasmodium* parasite prevalence rates (objective 2), a community cross-sectional survey was conducted among children aged 2 months to 9 years inclusively. This community-based survey was also used to recruit asymptomatic *Plasmodium* parasitemia children (controls) for the case-control study below. The prevalence of *Plasmodium* parasitemia was around 10% within the study area and thus around 1,000 children from the community were screened and enrolled.
To investigate the impact of STH on malaria clinical presentation and treatment outcome (objective 3), a case-control study with a semi longitudinal follow-up was conducted. Cases were enrolled in two different groups, cases with severe malaria and cases with uncomplicated malaria. Controls were children with asymptomatic *Plasmodium* parasitemia. The longitudinal short term observational part of the study consisted of the assessment of response to anti-malaria treatment in the three groups according to World Health Organization (WHO) procedures (day 0, 1, 2, 3, 7, 14, 28, 42). The exposure in this case-control study was taken as presence or absence of an infection with at least one of the helminth species investigated. Included children were assessed at each visit (recruitment and follow up visits) by a qualified, trained study clinician for signs and symptoms of malaria and other common diseases using a structured questionnaire designed for the study.

In both community survey and case-control study, stool, urine, adhesive tapes and blood samples were collected and examined using a broad set of quality controlled diagnostic methods for common STH

(*Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Enterobius vermicularis*, *and Trichuris trichiura*), schistosoma species and *Wuchereria bancrofti*. Blood slides and malaria rapid tests (mRDTs) were utilized for *Plasmodium* diagnosis. Stool, urine and adhesive tapes were collected at the initial screening (both studies) and during the day 28 follow up visit (case-control study). In order to investigate the impact of STH on malaria clinical presentation and treatment outcome, children diagnosed with STH received a delayed anti-helminthic treatment at the end of study follow up (day 42). To prevent unnecessary complication, children were closely followed up for safety and those with heavy helminth load and severe disease received treatment prior to day 42. Figure 9 summarizes the study population, study design and procedures.

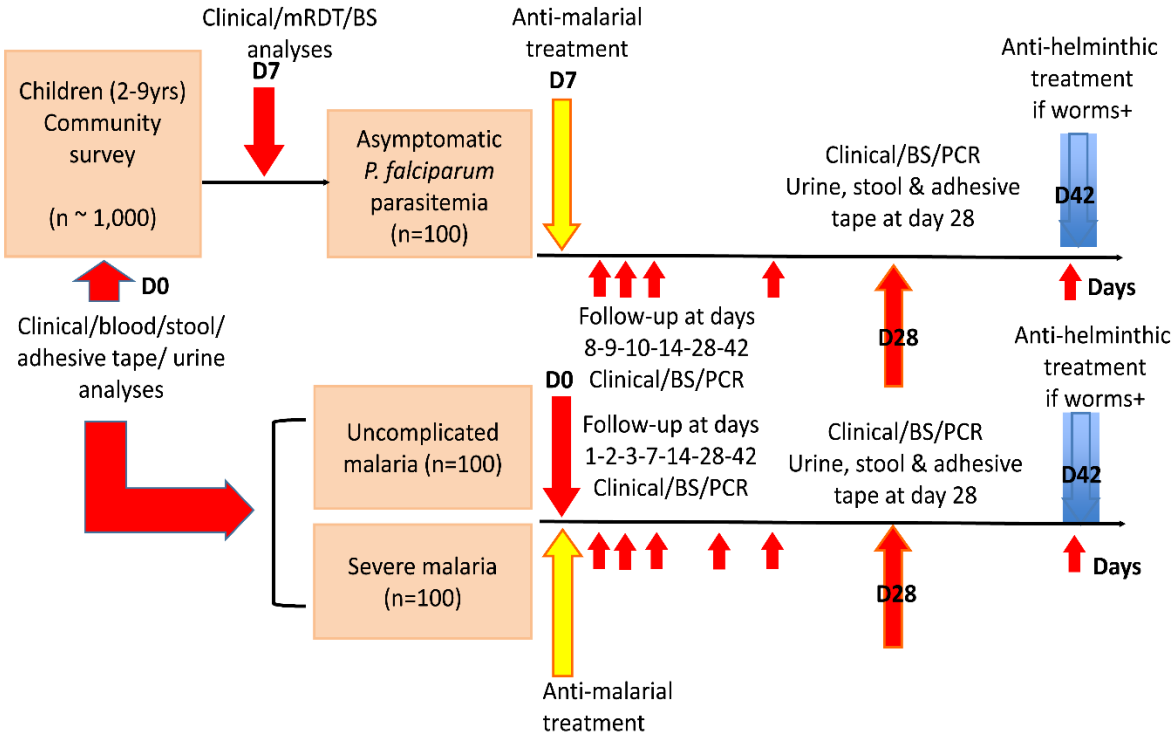


Figure 9. Study population, design and procedures

4.3 Statistical methods

Study findings derive from descriptive and analytical methods. The burden of *Plasmodium*, STH infection and co-infections are presented as prevalence rates, the strength of association between the different parasites as odds ratio and 95% confidence interval (CI) with adjustment of other covariates using different multivariate analyses. The covariates included age, gender, location (village/hamlets), education level, anthropometric measurements and specific interventions (use of antihelminthic and bednets).

A summary of statistical methods used for each objective is outlined in the below paragraph:

Objective 1: Agreement between the diagnostic methods used was assessed using kappa statistics. Wilcoxon rank sum (Mann-Whitney) test was applied to assess the differences of median hookworm egg per gram (EPG) or *S. stercoralis* larvae counts between the groups of samples identified as true positives or false negatives with methods used. The association between PCR cycle thresholds (Ct) and EPG values derived by different methods was assessed using Pearson correlation. The sensitivity and specificity including confidence intervals (95% CIs) were calculated by three different approaches i) direct comparison of the methods using McNemar exact test based on Yates X^2 considering only individuals who were helminth positive, ii) calculating sensitivity of the pooled results of the methods as diagnostic pseudo gold standard assuming 100% specificity of all the methods and iii) using Bayesian estimates.

Objective 2: Cross tabulation of helminth, *Plasmodium* and co-infections was performed. Observed and expected prevalence of *Plasmodium*, helminth and co-infections were calculated. Fisher's exact test was applied to assess differences between observed and expected prevalence. The age profile of *Plasmodium* and STH co-infections was investigated using a simple logistic model to see how the model fitted our observed values. Bivariate and multivariate analyses were used to assess the strength of association between mono-infections and co-infections using different models. Mantel-Haenszel analysis was applied to further investigate the age dependency relationship between STH and *Plasmodium* infections.

Objective 3: Prevalence of STH was compared according to clinical malaria status. Conditional logistic regression was used to assess the effect of STH on clinical presentation in the case control study. The relationship between STH and malaria clinical status was further explored using ordinal logistic regression model to better reflect the risk with increasing severity of malaria clinical status. Treatment response was analyzed according to adequate clinical and parasitological response (WHO, 2009b). Geometric mean time to first parasite clearance in the groups of asymptomatic parasitaemia, uncomplicated malaria and severe malaria was estimated using a simple regression model adjusted for age group.

Data were analyzed using STATA software (Stata Corp; College station, Texas, United States of America) and mapping performed using geographical information system (arcgis 10). More details of the specific statistical analytical methods applied in the thesis are presented in the respective chapters (chapter 5, 6, 7 and 8).

4.4 Ethical consideration

Studies included in this work were submitted and approved by the relevant ethical committees, including the Ethikkomission beider Basel (EKBB in Basel, Switzerland) and the Ifakara health institute, IHI; Dar es Salaam, United Republic of Tanzania. The ethical approval for the conduct of the study was granted by the EKBB; Basel Switzerland; reference number: 257/08 and the National institute for medical research, NIMR; Dar es Salaam, reference number NIMR/HQ/R.8a/Vol. IX/1098. The work was conducted according to Good Clinical Practice (GCP) guidelines as required by the International Conference on Harmonization (ICH). IDEA, an FP7 European funded project, was internally monitored by IHI through a Pan African Collaboration for the Evaluation of Anti-tuberculosis Antibiotics (PanACEA) capacity building support of clinical research associates (CRA) group.

Chapter 5: Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann, and PCR methods for the detection of light intensity hookworm and *Strongyloides stercoralis* infections in Tanzania

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The authors declare that they have no competing interests.

The article has been published in American Journal of Tropical Medicine and Hygiene, (Knopp et al., 2014)

Abstract

Abstract Sensitive diagnostic tools are crucial for an accurate assessment of helminth infections in low-endemicity areas. We examined stool samples from Tanzanian individuals and compared the diagnostic accuracy of a Real-Time polymerase chain reaction (PCR) with the FLOTAC technique and the Kato-Katz method for hookworm and with the Baermann method for *Strongyloides stercoralis* detection. Only FLOTAC had a higher sensitivity than the Kato-Katz method for hookworm diagnosis; the sensitivities of PCR and the Kato-Katz method were equal. PCR had a very low sensitivity for *S. stercoralis* detection. The cycle threshold values of the PCR were negatively correlated with the logarithm of hookworm egg and *S. stercoralis* larvae counts. The median larvae count was significantly lower in PCR false-negatives than in true-positives. All methods failed to detect very low intensity infections. New diagnostic approaches are needed for monitoring progressing helminth control programs, confirmation of elimination, or surveillance of disease recrudescence.

Background

Infections with intestinal nematodes are widespread in humans living in tropical and sub-tropical countries, where, as a consequence of a poor sanitary infrastructure, environmental contamination with feces is high. In 2010, the estimated global burden of intestinal helminthiases was 5.2 million (Mio) disability adjusted life years (DALYs) lost (Murray et al., 2012). Infections are commonly caused by the soil-transmitted helminths, namely *Ascaris lumbricoides*, hookworm (*Ancylostoma duodenale* and *Necator americanus*), and *Trichuris trichiura*. Hookworm disease accounts for the biggest part of the burden estimates (3.2 Mio DALYs), mainly since hookworms cause and contribute to iron deficiency anemia, which can negatively impact on the health of children and women in childbearing age, as well as on fetuses and newborn babies (Renggli et al., 2013, Bethony et al., 2006). *Strongyloides stercoralis*, an often neglected additional soil-transmitted helminth species, is infecting an estimated 30--100 Mio people (Bethony et al., 2006) but no DALY burden estimates exist. Recent prevalence estimates suggest that strongyloidiasis affects between 10% and 40% of the population in many tropical and subtropical countries, but that particularly in sub-Saharan Africa and Southeast Asia infection with *S. stercoralis* is highly underreported (Schär et al., 2013b). Strongyloidiasis can be asymptomatic or lead to cutaneous, gastrointestinal, or pulmonary symptoms like skin rashes, abdominal pain, and abnormal wheezing, respectively (Olsen et al., 2009, Becker et al., 2011, Ziegelbauer et al., 2012, Ericsson et al., 2001). Importantly, hyper-infections evolving in immunocompromised individuals can be potentially fatal (Marcos et al., 2008, Ziegelbauer et al., 2012, Segarra-Newnham, 2007, Schroeder and Banaei). The difficulties in correctly diagnosing this parasite are mainly responsible for its constant neglect in epidemiological mapping and burden estimations.

Recently, the will to control neglected tropical diseases has been boosted by the ambitious goal of the World Health Organization (WHO) to eliminate neglected tropical diseases or to reduce their impact to levels at which they are no longer considered public-health problems by 2020 (Zhou et al., 2012). The target for soil-transmitted helminths is to regularly treat 75% of preschool and school-aged children in need of treatment and to achieve 75% treatment coverage in all endemic countries. In support of this goal, a considerable number of public and private partners officially committed in the “London

Declaration on Neglected Tropical Diseases" from January 2012 to help controlling soil-transmitted helminthiases by supplying drugs and other interventions.

Scaling up interventions to control soil-transmitted helminthiases will require a solid and timely assessment of the epidemiological situation on the basis of sensitive and specific diagnostic methods to i) guide the initiation of interventions, ii) monitor and evaluate the impact of interventions, iii) detect anthelminthic resistance at an early stage of development in the field, iv) confirm the interruption of transmission, and v) spot the recrudescence of infections and disease by surveillance (Lustigman et al., 2012, WHO, 2012, McCarthy et al., 2012). Currently applied diagnostic methods have, however, a number of drawbacks and technical limitations. The Kato-Katz thick smear method, which is the most widely used technique to assess soil-transmitted helminth prevalence and infection intensities in epidemiological surveys and helminth control programs, is a cheap and simple method but lacks sensitivity for the detection of low-intensity soil-transmitted helminth infections (Booth et al., 2003, Knopp et al., 2008b). The recently developed FLOTAC technique, which is also based on microscopic detection of helminth eggs in stool samples, has a higher sensitivity to identify light soil-transmitted helminth infections and the application gains popularity in research studies conducted across the world (Knopp et al., 2009, Glinz et al., 2010b, Jeandron et al., 2010, Ziegelbauer et al., 2010, Habtamu et al., 2011, Gualdieri et al., 2011, Utzinger et al., 2008). The disadvantages of the FLOTAC technique are that it requires a set of more sophisticated laboratory equipment such as a centrifuge and special chemicals, and that it is relatively low-throughput. For the diagnosis of *S. stercoralis* larvae in stool samples, the Kato-Katz method and FLOTAC are not suitable. For this purpose, the Baermann funnel (de Kaminsky, 1993) and stool culture techniques such as the Koga agar plate (Koga et al., 1991) or stool-charcoal or stool-vermiculate mixtures in petri dishes (Polderman et al., 1991, Yelifari et al., 2005) or best a combination thereof are recommended (Utzinger et al., 2012, Knopp et al., 2008b, Steinmann et al., 2007). Different polymerase chain reaction (PCR) based approaches for the detection of soil-transmitted helminth DNA or rRNA in stool samples have been developed and are increasingly promoted for monitoring and surveillance of control programs (Verweij et al., 2007, Verweij et al., 2009, Basuni et al., 2011, Taniuchi et al., 2011, Murray et al., 2012). It remains to be elucidated, however, if the sensitivity of PCR based diagnosis of helminth infections in stool samples is considerably better than that of direct parasitological methods, particularly if infection intensities are low.

Here, we compare and discuss multiple aspects of the diagnostic performance of the Kato-Katz method and FLOTAC, Kato-Katz method and PCR, Baermann method and PCR and, for the first time, of FLOTAC and PCR for the diagnosis of hookworm and *S. stercoralis* infections. Three different statistical approaches were used to render our results comparable to a broad set of previous and future studies. Stool samples were obtained from individuals living in the Bagamoyo district in the coastal region of the United Republic of Tanzania who participated in a screening for helminth infections for the IDEA project between June 2011 and November 2012. The IDEA project is an African-European research initiative, which aims to dissect the immunological interplay between poverty related diseases and helminth infections (http://ec.europa.eu/research/health/infectious-diseases/neglected-diseases/projects/014_en.html).

Materials and methods

Ethics Statement

The institutional research commissions of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland) and the Ifakara Health Institute (IHI; Dar es Salaam, United Republic of Tanzania) approved the protocol of the IDEA project conducted at the Bagamoyo Research and Training Center (BRTC) in the United Republic of Tanzania. The Ethikkomission beider Basel (EKBB; Basel, Switzerland; reference number: 257/08) and the National Institute for Medical Research of Tanzania (NIMR; Dar es Salaam, United Republic of Tanzania; reference number: NIMR/HQ/R.8a/Vol.IX/1098) granted ethical approval for the study.

The purpose and procedures of the study were detailed to the local district, community and health authorities, and explained to individuals eligible for screening and potential participation in one of the three study arms of IDEA. In brief, these study arms are investigating the immunological interplay between helminth infections and malaria (arm 1), tuberculosis (arm 2), or human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS; arm 3), respectively. Participants were informed that their participation was voluntary and that they could withdraw from the study at any time without further obligation before they were invited to sign a written informed consent sheet. From all participating adult individuals and from the parents or legal guardians of participating minors (children below the age of ten years), written informed consent was obtained. In case participants or their parents or guardians were illiterate, they signed by thumbprint.

Participants infected with soil-transmitted helminths were administered albendazole (400 mg single oral dose) against *A. lumbricoides*, hookworm or *T. trichiura*, or ivermectin (200 µg/kg single oral dose) against *S. stercoralis*, or praziquantel (40 mg/kg) against schistosome infections, according to the national treatment guidelines of the United Republic of Tanzania.

Study Area

The participants whose data were included in the present analysis were children and adults residing in rural villages within the Bagamoyo district, which is located north of Dar es Salaam in the Coast Region of the United Republic of Tanzania. Samples were collected between June 2011 and November 2012. The fresh stool specimen were examined in the Helminth Unit laboratory of the BRTC and preserved stool samples were analyzed with PCR in the laboratory of the National Institute for Medical Research - Mbeya Medical Research Center (NIMR-MMRC) in Mbeya, United Republic of Tanzania.

Field procedures

Potential candidates for the inclusion in one of the study arms of the IDEA project were i) children aged 6 months to 9 years and living in the west catchment areas of one of six health facilities in the Bagamoyo district, ii) children aged 6 months to 9 years who presented at one of the six health facilities with either asymptomatic or uncomplicated malaria, iii) children who presented at the Bagamoyo District Hospital with severe malaria, and iv) people of all age groups being part of a community health screening

conducted in remote villages in the Bagamoyo district to recruit new participants for any arm of the IDEA study. All candidates were screened for helminth infections as detailed below.

After written informed consent or thumbprint was obtained from the participant or in case of minors from the parent/legal guardian, the participant was registered, assigned a personal unique identification number and provided with a plastic container (100 ml) for collection of a fresh morning stool sample that was to be submitted the following day before noon to the consulted health facility or, in case of the village health survey, to a predefined meeting point in the village center. The samples were collected every day around noon from the health facilities or the central village points in the Bagamoyo area by a fieldworker and transported by motorbike to the Helminth Unit of the BRTC.

Laboratory Procedures

All stool samples were examined in the Helminth Unit of the BRTC right after arrival by experienced laboratory technicians. The Baermann method was applied for the detection of *S. stercoralis* larvae (García and Bruckner, 2001). In brief, a walnut-sized stool sample was placed on double layered gauze in a tea sieve within a glass funnel that was filled with tap water and exposed to electric light from below. Phototactic *S. stercoralis* larvae were collected after 2 hours of light exposure, visualized on microscope slides and their number recorded in the case report form (CRF) of the respective participant. Duplicate Kato-Katz thick smear slides were prepared from each stool sample for the detection of soil-transmitted helminth and *S. mansoni* eggs (Katz et al., 1972). For this purpose, filtered stool samples were filled in a 41.7 mg template and the stool smears were incubated for ~20 min before the slides were read under the microscope. The number of helminth eggs was counted and recorded species specifically. Moreover, the FLOTAC dual technique was performed for the diagnosis of soil-transmitted helminth and *S. mansoni* infections (Glinz et al., 2010b). A small sub-sample of each individual's stool (~1 g) was weighed and preserved in sodium acetate acetic acid formalin (SAF) for examination by FLOTAC the next day, and 0.5 g of stool were placed in cryotubes and frozen at -80°C for DNA extraction and examination with PCR at a later point in time. The FLOTAC dual technique was performed on the following morning, before new samples arrived. We used flotation solution 2 (FS2; saturated sodium chloride (NaCl) solution; specific gravity (s.g.): 1.20) and FS7 (zinc sulfate ($ZnSO4{\cdot}7H2O$) solution; s.g.: 1.35).

For the DNA isolation we followed the procedure described by Verweij and colleagues (Verweij et al., 2001). All DNA samples were stored at -20 °C and transferred on ice to the NIMR-MMRC, where PCR amplification and detection was conducted in June and November 2012.

A Multiplex Real-Time PCR was used for the simultaneous detection of *A. lumbricoides*, *N. americanus, S. mansoni* and *S. stercoralis* DNA in fecal samples (Verweij et al., 2007, Verweij et al., 2009, Obeng et al., 2008, Wiria et al., 2010). For DNA amplification, 5 µl of DNA extracted from 0.1 g stool specimens was used as a template in a final volume of 25 µl with PCR buffer (HotstarTaq master mix, 5mM $MgCl_2$, 2.5 µg bovine serum albumin; Roche Diagnostics, Almere, The Netherlands), 2 pmol of each *A. lumbricoides*-specific primer (Thermo Fisher, Ulm, Germany), 5 pmol of each *N. americanus*-specific primer (Thermo Fisher, Ulm, Germany), 5 pmol of each *Schistosoma*-specific primer (Thermo Fisher, Ulm, Germany), and 2.5 pmol of each *S. stercoralis* specific primer (Thermo Fisher, Ulm, Germany), 1.25 pmol of each

N. americanus-specific double-labeled probe (Biolegio, Nijmegen, The Netherlands), *A. lumbricoides*-specific double-labeled probe (Thermo Fisher, Ulm, Germany), *S. stercoralis*-specific double-labeled probe (Biolegio, Nijmegen, The Netherlands), and *Schistosoma*-specific double-labeled probe (Thermo Fisher, Ulm, Germany). Amplification consisted of 15 min at 95°C followed by 50 cycles of 15 s at 95°C, 30 s at 60°C, and 30 s at 72°C. Amplification, detection and data analysis were performed with the Corbett Rotor-Gene 6000 Real-Time PCR system (Corbett Research, Mortlake, New South Wales, Australia) and Corbett Rotor-Gene 6000 Application Software, version 1.7. Negative and positive external control samples were included in each amplification run. The details of all primers and detection probes used in our study are described elsewhere (Verweij et al., 2009, Obeng et al., 2008, Wiria et al., 2010, Verweij et al., 2007).

Data Management and Statistical Analysis

The helminth species specific results derived by each method were entered manually in the participant's CRF and subsequently transferred into a Microsoft Access 2010 electronic database (Microsoft Corporation 2010, Redmond, Washington, USA). Data were analyzed using STATA version 12 (StataCorp.; College Station, Texas, USA) and R version 2.15.2 (R Foundation for Statistical Computing; Vienna, Austria).(R_Development_Core_Team, 2012)

For the comparison of diagnostic methods, the diagnostic results of the first stool sample collected and examined from each participant were included into the analysis. Eligible for inclusion were participants with results on i) duplicate Kato-Katz thick smears and one FLOTAC dual examination, ii) duplicate Kato-Katz thick smears and one PCR measurement, iii) one FLOTAC dual and one PCR examination, or iv) one Baermann and one PCR examination. The prevalence of each helminth species investigated is indicated per method and method combination. One must be aware, however, that the participants who submitted stool samples that were included into the present analysis were no random population sample, since children were recruited in part when they visited a health facility or hospital due to asymptomatic, uncomplicated or severe malaria and because stool samples examined with PCR were selected on purpose and not randomly from individuals who participated in the immunological investigations of the IDEA-malaria study arm.

Among the 215 stool samples tested with PCR, 123 were selected from children who participated in the immunological investigations of the IDEA-malaria study arm (i.e., from children selected according to their infection status with helminths based on the Kato-Katz thick smear, FLOTAC, and Baermann method results and according to asymptomatic or symptomatic malaria). The additional 92 stool samples were selected randomly from the list of study participants providing stool samples.

Helminth infection intensities were determined by multiplying the species specific average egg counts from duplicate Kato-Katz thick smears by factor 24 and by dividing the species specific sum of eggs counted in the two floatation chambers by the measured weight of the preserved stool sample and multiplying the result by factor 1.2 to derive eggs per gram of stool (EPG). Subsequently, the infection intensity thresholds recommended by the World Health Organization were applied for EPG derived with the Kato-Katz method (Montresor et al., 1998). The lower limits of moderate and heavy infections were

5,000 and 50,000 EPG for *A. lumbricoides*, 1,000 and 10,000 EPG for *T. trichiura*, 2,000 and 4,000 EPG for hookworm, and 99 and 399 EPG for *S. mansoni*, respectively.

The agreement between the diagnostic methods was assessed using kappa (κ)-statistics. The κ-statistics were interpreted as follows: < 0.00, poor agreement; 0.00--0.20, slight agreement; 0.21--0.40, fair agreement; 0.41--0.60, moderate agreement; 0.61--0.80, substantial agreement; 0.81--1.00, almost perfect agreement (Landis and Koch, 1977).

High PCR cycle-threshold (Ct-) values reflect low parasite-specific DNA loads and vice versa. In addition to PCR assays where no amplification curve was obtained, all Ct-values above 40 were considered as negative test results (Basuni et al., 2011). To assess if the median of positive Ct-values from PCR, the median of positive EPG values derived with the Kato-Katz thick smear method or FLOTAC, or the median of positive larvae counts from Baermann differed between the groups of samples identified as true-positive or false-negative with any other method, we used the Wilcoxon rank-sum (Mann-Whitney) test. We used a statistical significance level of 5%.

The Pearson's correlation was applied to assess an association between PCR Ct-values and EPG values derived by the Kato-Katz thick smear method and FLOTAC, respectively, or *S. stercoralis* larvae counts determined by the Baermann method. In line with codes used in a previous publication about the same topic from another research group (Verweij et al., 2007). PCR assays where no amplification curve was obtained and all Ct-values above 40 were considered as negative and coded 45, negative EPG results from duplicate Kato-Katz thick smears were coded 10, negative EPG results from the FLOTAC dual technique were coded 0.1 and negative larvae counts from Baermann were coded 0.5.

The diagnostic accuracy parameters including 95% confidence intervals (95% CI) were calculated by three different approaches. Firstly, we directly compared the above mentioned methods with each other to calculate the sensitivity and specificity for each test. The sensitivities of the tests were compared using the McNemar exact test based on Yates chi2 and considering only individuals who were identified as helminth positive (Hawass, 1997). Secondly, we calculated the sensitivity considering the pooled results from any of the above mentioned dual method combinations as well as the triple combination of Kato-Katz thick smear method, FLOTAC and PCR as diagnostic pseudo 'gold' standard. Here, an individual was considered as true-positive, if any of the applied method detected eggs, larvae, or DNA, respectively, of the species under investigation. Specificity was estimated at 100% for each method. Thirdly, since results from stool examinations generally underestimate the prevalence (Joseph et al., 1995). We additionally used a Bayesian approach to estimate the prevalence, sensitivity, and specificity for all applied diagnostic methods in the absence of a true 'Gold' standard (Joseph et al., 1995, Dendukuri and Joseph, 2001). Assuming that the PCR follows a different biological process than the Kato-Katz thick smear, FLOTAC, and Baermann method (i.e. DNA detection *versus* visual egg/larvae detection by microscopy), we incorporated conditional dependence on the true infection status between microscopy based diagnostic tests (FLOTAC and Kato-Katz thick smear) into our models as suggested by Branscum and colleagues (Branscum et al., 2005). Based on 2x2 tables (Table 1) the vector $y = (y_{11}, y_{12}, y_{21}, y_{22})$ follows a multinomial distribution with a probability vector $p = (p_{11}, p_{12}, p_{21}, p_{22})$ where:

$$p_{11} = \pi(S_K S_F + d_1) + (1-\pi)((1-C_K)(1-C_F) + d_2)$$
$$p_{12} = \pi((1-S_K)S_F - d_1) + (1-\pi)(C_K(1-C_F) - d_2)$$
$$p_{21} = \pi(S_K(1-S_F) - d_1) + (1-\pi)((1-C_K)C_F - d_2)$$
$$p_{21} = \pi((1-S_K)(1-S_F) + d_1) + (1-\pi)(C_K C_F + d_2)$$

S, C, and π denote the specificity, sensitivity and prevalence, respectively, while *d1* and *d2* quantify the conditional dependence of the two tests. In our analysis all parameters were assigned uninformative uniform distributions. The bounds of the uniform priors for *d1* and *d2* were derived as described by Branscum and colleagues (Branscum et al., 2005). Posterior inference was based on Markov chain Monte Carlo simulations implemented in OpenBUGS (Lunn et al., 2009) and all simulations were run for at least 1 million iterations and 4 chains. Convergence was assessed using the Gelman-Rubin Statistics (Brooks and Gelman, 1998, Gelman and Rubin, 1992).

Results

Operational results and baseline infections

Between July 2011 and November 2012, a total of 1460 participants consented to participate in the screening for helminth infections and to be included into one of the study arms of the IDEA project if eligible. Among them, 1357 and 1453 had their gender and age recorded, respectively, with 50.3% being male and 49.7% being female and a median age of 5 years (range: 0--98 years). Stool samples of sufficient size for duplicate Kato-Katz thick smears, FLOTAC and Baermann were submitted by 1195, 1179, and 1128 individuals, respectively. PCR was applied on 215 stool samples (Figure 10).

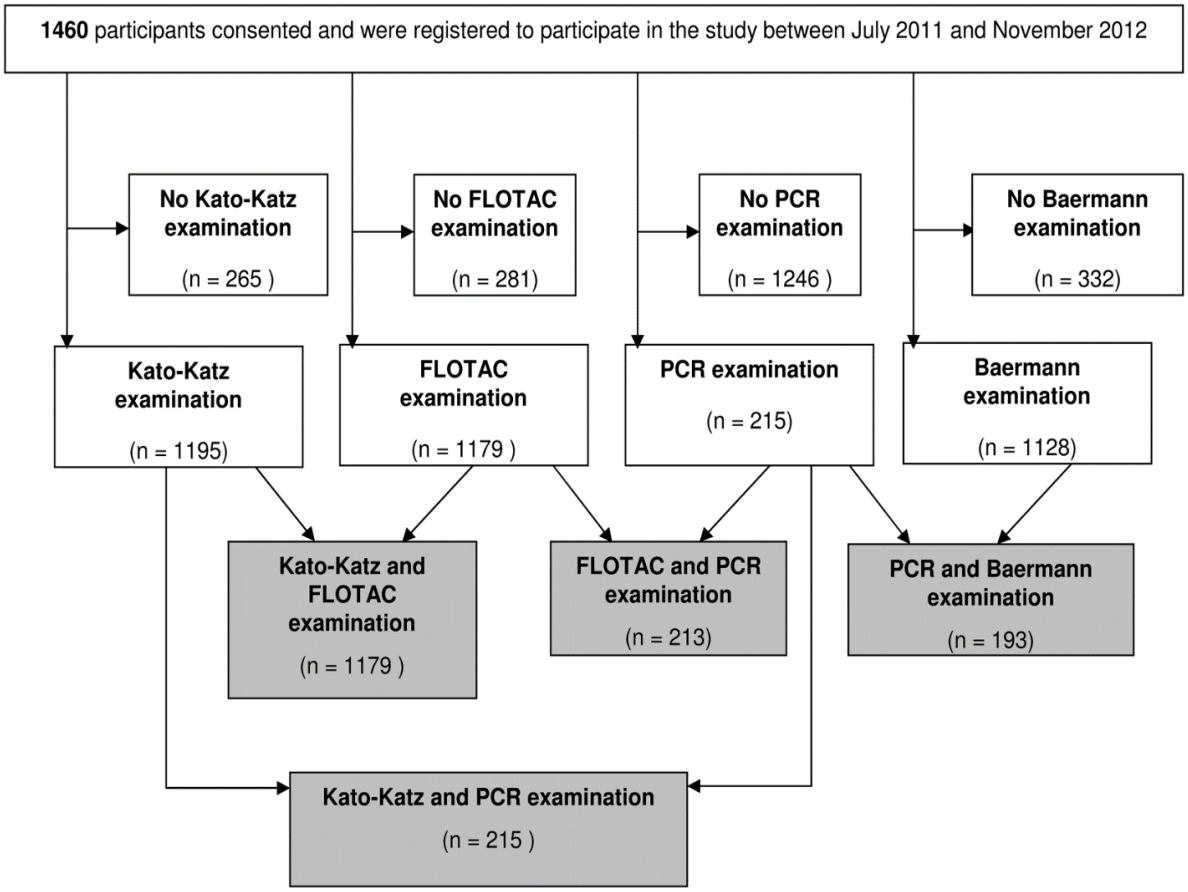

Figure 10. Flowchart indicating the number of study participants invited to participate in a helminth screening for the IDEA project in the United Republic of Tanzania between June 2011 and November 2012, and the number of stool samples examined with the Kato-Katz thick smear, FLOTAC, Baermann und PCR methods or a combination thereof for the diagnosis of helminth infections

The following overall prevalences were detected by combining the results from Kato-Katz and FLOTAC (n = 1179). Hookworm: 10.0%, *T. trichiura*: 1.9%, *A. lumbricoides*: 0.2%, and *S. mansoni*: 0.2%. Applying the Baermann method (n = 1128), *S. stercoralis* infections were detected in 7.4% of the participants.

According to the Kato-Katz thick smear method egg count results and WHO thresholds, 84.0% of the hookworm infections were light, 7.0% moderate, and 9.0% heavy. Light and moderate *T. trichiura* infection intensities were observed in 86.4% and 13.6% of infected participants, respectively. One among two *A. lumbricoides* infected participants had a light and the second a moderate intensity of infection, and both *S. mansoni* infections were light.

Because of the low number of infected individuals, the method comparisons between Kato-Katz and FLOTAC (n = 1179), Kato-Katz and PCR (n = 215), FLOTAC and PCR (n = 213) and Baermann and PCR (n = 193), were only conducted for hookworm and *S. stercoralis* infections, respectively.

Agreement of diagnostic methods and parameters

Table 3 shows that the agreement between duplicate Kato-Katz thick smears and the FLOTAC dual technique for hookworm egg detection was almost perfect (κ = 0.86). The 21 individuals that were identified as negative by the Kato-Katz method, but positive by FLOTAC had a median egg count of 4 EPG (range: —1--430 EPG). The 6 false-negatives from FLOTAC had a median egg count of 12 EPG (range: 12--24 EPG) in Kato-Katz. The median EPG values were significantly lower in the false-negative group than in the true-positive group for either method (Figure 11 A and B).

The agreement between PCR and FLOTAC (κ = 0.68) and PCR and Kato-Katz (κ = 0.63) for hookworm diagnosis was substantial. The 17 individuals that were not identified as positive by PCR, but only by FLOTAC had a median egg count of 84 EPG (range: 1--4603 EPG) and the 15 false-negatives by PCR that were detected by Kato-Katz had a median egg count of 480 EPG (range: 12--14064). For both FLOTAC and Kato-Katz, the median EPG values in the PCR false-negative group were not significantly lower than in the PCR true-positive group (Figure 11 C and D).

A slight agreement (κ = 0.14) was found between PCR and the Baermann method for the detection of *S. stercoralis*. The 38 individuals with *S. stercoralis* larvae found by the Baermann method but not by PCR had a median of 1 larva identified (range: 1--314). The median larvae count in the PCR false-negative group was significantly lower than in the PCR true-positive group (Figure 11 E).

Correlation between PCR Ct-values and microscopic egg/larvae counts

The median Ct-value was 31.4 (range: 24.6--39.3) in the samples with hookworm true-positive egg counts in FLOTAC, and 37.8 (range: 26.6--39.2) in false-negative FLOTAC samples. The median Ct-value was 31.5 (range: 24.6--39.3) in true-positive Kato-Katz samples and 34.8 (range: 26.6.--39.6) in false-negative samples. For both, Kato-Katz and FLOTAC, there was no significant difference between the median Ct-values of the false-negative and true-positive groups (Figure 11 F and G).

As shown in Figure 12, there was a significant negative correlation between PCR Ct-values and hookworm EPG values derived with either FLOTAC (ρ = -0.30; $p < 0.001$) or Kato-Katz (ρ = -0.36; $p < 0.001$).

In true-positive and false-negative Baermann samples, the median Ct-value was 34.7 (range: 28.6--39.1) and 31.7 (range: 19.7--38.5), respectively. The difference was not significant (Figure 11 H). A negative correlation was found between Ct-values and the number of *S. stercoralis* larvae (ρ = -0.14; p = 0.049).

Table 3. Two-way contingency tables showing the agreement between methods for the diagnosis of hookworm and *S. stercoralis* infections in stool samples from individuals participating in our study conducted in the United Republic of Tanzania between June 2011 and November 2012. The 2x2 table was also used for the Bayesian approach (vectors indicated in brackets) to estimate diagnostic parameters.

	Duplicate Kato-Katz		
Single FLOTAC	Positive	Negative	Total
Positive	91 (y_{11})	21 (y_{12})	112
Negative	6 (y_{21})	1061 (y_{22})	1067
Total	97	1082	1179
kappa-agreement	0.86		
	Duplicate Kato-Katz		
PCR	Positive	Negative	Total
Positive	40	15	55
Negative	15	145	160
Total	55	160	215
kappa-agreement	0.63		
	Single FLOTAC		
PCR	Positive	Negative	Total
Positive	43	10	53
Negative	17	143	160
Total	60	153	213
kappa-agreement	0.68		
	Baermann		
PCR	Positive	Negative	Total
Positive	8	9	17
Negative	38	138	176
Total	46	147	193
kappa-agreement	0.14		

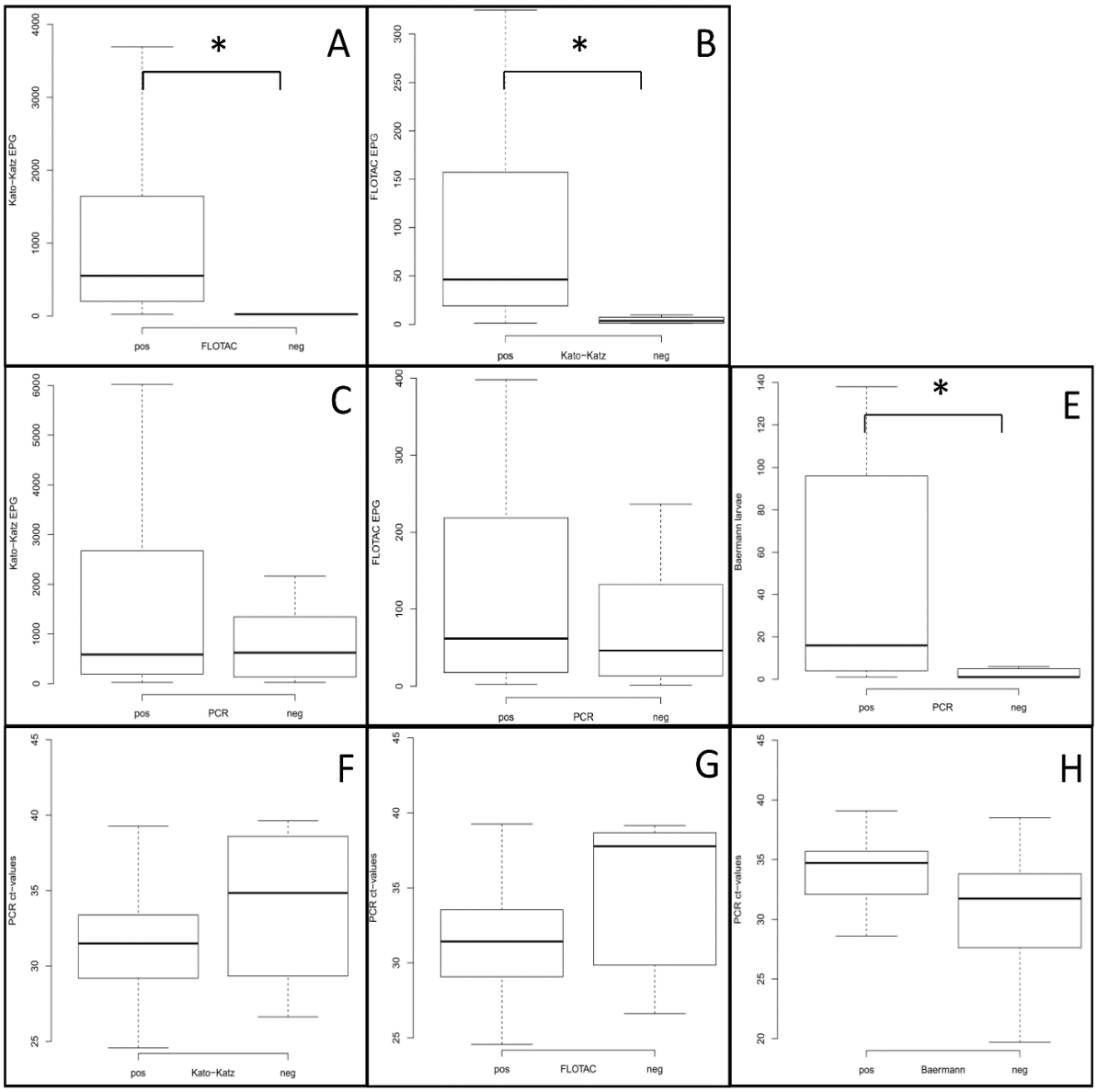

Figure 11. Differences in the median of hookworm positive eggs per gram of feces (EPG) values, median *S. stercoralis* larvae positive counts, or median positive cycle threshold (Ct-) values, in groups of samples identified as true-positive or false-negative with any other diagnostic method in a study conducted in the United Republic of Tanzania between June 2011 and November 2012. * = significant difference ($p \leq 0.05$) in the median determined by the Wilcoxon rank-sum (Mann-Whitney) test.

(A) Difference between hookworm median EPG in true-positive (n = 91) and false-negative (n = 6) FLOTAC samples identified as positive with Kato-Katz ($p < 0.001$).

(B) Difference between hookworm median EPG in true-positive (n = 91) and false-negative (n = 21) Kato-Katz samples identified as positive with FLOTAC ($p < 0.001$).

(C) Difference between hookworm median EPG in true-positive (n = 40) and false-negative (n = 15) PCR samples identified as positive with Kato-Katz (p = 0.4382).

(D) Difference between hookworm median EPG in true-positive (n = 43) and false-negative (n = 17) PCR samples identified as positive with FLOTAC (p = 0.6226).

(E) Difference between *S. stercoralis* median larvae in true-positive (n = 8) and false-negative (n = 38) PCR samples identified as positive with Baermann (p = 0.0227).

(F) Difference between hookworm median Ct-values in true-positive (n = 40) and false-negative (n = 15) Kato-Katz samples identified as positive with PCR (p = 0.0821).

(G) Difference between hookworm median Ct-values in true-positive (n = 43) and false-negative (n = 10) FLOTAC samples identified as positive with PCR (p = 0.0562).

(H) Difference between *S. stercoralis* median Ct-values in true-positive (n = 8) and false-negative (n = 9) Baermann samples identified as positive with PCR (p = 0.1937).

Accuracy estimates of diagnostic methods without pseudo 'gold' standard

When directly comparing two methods, the FLOTAC had a significantly higher sensitivity than the Kato-Katz method for detecting hookworm infections (93.8% *versus* 81.3%; p = 0.006), and the specificity of both methods was almost 100% (Table 4). The sensitivity of the PCR for hookworm infections was equal to the sensitivity of duplicate Kato-Katz thick smears and lower than the sensitivity of FLOTAC. The specificity of the PCR was 93.5% when compared to FLOTAC as reference test and 90.6% when compared to duplicate Kato-Katz thick smears. The sensitivity of the Baermann method for *S. stercoralis* detection was significantly higher than that of the PCR (47.1% *versus* 17.4%; p< 0.001). The specificity of Baermann was 78.4% and the one of PCR was 93.9%.

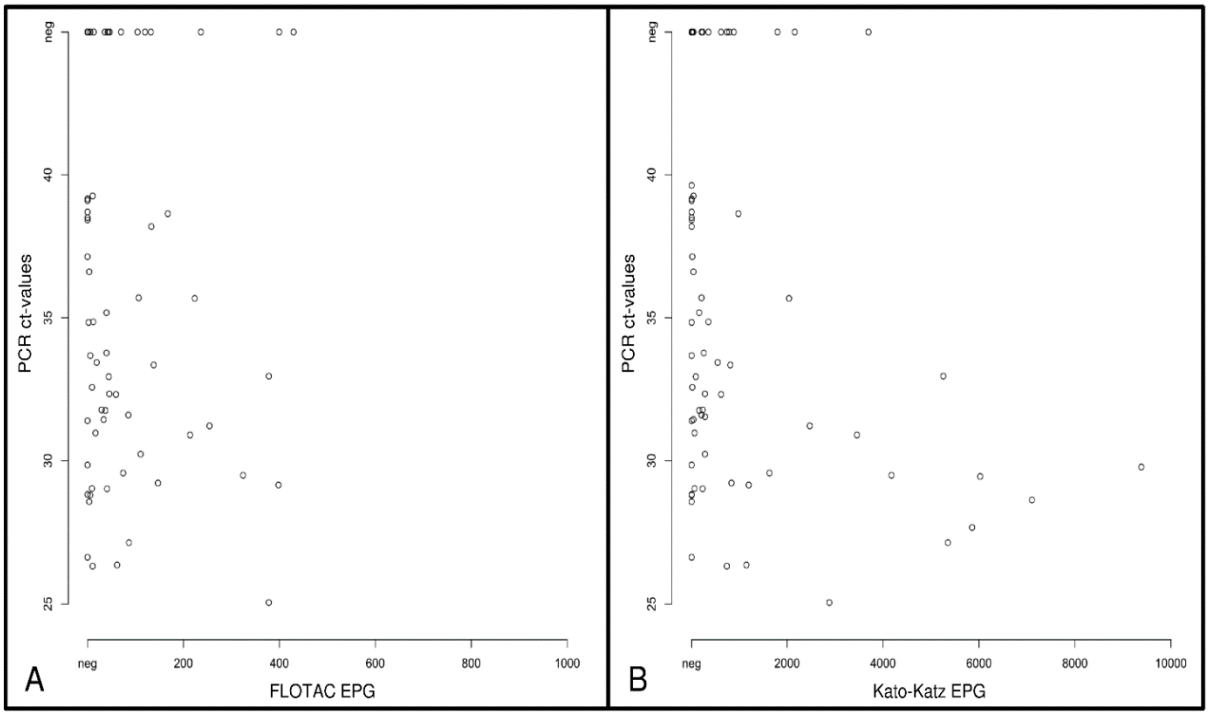

Figure 12. Correlation between hookworm eggs per gram of feces (EPG) measured with FLOTAC or duplicate Kato-Katz thick smears and cycle threshold (Ct-) values of hookworm Real-Time PCR in a study conducted in the United Republic of Tanzania between June 2011 and November 2012.

(A) Correlation between hookworm EPG values measured with FLOTAC and Ct-values of hookworm Real-Time PCR for the detection of *N. americanus* in fecal samples (n = 211) from coastal Tanzania (Pearson's correlation, $\rho = -0.30$; $p < 0.001$).

(B) Correlation between hookworm EPG values measured with duplicate Kato-Katz thick smears and PCR Ct-values of hookworm Real-Time PCR for the detection of *N. americanus* in fecal samples (n = 215) from coastal Tanzania ($\rho = -0.36$; $p < 0.001$).

Accuracy estimates of diagnostic methods using a pseudo 'gold' standard

As shown in Table 4, applying a combination of the available test results (duplicate Kato-Katz thick smears, FLOTAC and PCR) as diagnostic pseudo 'gold' standard, the sensitivity for hookworm diagnosis was highest for FLOTAC (83.3%), followed by Kato-Katz (75.0%), and PCR (73.6%), respectively. For the diagnosis of *S. stercoralis*, the Baermann method showed a better sensitivity (83.6%) than the PCR (30.9%).

Accuracy estimates of diagnostic methods in the absence of a true 'gold' standard using a Bayesian approach

For the comparison of FLOTAC and Kato-Katz the two dependence parameters were close to zero, and therefore we report the following results under the assumption of conditional independence. As shown in Table 4, in the absence of a 'gold' standard, FLOTAC had the highest sensitivity for hookworm detection when compared to Kato-Katz (96.3% *versus* 89.6%) or PCR (88.8% *versus* 83.3%). The sensitivities of Kato-Katz (79.2%) and PCR (78.8%) were estimated to be almost equal. The estimated specificity of the PCR was 96.2% when compared to FLOTAC and 92.7% when compared to duplicate Kato-Katz thick smears. For the diagnosis of *S. stercoralis*, both the Baermann method and PCR showed a low sensitivity of 28.3% and 11.6%, respectively. The specificity of the PCR was higher than that of the Baermann method (90.6% *versus* 75.2%).

Table 4. Diagnostic accuracy of duplicate Kato-Katz thick smears, the FLOTAC dual technique and Real-Time PCR for hookworm and of the Baermann method and PCR for *S. stercoralis* detection, and prevalences according to three different statistical approaches applied in our study conducted in the United Republic of Tanzania between June 2011 and November 2012.

Statistical approach	n	Test	Sensitivity % [95% CI]	Specificity % [95% CI]	McNemar p-value	Prevalence [95% CI]
Direct method comparison	1179	FLOTAC	93.8 [87.0--97.7]	98.1 [97.0--98.8]		9.5 [7.9--11.3]
		Kato-Katz	81.3 [72.8--88.0]	99.4 [98.8--99.8]	0.006	8.2 [6.7--9.8]
Direct method comparison	215	PCR	72.7 [59.0--83.9]	90.6 [85.0--94.7]		25.6 [19.9--32.0]
		Kato-Katz	72.7 [59.0--83.9]	90.6 [85.0--94.7]	1.000	25.6 [19.9--32.0]
Direct method comparison	213	PCR	71.7 [58.6--82.5]	93.5 [88.3--96.8]		24.9 [19.2--31.2]
		FLOTAC	81.1 [68.0--90.6]	89.4 [83.5--93.7]	0.248	28.2 [22.2--34.7]
Direct method comparison	193	PCR	17.4 [7.8--31.4]	93.9 [88.7--97.2]	<0.001	8.8 [5.2--13.7]
		Baermann	47.1 [23.0--72.2]	78.4 [71.6--84.2]		23.8 [18.0--30.5]
Combination of methods as gold standard	212	PCR	73.6 [61.9--83.3]	100*		
		FLOTAC	83.3 [72.7--91.1]	100*		
		Kato-Katz	75.0 [63.4--84.5]	100*		33.8 [27.5--40.6]
Combination of methods as gold standard	1179	FLOTAC	94.9 89.3--98.1]	100*		
		Kato-Katz	82.2 [74.1--88.6]	100*		10.0 [8.4--11.9]
Combination of methods as gold standard	215	PCR	78.6 [67.1--87.5]	100*		
		Kato-Katz	78.6 [67.1--87.5]	100*		32.6 [26.3--39.3]

Combination of methods as gold standard	213	PCR	75.7 [64.0--85.2]	100*	
		FLOTAC	85.7 [75.3--92.9]	100*	32.9 [26.6--39.6]
Combination of methods as gold standard	193	PCR	30.9 [19.1--44.8]	100*	
		Baermann	83.6 [71.2--92.2]	100*	28.5 [22.2--35.4]
Bayesian modelling	1179	FLOTAC	96.3 [89.3--99.8]	98.9 [97.6--100]	
		Kato-Katz	89.6 [77.2--99.5]	99.7 [99.0--100]	8.9 [7.0--11.0]
Bayesian modelling	215	PCR	78.8 [1.2--98.8]	92.7 [3.6--99.6]	
		Kato-Katz	79.2 [1.2--98.8]	92.8 [3.4--99.6]	28.1 [17.6--80.1]
Bayesian modelling	213	PCR	83.3 [64.5--99.1]	96.2 [90.3--99.8]	
		FLOTAC	88.8 [73.1--99.4]	93.7 [86.1--99.7]	26.7 [18.4--36.5]
Bayesian modelling	193	PCR	11.6 [0.7--89.3]	90.6 [11.5--99.3]	
		Baermann	28.3 [3.3--95.0]	75.2 [6.0--96.8]	43.1 [2.6--97.1]

95% CI = 95% confidence interval

P-value = differences for sensitivities determined by the McNemar test on positive individuals

* = we assumed 100% specificity

** = in the Bayesian approach the intervals correspond to credible intervals

Discussion

The upscale of control interventions against neglected tropical diseases over the next years in accordance with the WHO goals set for the year 2020 will likely reduce the prevalence and intensities of soil-transmitted helminth infections in endemic countries. For the decision where to implement and particularly when to stop control interventions and for adequate surveillance to avoid the recrudescence of soil-transmitted helminthiases sensitive diagnostic methods are needed (Bergquist et al., 2009).

We compared, to our knowledge for the first time, the diagnostic accuracy of the FLOTAC and a previously described Real-Time PCR assay for hookworm diagnosis, applying three different statistical approaches, and found that FLOTAC was slightly more sensitive than the PCR. When directly comparing each of the techniques to the Kato-Katz method, only FLOTAC but not the PCR had a significantly higher sensitivity. The sensitivity of the PCR for *S. stercoralis* diagnosis was significantly lower than that of the Baermann method.

The direct method comparison revealed an equal sensitivity of the PCR and Kato-Katz for hookworm diagnosis of 73%. This result of the PCR sensitivity is very much in line with findings from another research group that identified sensitivities of 79% and 54%, for PCR and Kato-Katz, respectively (Schär et al., 2013a). The considerably higher sensitivity of the Kato-Katz thick smear technique in our study is likely explainable by i) that we performed duplicate and no single thick smears per stool sample per person, and ii) that we examined the Kato-Katz slides exactly after 20 min, which avoided the over-clearance of hookworm eggs by glycerol. A study conducted in Ghana in 2007, revealed higher sensitivities of 100% and 81% for PCR and Kato-Katz, respectively (Verweij et al., 2007). However, the Ghanaian study participants had higher hookworm infection intensities (median: 720 EPG by Kato-Katz) than our Tanzanian population sub-sample (median: 516 EPG). A decrease of sensitivity with lower infection intensities has been postulated for Kato-Katz and FLOTAC before, (Booth et al., 2003, Knopp et al., 2009) and might also be true for PCR, since Ct-values are correlated to the number of eggs detected in FLOTAC and Kato-Katz. The group of individuals false-negatively diagnosed with PCR that were found positive using Kato-Katz or FLOTAC, had, however, no significantly lower EPG values than the group of correctly identified positives, and hence there must be additional factors that impacted on the sensitivity of the PCR. Inhibition of the PCR by substances present in stool samples might be one possible explanation. Since the external control was always amplified, there might have been stool sample specific enzymes or other factors that inhibited the DNA amplification in some cases resulting in false-negative results. The absence of an internal control is a clear limitation of our study. The inclusion of an internal control in each sample, for example by adding 10^3 PFU/mL phocin herpes virus 1 (PhHV-1) into the isolation lysis buffer,(Verweij et al., 2007) would have shown if present DNA was amplified or not and hence, if samples were correctly diagnosed as negatives. Another explanation for the non-detection of hookworm positives with PCR might be that the hookworm eggs detected with Kato-Katz and FLOTAC were from *A. duodenale*, a hookworm species that would not have been identified with the *N. americanus*-specific primers we used in our PCR. Only the third-stage larvae of these helminths but not the morphological identical eggs allow a microscopic differentiation between *A. duodenale* and *N. americanus* (Blotkamp et al., 1993). Studies that have undertaken differential diagnosis using coproculture in East Africa have shown that both *A. duodenale* and *N. americanus* do occur in East

Africa, but that the latter is the predominant species in the region (Sturrock, 1966b, Sturrock, 1966a, Chunge et al., 1986).

The lack of accuracy of the PCR for the detection of light helminth infections is reflected in our study with the very low sensitivity (17%, 31%, or 12%, depending on statistical approach) of the PCR for *S. stercoralis* diagnosis, and the observation that the group of individuals with false-negative PCR results had a significantly lower median larvae count than the correctly identified positives (1 larva *versus* 16 larvae). We found a borderline correlation between Ct-values and the number of *S. stercoralis* larvae detected in Baermann, and the PCR was not able to detect all cases and missed out light infections. Noteworthy, the PCR sensitivities for *S. stercoralis* detection were considerably higher in previous studies conducted by other research groups in Ghana (86%)(Verweij et al., 2009) and Cambodia (88%)(Schär et al., 2013a) when compared with the Baermann method, but for example the median larvae count in the positive Baermann samples from Cambodia was considerably higher than the median of 1.5 larvae found in our study (Schär, personal communication). The specificity of the PCR for hookworm and *S. stercoralis* in our study was above 90% regardless of the statistical approach used and therefore in agreement with what was found elsewhere (Verweij et al., 2009, Schär et al., 2013a).

The higher sensitivity of the FLOTAC compared with the Kato-Katz method for the detection of hookworm infection is in line with the results of previous studies (Knopp et al., 2009, Glinz et al., 2010b, Habtamu et al., 2011, Utzinger et al., 2008). The agreement of the two methods was almost perfect. The small group of FLOTAC tested individuals with false-negative results had a very low median EPG value when they were tested positive with the Kato Katz method (12 EPG), and also the group of individuals with positive FLOTAC but false-negative Kato-Katz results had a very low median of 4 EPG. This observation confirms a previous assumption in that the sensitivity only drops considerably if the egg counts fall under the lower detection limit of the FLOTAC dual technique (2 EPG) and duplicate Kato-Katz thick smears (12 EPG) (Knopp et al., 2011). Also the PCR was able to identify some cases that were either not detected with the Kato-Katz method and had a low EPG values with FLOTAC (n = 5; median: 10 EPG), or were not detected with FLOTAC and had borderline EPG values with the Kato-Katz method (n = 1; median: 12 EPG).

We used three statistical approaches to determine the diagnostic accuracy, mainly to render our results comparable to a set of previously conducted studies, where the sensitivity and specificity of Kato-Katz, Baermann and PCR were determined by direct method comparison (Schär et al., 2013a, Verweij et al., 2007, Verweij et al., 2009) or by using the combined results of Kato-Katz and FLOTAC as diagnostic pseudo gold-standard (Glinz et al., 2010b, Knopp et al., 2009, Utzinger et al., 2008). The Bayesian approach was chosen since it is expected to give a more accurate picture of the true prevalence and might be used in future studies, particularly in light of increased helminth control and elimination efforts, when accurate prevalence estimates will be very important for program decisions. While the diagnostic sensitivity and specificity and the prevalence estimates for hookworm infections was similar in all three approaches, the sensitivity of the PCR for *S. stercoralis* infection was estimated considerably lower when using the Bayesian model. The substantial proportion of extra positives identified by either the Baermann method or the PCR, respectively, resulted in this very high estimate of the “true” prevalence when using the Bayesian model.

Generally, it must be noted that the standardization and adherence to protocols and procedures particularly for molecular but also for conventional diagnosis of helminth infections in different laboratories and the implementation of external quality assurance systems would help to render results more readily comparable, to evaluate the quality of the PCR and other diagnostic systems used by each respective laboratory, and to draw a clearer picture of the sensitivity and specificity of the tests applied for the diagnosis of helminth infections.

Conclusion

We conclude that the diagnostic accuracy of the Real-Time PCR for hookworm identification is similar to that of the FLOTAC and Kato-Katz method, if the infection intensity is considerably light, as it was in our study. Whether PCR is suitable to give a more accurate picture of hookworm prevalence than the Kato-Katz or FLOTAC method in areas targeted by control interventions against soil-transmitted helminthiases, where infection intensities drop to very low levels, remains to be elucidated. Since Ct-values seem to be associated with the number of eggs detected in feces and since the amount of stool used for DNA extraction is small, also the currently used PCR protocol might fail to detect very light infections and can therefore not readily be applied to monitor progressing control programs, confirmation of elimination or surveillance of disease recrudescence. For these scenarios, innovative diagnostic assays are required that do not only reliably detect very light infections but can also be performed in a high throughput format on large population samples and detect a number of parasite species simultaneously. Novel protocols and approaches should be developed that meet these requirements and allow an accurate, standardized, and quality controlled assessment of the achievements made through intensified helminth control and elimination efforts in light of the WHO goals for the year 2020.

Acknowledgements

We thank all individuals who participated in the IDEA project and all motivated staff from the Bagamoyo Research and Training Centre, United Republic of Tanzania, who helped in the field and laboratory with the collection and examination of the samples. We are particularly indebted to Rehema Mussa Mangoli, Tatu Nassoro Matuzya, John Masimba, Kwaba Denis, Shabani Halfani Ally, and Wambura Wanchoke for their great support, commitment and dedication to work within the IDEA project. Moreover, we would like to thank Professor Giuseppe Cringoli from the University of Naples Federico II, Italy, for the free provision of multiple FLOTAC apparatuses for this study.

Chapter 6: Enterobiasis and Strongyloidiasis and associated co-infections and morbidity markers in infants, preschool and school aged children from rural coastal Tanzania: a cross sectional study

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The authors declare that they have no competing interest.

The article has been published in BMC Infectious Disease journal, (Salim et al., 2014)

Abstract

Background: There is a paucity of data pertaining to the epidemiology and public health impact of *Enterobius vermicularis* and *Strongyloides stercoralis* infections. We aimed to determine the extent of enterobiasis, strongyloidiasis, and other helminth infections and their association with asymptomatic *Plasmodium* parasitaemia, anaemia, nutritional status, and blood cell counts in infants, preschool-aged (PSAC), and school-aged children (SAC) from rural coastal Tanzania.

Methods: A total of 1,033 children were included in a cross-sectional study implemented in the Bagamoyo district in 2011/2012. Faecal samples were examined for intestinal helminth infections using a broad set of quality controlled methods. Finger-prick blood samples were subjected to filariasis and *Plasmodium* parasitaemia testing and full blood cell count examination. Weight, length/height, and/or mid-upper arm circumference were measured and the nutritional status determined in accordance with age.

Results: *E. vermicularis* infections were found in 4.2% of infants, 16.7%, of PSAC, and 26.3% of SAC. *S. stercoralis* infections were detected in 5.8%, 7.5%, and 7.1% of infants, PSAC, and SAC, respectively. Multivariable regression analyses revealed higher odds of enterobiasis in children of all age-groups with a reported anthelminthic treatment history over the past six months (odds ratio (OR): 2.15; 95% confidence interval (CI): 1.22 - 3.79) and in SAC with a higher temperature (OR: 2.21; CI: 1.13 - 4.33). Strongyloidiasis was associated with eosinophilia (OR: 2.04; CI: 1.20-3.48) and with *Trichuris trichiura* infections (OR: 4.13; CI: 1.04-16.52) in children of all age-groups, and with asymptomatic *Plasmodium* parasitaemia (OR: 13.03; CI: 1.34 - 127.23) in infants. None of the investigated helminthiases impacted significantly on the nutritional status and anaemia, but moderate asymptomatic *Plasmodium* parasitaemia was a strong predictor for anaemia in children aged older than two years (OR: 2.69; 95% CI: 1.23 – 5.86).

Conclusions: *E. vermicularis* and *S. stercoralis* infections were moderately prevalent in children from rural coastal Tanzania. Our data can contribute to inform yet missing global burden of disease and prevalence estimates for strongyloidiasis and enterobiasis. The association between *S stercoralis* and asymptomatic *Plasmodium* parasitaemia found here warrants further comprehensive investigations.

Background

Soil-transmitted helminths belong to the neglected tropical diseases (Utzinger et al., 2012). The most common soil-transmitted helminths are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*). Infections are acquired via the ingestion of the parasites' eggs, for example with contaminated food, or in case of hookworms via larvae penetrating bare skin (Knopp et al., 2012, Bethony et al., 2006). The adult worms live in the intestines of humans, and eggs or larvae are excreted with faeces and thrive in the soil. Hence, infections are particularly prevalent in areas, where hygiene and sanitation are low and where the environment favours a rapid transmission. It is estimated that globally around 1.45 billion people are infected with at least one intestinal helminth species, and a global burden of 5.2 million disability adjusted life years (DALYs) is attributed to these infections (Pullan et al., 2014, Hotez et al., 2014, Murray et al., 2012).

In addition to the four major species, there are two intestinal nematodes infecting humans, the threadworm *Strongyloides stercoralis* and the pinworm *Enterobius vermicularis* that are mostly neglected in prevalence reports and global burden of disease estimates due to their rather unpleasant and cumbersome diagnosis with the Baermann and/or Koga agar plate methods or adhesive tape test, respectively, and due to difficulties to assess associated morbidity (Knopp et al., 2012, Utzinger et al., 2012, Krolewiecki et al., 2013, Olsen et al., 2009, Knopp et al., 2014, Becker et al., 2011).

S. stercoralis has an intra- and extra-human lifecycle during which the parasite can reproduce asexually and sexually, respectively. Larvae that hatched from eggs within the intestines are able to penetrate the intestinal wall and can perpetuate the lifecycle within the human host for decades after initial exposure (Montes et al., 2010, Knopp et al., 2012). Infections are mostly mild and often asymptomatic in otherwise healthy individuals (Greaves et al., 2013). Skin lesions, pulmonary and gastro-intestinal symptoms, and blood eosinophilia are reported as unspecific disease markers (Leder and Weller, 2000, Grove, 1996, Khieu et al., 2013b, Becker et al., 2011, Ardic, 2009, Nuesch et al., 2005). Chronically infected immunocompromised patients, however, are at high risk of developing a lethal hyper-infection syndrome, caused by proliferating tissue-invasive larvae that might carry bacteria from the intestines to organs, leading to systemic infections, multiorgan failure and systemic sepsis (Greaves et al., 2013, Segarra-Newnham, 2007, Jia et al., 2012). It is estimated that *S. stercoralis* affects between 10% and 40% of the population in tropical- and sub-tropical countries, but adequate information is lacking, particularly for the high-risk areas including Sub-Saharan Africa and Southeast Asia (Schär et al., 2013b). Global burden of disease estimates do not exist for this parasite (Hotez et al., 2014, Khieu et al., 2013b, Utzinger et al., 2009) and strongyloidiasis remains an underestimated health problem (Bisoffi et al., 2013). Good reliable data on infection, morbidity, and indisputable causal links between them, are urgently needed to make the public health argument for a guided response against *S. stercoralis* (Krolewiecki et al., 2013).

E. vermicularis is transmitted via the ingestion of eggs contained in dust, water, or sticking on hands and food. The female adults live in the cecum and large intestines and migrate to the anus to deposit their eggs on the perianal skin (Knopp et al., 2012). Since eggs become infective within hours, autoinfection is possible (St Georgiev, 2001). Enterobiasis includes symptoms such as intense pruritus in the perianal area, which can lead to insomnia, restlessness, and irritability. Moreover, the adult pinworms can migrate into

the appendix or genital tract, causing appendicitis and genitourinary complications (Arca et al., 2004, Burkhart and Burkhart, 2005). Ectopic infections of liver, lung, kidneys, and other organs occur infrequently (Burkhart and Burkhart, 2005, Cook, 1994). Enterobiasis is considered the most common helminth infection worldwide (Fry and Moore, 1969). It is estimated that globally 4-28% of children are infected (Bethony et al., 2006), but recent and profound prevalence and burden estimates are missing, particularly for Sub-Saharan Africa.

The neglect of strongyloidiasis and enterobiasis in most studies pertaining to soil-transmitted helminth infections results in a knowledge gap about their potential impact on co-infections and the nutritional status of the host. While it is widely acknowledged that helminth infections modulate immune responses and might influence the acquisition and outcomes of diseases such as malaria, tuberculosis (TB), and human immunodeficiency virus-acquired immune deficiency syndrome (HIV-AIDS), the exact mechanisms are not known (Nacher, 2011, Adegnika and Kremsner, 2012, Rafi et al., 2012, Walson et al., 2009, Secor, 2012, Webb et al., 2012, Knopp et al., 2013, Moreau and Chauvin, 2010, McSorley et al., 2013, Kinung'hi et al., 2014, Salgame et al., 2013). Research on this topic has gained much interest over the past years, but results are inconsistent. Multiple dimensions seem to be involved, including helminth species, infection intensity, and the host's age, genetics, and immunological and nutritional status (Righetti et al., 2012, Mwangi et al., 2006).

The IDEA research program is designed to intensively study the immunological interplay between helminth infections and malaria, TB, and HIV-AIDS (http://ec.europa.eu/research/health/infectious-diseases/neglected-diseases/projects/014_en.html). Of course, to obtain reliable results, not only the presence of major helminth species, but also infections with *S. stercoralis* and *E. vermicularis* need to be taken into account. Here, we present, to our knowledge for the first time, the extend of strongyloidiasis and enterobiasis, besides other helminthiases, in infants, preschool-aged children (PSAC) and school-aged children (SAC) from rural coastal Tanzania, that participated in a cross-sectional study implemented to recruit participants for the IDEA project in the Bagamoyo district, United Republic of Tanzania. We also show anthropometric and haematological characteristics of the study group and associations found between the individual helminth species infections, between helminth infections and asymptomatic *Plasmodium* infections and between helminth infections and anthropometric status, anaemia and additional haematological parameters in different age-groups. With this report we aim to shed more light on the distribution and public health importance of two highly neglected soil-transmitted helminth species and therefore to contribute to inform yet missing global burden and prevalence estimates.

Materials and methods

Ethics statement

The protocol for the IDEA project conducted at the Bagamoyo Research and Training Center of the Ifakara Health Institute (IHI-BRTC) in Bagamoyo, United Republic of Tanzania, was approved by the institutional research commissions of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland) and the IHI. Ethical approval for the study was obtained from the Ethikkomission beider Basel (EKBB; Basel, Switzerland; reference number: 257/08) and the National Institution for Medical Research of Tanzania (NIMR; Dar es Salaam, United Republic of Tanzania; reference number: NIMR/HQ/R.8a/Vol.IX/1098). The

study was monitored by the IHI internal monitoring team in collaboration with the Pan African Collaboration for the Evaluation of Antituberculosis Antibiotics (PanACEA) and Swiss TPH.

Local district, community, school, and health authorities were informed about the purpose and procedures of the study and their acceptance for the implementation was sought. Individuals eligible to be screened and to potentially participate in one of the study arms of IDEA were informed in detail about the aims of the study, the risks and benefits of study participation, and the extend of time involvement in case of their participation (Knopp et al., 2014). It was pointed out that participation was voluntary and that participants could withdraw from the study at any time without further obligation. All adult participants and in case of children aged ten years or younger (ten years being the age-limit of children eligible for inclusion in our study) their parents or legal guardians, were asked to sign a written informed consent sheet if they agreed to participate. Participants' privacy was preserved by de-identification of the dataset. Participants' names were replaced by individual identification codes and all information pertaining to names, geographical locations, and dates of birth, sample collection, and sample examination was removed from the dataset.

Patients with helminthiases, malaria, TB, HIV-AIDS, and/or other medical conditions received treatment according to the national treatment guidelines of the United Republic of Tanzania. Accordingly, participants of our cross-sectional survey that were infected with helminths were treated with albendazole (400 mg single oral dose) against common soil-transmitted helminth infections, *S. stercoralis*, and *E. vermicularis*, or praziquantel (40 mg/kg) against *Schistosoma* infections. Participants with asymptomatic *Plasmodium* parasitaemia or clinical malaria were treated with Artemether Lumefantrine and children with severe malaria received Quinine.

Study area

All participants included in the present analysis resided in one among 12 villages or small towns in the Bagamoyo district, belonging to the Pwani region in the United Republic of Tanzania. These settlements are located in a rural environment approximately 20-60 kilometres from Bagamoyo, which is a historical town located directly at the coast along the Indian Ocean, 75 kilometres north of Dar es Salaam. The population of the Bagamoyo district was estimated at 311,740 inhabitants in the 2012 census (OCGS, 2013). The IHI-BRTC, where the laboratory work was conducted, is located right beside the Bagamoyo District Hospital in the heart of the town.

The average temperature for the region is 28°C and there are two annual rainy seasons, the heavy Masika rains from March to June and the light Vuli rains from October to December. The leading source of income of the region is cash crop production of coconuts, fruits and cashew nuts, livestock farming and, yet to a lesser extent, fishing and salt mining (NBS, 2007). The inhabitants of the region are mainly smallholder farmers engaged in food crop production such as vegetables, cassava, pulses, maize, and paddy (NBS, 2007).

Study design and participant recruitment

The IDEA study is designed as longitudinal short-term study with three study arms (Knopp et al., 2014). In the present study, we focused on results of a cross-sectional study, implemented to recruit children aged two months to ten years with asymptomatic *Plasmodium* parasitaemia for the IDEA-malaria study arm. In that arm, a sample size of 100 children with asymptomatic *Plasmodium* parasitaemia was required. Expecting a prevalence of 10% of asymptomatic *Plasmodium* parasitaemia (as found in previous, yet unpublished studies in the Bagamoyo area), we had to enrol about ~1,000 children living in the Bagamoyo district in the cross-sectional study. The fieldwork for recruitment and data collection started in early 2011 and was concluded in November 2012.

Field procedures

Before the onset of the study, sensitization and information meetings were hold with community leaders, village health care workers (VHCW), teachers and local clinicians. Parents were informed by VHCW about the study and asked to bring the children that they considered as healthy to meeting points to participate in the health screening to recruit children into the asymptomatic IDEA-malaria study arm on a specific day. At the meeting point, the study aims and procedures were explained in detail and in lay terms to the children and their accompanying parent or legal guardian. If the patient or parent/legal guardian orally assented to participation, the parent/legal guardian was given an information sheet and asked to provide a written informed consent for the child to participate. In case of illiteracy, a thumbprint signature was obtained.

Upon submission of the signed consent sheet, the participant's demographics, vitals, clinical signs and symptoms, and reported anthelmintic treatment history (treatment with albendazole or mebendazole within the past 6 months) and use of bed nets were entered in standardized forms. Children's axillary temperature, height or length (measured with standardized height/length boards from the World Health Organization (WHO)/United Nations Children's Fund (UNICEF)), weight (measured with a 25 kilogram hanging scale (C. M. S Weighing Equipment Ltd., London, United Kingdom) for light children and with a seca weighing scale (seca Deutschland, Hamburg, Germany) for heavier children), and mid-upper arm circumference (MUAC; measured with the standard UNICEF MUAC tape (S0145620 MUAC, Child 11.5 Red/PAC-50)) were recorded. A finger-prick blood sample (1.0 ml) was collected and stored on ice immediately after collection until examination in the laboratory at IHI-BRTC. Each participant was provided with (i) two plastic containers (100 ml) with lid for urine and stool collection, respectively, and (ii) an adhesive tape (50 x 20 mm) and a microscope slide, all labelled with an individual identifier code. The participants were invited to collect an own urine and morning stool sample of sufficient size (i.e. to fill half of the container) to perform all laboratory examinations. Additionally, they were instructed to wash their buttocks before going to bed and, in the morning after wake up and before taking a shower, to apply the adhesive tape on their anus before sticking it to the microscope slide. The filled containers and adhesive tape slides were collected by a VHCW the next day around noon and transferred to the Helminth Unit laboratory of the IHI-BRTC where all stool and urine samples were examined.

Laboratory procedures

The blood samples were examined for a full blood cell count including a differential white blood cell count using an externally quality controlled Sysmex XS-800 Haematology Analyser (Sysmex Deutschland GmbH, Norderstedt, Germany). Malaria parasitaemia was assessed using the SD Bioline Malaria Ag Pf/Pan 05FK60 rapid test (Standard Diagnostics, Kyonggi, Republic of Korea) and by microscopy. For the latter purpose, two thick blood smear slides (using 6 µl of blood) per patient were prepared, stained with Giemsa, and examined by two certified malaria microscopists who independently counted the number of asexual *Plasmodium* parasite stages per up to 500 white blood cells counts, depending on the level of parasitaemia (Greenwood and Armstrong, 1991). *Plasmodium* species were not differentiated, but it is widely assumed that *P. falciparum* is the major cause of malaria in Tanzania (NMCP, 2013). Blood samples from children who entered the study from July 2012 onwards were additionally examined for *Wuchereria bancrofti* antigen using the Binax NOW Filariasis rapid immunochromatic test (ICT)-card (Inverness Medical Professional Diagnostics; Scarborough, Maine, United States of America). In brief and according to the manufacturer's manual, 100 µl of whole blood were transferred on the ICT-card. Subsequently, the test card was closed and incubated for 10 min before the result was read.

Stool and urine samples were processed by experienced laboratory technicians as described in detail elsewhere (Knopp et al., 2014). Firstly, the Baermann method was applied for the detection of *S. stercoralis* larvae (García and Bruckner, 2001). Secondly, during the Baermann incubation time, from the remainder of the stool sample of each participant duplicate Kato-Katz thick smear slides were prepared using a 41.7 mg template (Katz et al., 1972) for the diagnosis and number of *A. lumbricoides*, hookworm, *T. trichiura* and *S. mansoni* eggs. Thirdly, adhesive tape slides were microscopically examined for the presence of *E. vermicularis* eggs. Fourthly, the urine sample of each participant was investigated for microhaematuria using a dipstick (Haemastix; Siemens Healthcare Diagnostics, Eschborn, Germany) and for *S. haematobium* eggs by duplicate urine filtration slides (hydrophilic polycarbonate membrane filter; pore size 20 micron, diameter 13mm; Sterlitech, Kent, WA, United States of America) (Peters et al., 1976). Fifthly, the following morning, before new samples arrived, a subsample of each individual's stool (~1 g), which had been preserved in sodium acetate acetic acid formalin (SAF) over night, was examined for the presence and number of helminth eggs with the FLOTAC dual technique (Glinz et al., 2010b) using flotation solution 2 (FS2; saturated sodium chloride (NaCl) solution; specific gravity (s.g.): 1.20) and FS7 (zinc sulfate ($ZnSO_4 \cdot 7H_2O$) solution; s.g.: 1.35). At least 10% of the blood smear, Kato-Katz thick smear, adhesive tape and urine filtration slides were subjected to re-examination for quality control by independent trained microscopists blinded to the initial result.

Data management and statistical analysis

Each participant's data were entered manually in the participant's case report form (CRF), subsequently transferred into an electronic data base (Microsoft Access 2010, Microsoft Corporation; Redmont, WA, United States of America; and DMSys, SigmaSoft International; Chicago, IL, United States of America) and finally transferred, cleaned, and analysed using STATA version 9.2 (StataCorp.; College Station, TX, United States of America). Anthropometric z-scores were calculated using the WHO Anthro and AnthroPlus softwares (WHO; Geneva, Switzerland).

Children were grouped according to their age into infants (0-2 years), PSAC (3-4 years), and SAC (5-10 years). According to WHO guidelines, moderate and severe malnutrition were defined at a MUAC cut-off of 12.5 cm and 11.5 cm, respectively, for infants and PSAC (Mogeni et al., 2011, WHO, 2009c). Moderate and severe wasting were defined by applying a weight-for-height z-score cut-off below -2 and -3, respectively, for infants and PSAC and for combined sexes (WHO, 2009c). The weight-for-height of infants whose length was under 45 cm was not calculated. In the same age-group, moderate and severe underweight were defined at height-for-age z-scores of -2 and -3, respectively. For SAC, thinness and severe thinness were defined by a body mass index (BMI)-for-age z-score cut-off below -2 and -3, respectively, and stunting at a height-for-age z-score cut-off below -2.

Anaemia thresholds were applied according to WHO recommendations as follows: haemoglobin values >10.9 mg/l non-anaemia, 10.0-10.9 mg/l mild anaemia, 7.0-9.9 mg/l moderate anaemia, and <7.0 mg/l severe anaemia for infants and PSAC, and haemoglobin values >11.5 mg/l non-anaemia, 11.0-11.4 mg/l mild anaemia, 8.0-10.9 mg/l moderate anaemia, and <8.0 mg/l severe anaemia for SAC (WHO, 2011a). Reference values for the full blood cells counts were taken from Buchanan and colleagues (2004) who provide haematology reference values for healthy Tanzanian children from the Kilimanjaro region stratified by age, including infants, PSAC and SAC (Buchanan et al., 2010). The cell counts of our children were classified as low, when they were below the 95% confidence interval (CI) limit and as high when they were above the 95% CI limit of the Kilimanjaro reference group. Elevated body temperature was considered as axillary temperature of $\geq$38.0°C as suggested by the Brighton Collaboration (Kohl et al., 2004).

A patient was considered to be infected with a helminth species, if the infection was detected with one or several diagnostic methods. For each individual, helminth infection intensity was determined according to Kato-Katz thick smear results as suggested by the WHO (Montresor et al., 1998). For this purpose, faecal egg counts (FEC) as recorded from each Kato-Katz thick smear microscopic examination were transferred into eggs per gram of stool (EPG) by multiplying the average FEC from duplicate Kato-Katz thick smears of each individual by a factor 24. The lower limits of moderate and heavy infections were 5,000 and 50,000 EPG for *A. lumbricoides*, 1,000 and 10,000 EPG for *T. trichiura*, 2,000 and 4,000 EPG for hookworm and 99 and 399 EPG for *S. mansoni*, respectively. Microhaematuria was classified according to the manufacturer's suggestion into negative, trace, +, ++, or +++ and *S. haematobium* egg counts into light (1–49 eggs/10 ml of urine) and heavy (≥50 eggs/10 ml of urine). Asymptomatic *Plasmodium* parasitaemia was defined by a positive malaria rapid diagnostic test result and/or by *Plasmodium* parasites detected microscopically plus the absence of unspecific symptoms of malaria at the day of enrolment or over the past seven days (i.e., fever, flue, cough, difficult breathing, and/or abdominal discomfort). Counts of below 10 parasites per 200 white blood cells (i.e. less than 400 parasites per µl blood) were defined as low grade parasitaemia, and counts of 100 and more parasites per 200 white blood cells (i.e. 400 or more parasites per µl blood) as moderate parasitaemia.

To assess a direct interaction between helminth and helminth co-infections, and between helminth and asymptomatic *Plasmodium* parasitaemia co-infections we calculated observed and expected prevalences for co-infections (Raso et al., 2006). The expected co-infection prevalences were calculated as the product of the observed prevalence of one infection (regardless of a co-infection) and the observed prevalence of

the second infection (regardless of a co-infection). For comparison of the observed *versus* expected prevalences, the Fisher's exact test (two-sided) was applied.

Multivariable logistic regression analyses were used for estimating odds ratios (ORs), including CIs, to determine associations between different helminth species infections or asymptomatic *Plasmodium* parasitaemia or anaemia (binary outcome variables), and nutritional measures (ordinal or binary explanatory variable), anaemia (ordinal or binary explanatory variable), specific full blood cell count variables including haemoglobin (continuous explanatory variable), helminth co-infections (binary explanatory variable), asymptomatic *Plasmodium* parasitaemia (binary explanatory variable), or fever (binary explanatory variable). In all multivariable analyses we adjusted for age in months (continuous explanatory variable), sex (binary explanatory variable), and reported anthelmintic treatment in the past six months (binary explanatory variable) and included any significant explanatory variable from univariable models pertaining to the same outcome, excluding co-linear variables. For the multivariable logistic regression, we applied a backward stepwise procedure removing non-predicting covariates up to a significance level of 0.2 and allowed for possible clustering within houses by using the sandwich estimator robust cluster option in STATA. Both, univariable and multivariable regression analyses were run (i) for all ages and (ii) stratified by age-group.

Results

Study group

Written informed consent to participate in the cross-sectional survey was provided for 1,033 children. Among them, 519 were girls and 514 were boys. According to their month and year of birth, 225 were grouped as infants, 336 as PSAC and 472 as SAC. The numbers of children with parasitological, haematological, and anthropometric examinations in each age-group are shown in Figure 13.

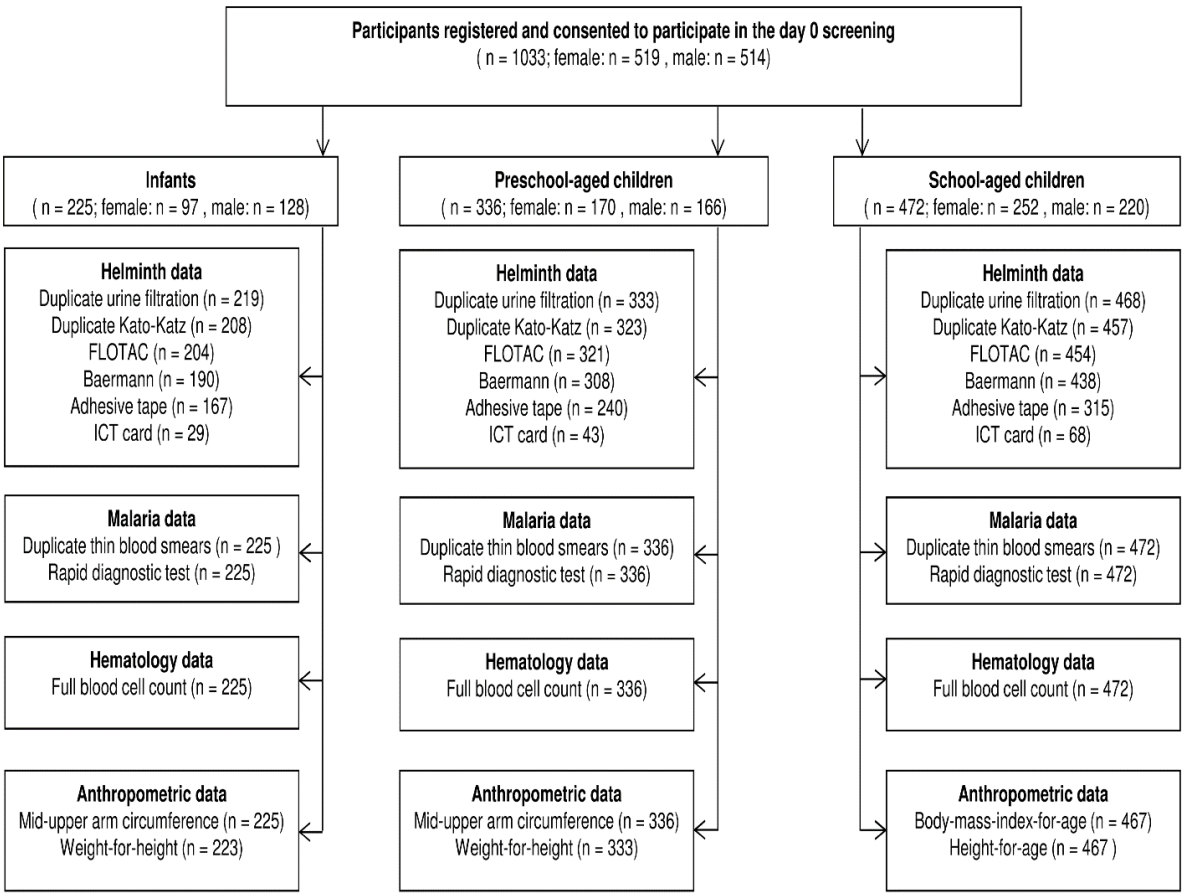

Figure 13. Characteristics of the IDEA-malaria study group consisting of children from the Bagamoyo district, United Republic of Tanzania

Anthropometric and haematological characteristics

In our study cohort, 1.3% of infants were wasted and 2.2% were underweight (Table 5). Among the PSAC, 0.3% were wasted and 2.4% were underweight. Thinness and stunting were detected in 3.2% and 18.7% of SAC, respectively. Anaemia was observed in 85.6%, 47.3%, and 45.6% of infants, PSAC, and SAC, respectively.

Table 5. Anthropometric and anaemia status of infants, preschool-aged (PSAC), and school-aged children (SAC) from the Bagamoyo district, United Republic of Tanzania, calculated in line with guidelines and thresholds provided by the World Health Organization
(Mogeni et al., 2011, WHO, 2009c, WHO, 2011a)

	Age group								
	Infants			PSAC			SAC		
Anthropometric aspects	**total**	**n**	%	**total**	**n**	%	**total**	**n**	%
Mid-upper arm circumference	225			336					
normal		222	98.7		335	99.7			
moderately wasted		3	1.3		1	0.3			
severely wasted		0	0		0	0			
Weight-for-height	223			333					
normal		218	97.8		325	97.6			
moderate underweight		2	0.9		7	2.1			
severe underweight		3	1.3		1	0.3			
Body-mass-index-for-age							467		
normal								452	96.8
moderate thinness								10	2.1
severe thinness								5	1.1
Height-for-age							467		
normal								380	81.4
moderate stunting								68	14.6
severe stunting								19	4.1
Anaemia	221			334			471		
normal		32	14.5		176	52.7		256	54.4
mild anaemia		74	33.5		108	32.3		81	17.2
moderate anaemia		106	48.0		47	14.1		127	27.0
severe anaemia		9	4.1		3	0.9		7	1.5

As shown in Table 6, the full blood cell counts from children belonging to our study group revealed that more than 70% of the children had haematocrit, mean corpuscular volume, platelets, white blood cell counts, neutrophil and lymphocyte counts within the normal range, when compared to haematological reference values derived from children of the same age-groups residing in the Kilimanjaro district, United Republic of Tanzania (Buchanan et al., 2010). However, more than 10% of our children in any age-group had elevated white blood cell and lymphocyte counts and more than 20% had high neutrophil counts. High monocyte, eosinophil and basophil counts were detected in more than 30% of our children regardless of age-group.

Parasitic infections, fever, and microhaematuria

Among all children examined for helminth infections, *E. vermicularis* was found in 18.0%, hookworm in 9.1%, *S. stercoralis* in 6.9%, *T. trichiura* in 2.5%, *W. bancrofti* in 1.4%, *S. haematobium* in 0.3%, and *A. lumbricoides* in 0.1%. No child was diagnosed with a *S. mansoni* infection.

Stratified by age-group, infections with any investigated helminth species were found in 10.2% of infants, 25.0% of PSAC, and 33.5% of SAC. As shown in Figure 14, the most prevalent helminth infections in infants were with *S. stercoralis* (5.8%) and *E. vermicularis* (4.2%), followed by *W. bancrofti* (3.4%), hookworm

(2.5%) and *T. trichiura* (0.5%). The youngest children infected with *T. trichiura*, *W. bancrofti*, *S. stercoralis*, *E. vermicularis*, and hookworms were aged six, seven, ten, eleven, and 15 months, respectively. PSAC and SAC were mostly infected with *E. vermicularis* (16.7% and 26.3%, respectively), hookworm (8.7% and 12.3%, respectively), *S. stercoralis* (7.5% and 7.1%, respectively), and *T. trichiura* (2.5% and 3.3%, respectively).

Table 6. Haematological values derived from a full blood cell count from infants, preschool-aged, and school-aged children from the Bagamoyo district, United Republic of Tanzania

Age group	Infants			PSAC			SAC		
	total	n	%	total	n	%	total	n	%
Haematocrit (%)	225			336			472		
low		12	5.3		5	1.5		60	12.7
normal		190	84.4		305	90.8		372	78.8
high		23	10.2		26	7.7		40	8.5
Mean corpuscular volume (**pg**)	225			336			472		
low		19	8.4		2	0.6		28	5.9
normal		186	82.7		308	91.7		408	86.4
high		20	8.9		26	7.7		36	7.6
Platelets (**10^9/l**)	**225**			336			472		
low		1	0.4		5	1.5		15	3.2
normal		202	89.8		305	90.8		420	89.0
high		22	9.8		26	7.7		37	7.8
White blood cells (**10^9/l**)	**225**			336			472		
low		0	0.0		0	0.0		4	0.8
normal		182	80.9		288	85.7		351	74.4
high		43	19.1		48	14.3		117	24.8
Neutrophils (**10^9/l**)	**225**			336			472		
low		2	0.9		0	0.0		6	1.3
normal		176	78.2		266	79.2		350	74.2
high		47	20.9		70	20.8		116	24.6
Lymphocytes (**10^9/l**)	**225**			336			472		
low		6	2.7		6	1.8		5	1.1
normal		183	81.3		292	86.9		374	79.2
high		36	16.0		38	11.3		93	19.7
Monocytes (**10^9/l**)	**225**			336			472		
low		0	0.0		0	0.0		0	0.0
normal		114	50.7		198	58.9		249	52.8
high		111	49.3		138	41.1		223	47.2
Eosinophils (**10^9/l**)	**225**			336			472		
low		11	4.9		12	3.6		12	2.5
normal		127	56.4		187	55.7		276	58.5
high		87	38.7		137	40.8		184	39.0
Basophils (**10^9/l**)	**225**			336			472		
low		2	0.9		0	0.0		0	0.0
normal		143	63.6		221	65.8		252	53.4
high		80	35.6		115	34.2		220	46.6

Reference values are derived from children living in the Kilimanjaro district (Buchanan et al., 2010).

Among the 70 children diagnosed with a hookworm infection by the Kato-Katz method, 60 (85.7%) showed a light, 6 (8.6%) a moderate, and 4 (5.7%) a heavy infection intensity. Among the 21 *T. trichiura* positive children, 19 (90.5%) had a light and 2 (9.5%) a moderate infection intensity. The only child infected with *A. lumbricoides* had moderate infection intensity. All three children infected with *S. haematobium* had light infection intensities.

Asymptomatic *Plasmodium* parasitaemia was diagnosed in 7.6% of infants, 9.8% of PSAC, and 18.9% of SAC, respectively. Among all children with asymptomatic *Plasmodium* parasitaemia 66.3% had a moderate parasitaemia and 33.7% had a low parasitaemia. Fever at the day of examination was measured in 1.3% of infants and 0.6% of SAC without *Plasmodium* parasitaemia. Microhaematuria was detected in 10.5% of infants, 3.9% of PSAC, and 2.4% of SAC, respectively.

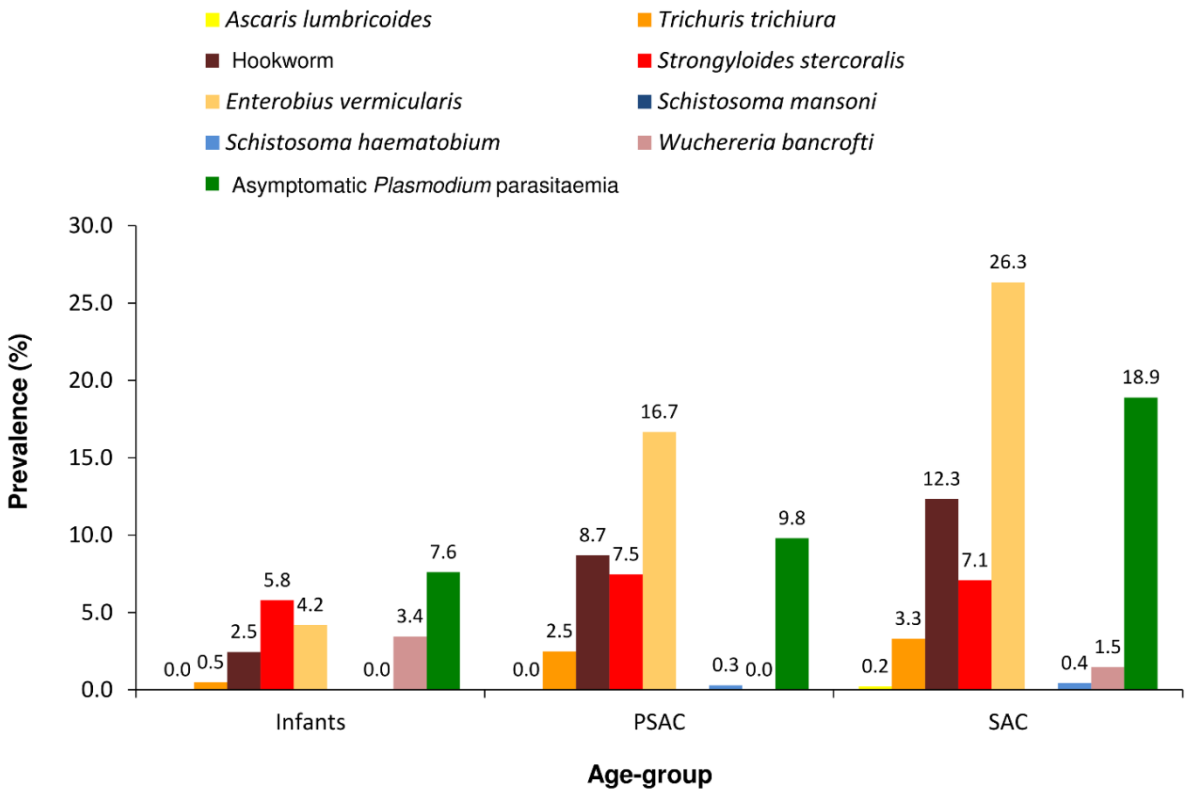

Figure 14. Prevalence of helminth infections and asymptomatic *Plasmodium* parasitaemia in infants, preschool-aged children (PSAC) and school-aged children (SAC) from the Bagamoyo district, United Republic of Tanzania

Comparison between observed and expected co-infection prevalences

A summary of observed *versus* expected parasite co-infection prevalences is presented in Table 7. Significant differences in prevalence, suggestive for non-chance findings, were detected for co-infection with *S. stercoralis* and asymptomatic *Plasmodium* parasitaemia in children of all age-groups (p = 0.039), but particularly in infants (p = 0.006). Moreover, observed co-infections with *S. stercoralis* and hookworm in all age-groups (p = 0.038), *S. stercoralis* and *T. trichiura* in all age-groups (p = 0.018), but particularly in SAC (p = 0.016), and hookworm and *T. trichiura* in all age-groups (p = 0.004), but particularly in PSAC (p = 0.022) were significantly higher than expected by chance.

Association of helminth infections with anthropometric measures, haematology, and parasitic co-infections

Results of the multivariable regression models stratified by helminth infection and age-group are shown in detail in Table 8. After adjusting for potential confounders in multivariable analyses and here only presenting OR higher than 2.00, strongyloidiasis was associated with asymptomatic *Plasmodium* parasitaemia in infants (*S. stercoralis* as outcome: OR: 13.03; 95% CI: 1.34 – 127.23; asymptomatic *Plasmodium* parasitaemia as outcome: OR: 5.75; 95% CI: 1.21 – 27.41).

Table 7. Infants', preschool-aged children's (PSAC) and school-aged children's (SAC) co-infection status with hookworm, *S. stercoralis*, *E. vermicularis*, *T. trichiura*, and/or asymptomatic *Plasmodium* parasitaemia in the Bagamoyo region, United Republic of Tanzania, and comparison between observed and expected co-infection prevalence at the unit of age-group

	Examined children (n)	Observed co-infection (%)	Expected co-infection (%)*	P-value**
Asymptomatic *Plasmodium* and *E. vermicularis* infection				
Infants	167	0.60	0.30	0.413
PSAC	240	1.67	1.46	0.760
SAC	315	5.71	5.44	0.755
All age-groups	722	3.19	2.44	0.156
Asymptomatic *Plasmodium* and hookworm infection				
Infants	204	0.49	0.19	0.338
PSAC	322	0.93	0.86	0.749
SAC	454	2.42	2.28	0.854
All age-groups	980	1.53	1.22	0.329
Asymptomatic *Plasmodium* and *S. stercoralis* infection				
Infants	190	2.11	0.46	0.006
PSAC	308	0.97	0.75	0.715
SAC	438	1.83	1.36	0.345
All age-groups	936	1.60	0.96	0.039
Asymptomatic *Plasmodium* and *T. trichiura* infection				
Infants	204	0.00	0.04	1.000
PSAC	321	0.00	0.25	1.000
SAC	454	0.44	0.61	1.000
All age-groups	979	0.20	0.33	0.760
Hookworm and *E. vermicularis* infection				
Infants	155	0.65	0.12	0.181
PSAC	229	2.18	1.93	0.782
SAC	305	3.61	3.23	0.693
All age-groups	689	2.47	1.80	0.138
Hookworm and *S. stercoralis* infection				
Infants	188	0.53	0.12	0.216
PSAC	307	0.98	0.59	0.406
SAC	436	1.61	0.90	0.093
All age-groups	931	1.18	0.62	0.038
Hookworm and *T. trichiura* infection				
Infants	204	0.00	0.01	1.000
PSAC	321	0.93	0.21	0.022
SAC	454	0.88	0.41	0.100
All age-groups	979	0.72	0.22	0.004
***S. stercoralis* and *E. vermicularis* infection**				
Infants	144	0.00	0.19	1.000
PSAC	215	1.40	1.48	1.000
SAC	292	1.37	1.67	0.789
All age-groups	651	1.08	1.26	0.841

***S. stercoralis* and *T. trichiura* infection**				
Infants	188	0.00	0.03	1.000
PSAC	307	0.33	0.17	0.423
SAC	436	0.92	0.24	0.016
All age-groups	931	0.54	0.17	0.018
***E. vermicularis* and *T. trichiura* infection**				
Infants	155	0.00	0.02	1.000
PSAC	228	0.44	0.53	1.000
SAC	305	1.31	0.93	0.484
All age-groups	688	0.73	0.50	0.361

* Expected co-infection prevalence is the product of the observed infection prevalence of one species (regardless of a co-infection) and the observed infection prevalence of the other species (irrespective of co-infection).

** Comparison of observed and expected co-infection proportions, p-value based on a Fisher's Exact-test.

Elevated eosinophil counts in infants were a predictor for *S. stercoralis* (OR: 4.00; 95% CI: 1.10 – 14.58) and hookworm infections (OR: 16.60; CI: 1.39 - 198.32). Infants with a reported anthelmintic treatment in the past 6 months were more likely to be infected with hookworm (OR: 27.91; 95% CI: 5.52 – 141.21).

Preschool-aged children with increased temperature had higher odds of presenting with a hookworm infection (OR: 3.74; 95% CI: 1.14 – 12.28). Hookworm and *T. trichiura* infections were positively associated in this age-group (hookworm as outcome OR: 22.78%; 95% CI: 4.43 – 117.23; *T. trichiura* as outcome (OR: 11.53; 95% CI: 2.30 – 57.77). Elevated monocyte counts were a predictor for asymptomatic *Plasmodium* parasitaemia in PSAC (OR: 13.88; 95% CI 3.42 - 56.30).

School-aged children with *S. stercoralis* infection had higher odds of being co-infected with *T. trichiura* (OR: 6.63; 95% CI: 1.52 – 28.93). In this age-group, children presenting with increased temperature were more likely to be infected with *E. vermicularis* (OR: 2.21; 95% CI: 1.13 – 4.33). Increased eosinophil counts were predictors for infections with *T. trichiura*, hookworm, and *S. stercoralis* (OR: 3.23; 95% CI: 1.73 – 6.02, OR: 2.71; 95% CI: 1.39 – 5.31; and OR: 2.15; 95% CI: 1.10 – 14.58, respectively).

Grouping children according to WHO anaemia thresholds, in children below the age of two years asymptomatic *Plasmodium* parasitaemia was associated with light (OR: 1.84; 95% CI 1.04 – 3.27), moderate (OR: 5.36; 95% CI 2.92 – 9.82), or severe anaemia (OR: 11.20; 4.20 – 29.86). Also children aged older than two years presenting with moderate (OR: 3.07; 95% CI 1.96 – 4.81) or severe anaemia (OR: 7.27; 95% CI 2.04 – 25.96) were more likely to have an asymptomatic *Plasmodium* parasitaemia. In turn, children in the latter age-range presenting with moderate asymptomatic *Plasmodium* parasitaemia were more likely to be anaemic (OR: 2.69; 95% CI: 1.23 – 5.86).

Wasting, underweight, stunting, thinness, fever and microhaematuria were not associated with any helminth infection, asymptomatic *Plasmodium* parasitaemia or anaemia in our study population.

Table 8. Helminth infections and significantly associated factors according to stepwise backwards multivariable regression analyses in infants, preschool-aged (PSAC), and school-aged children (SAC) from the Bagamoyo district, United Republic of Tanzania

Helminth infection	Age-group	Explanatory variable	n	OR	95% Confidence interval	p-value	Original model run with*
E. vermicularis	**all age-groups**	Gender	585	0.70	(0.45 - 1.11)	0.127	9,1,2,3,4,6,7,16,17,20
		Age (month)		1.03	(1.02 - 1.05)	<0.001	
		Temperature		1.60	(0.96 - 2.67)	0.071	
		Weight		0.91	(0.82 - 1.00)	0.051	
		Anthelmintic treatment		2.15	(1.22 - 3.79)	0.008	
		Neutrophil counts		1.10	(1.01 - 1.20)	0.034	
	Infants	Gender	158	3.72	(0.68 - 20.36)	0.129	9,1,2,3,21
		Age (month)		1.13	(1.01 - 1.27)	0.027	
	PSAC	Gender	201	0.51	(0.23 - 1.16)	0.108	9,1,2,3,16
		Anthelmintic treatment		3.60	(1.01 - 12.86)	0.049	
		Neutrophil counts		1.09	(1.00 - 1.19)	0.041	
	SAC	Temperature	315	2.21	(1.13 - 4.33)	0.021	9,1,2,3,4
		Anthelmintic treatment		1.78	(0.94 - 3.36)	0.076	
S. stercoralis	**all age-groups**	Gender	624	0.62	(0.32-1.21)	0.163	11,1,2,3,4,10,12,13,14,19
		Anthelmintic treatment		1.65	(0.80-3.41)	0.178	
		T. trichiura		4.13	(1.04-16.52)	0.045	
		Asymptomatic *Plasmodium* parasitaemia		2.10	(0.97-4.51)	0.058	
		Eosinophil counts		2.04	(1.20-3.48)	0.008	
		Platelet counts		1.00	(0.99-1.00)	0.064	
	Infants	Gender	132	0.13	(0.01 - 1.38)	0.091	11,1,2,3,6,7,13,14
		Age (month)		1.20	(0.97 - 1.49)	0.086	
		Asymptomatic *Plasmodium* parasitaemia		13.03	(1.34 - 127.23)	0.027	
		Eosinophil counts		4.00	(1.10 - 14.58)	0.036	
	PSAC	Light anaemia	303	0.19	(0.04 - 0.82)	0.026	11,1,2,3,21
	SAC	*T. trichiura*	290	3.59	(0.80 - 16.08)	0.095	11,1,2,3,4,12,14,19
		Eosinophil counts		2.15	(1.10 - 4.23)	0.026	
		Platelet counts		0.99	(0.98 - 1.00)	0.011	
Hookworm	**all age-groups**	Temperature	623	1.77	(0.83 - 3.78)	0.143	10,1,2,3,4,6,7,11,12,14,16,20
		Weight		1.12	(1.05 - 1.18)	<0.001	
		T. trichiura		3.33	(0.77 - 14.39)	0.107	
		Eosinophil counts		2.30	(1.29 - 4.09)	0.005	

	Infants	Anthelmintic treatment	138	27.91	(5.52 - 141.21)	<0.001	10,1,2,3,6,7,14,16,20
		Eosinophil counts		16.60	(1.39 - 198.32)	0.026	
		Basophil counts		0.10	(0.01 - 0.86)	0.036	
		Haemoglobin		1.96	(1.13 - 3.41)	0.016	
	PSAC	Gender	272	1.97	(0.72 - 5.38)	0.185	10,1,2,3,4,12,16
		Temperature		3.74	(1.14 - 12.28)	0.030	
		Anthelmintic treatment		0.52	(0.2 - 1.34)	0.174	
		T. trichiura		22.78	(4.43 - 117.23)	<0.001	
		Neutrophil counts		1.11	(1.01 - 1.21)	0.03	
	SAC	Eosinophil counts	295	2.71	(1.39 - 5.31)	0.004	10,1,2,3,14,27
T. trichiura	**all age-groups**	Anthelmintic treatment	623	0.12	(0.03 - 0.54)	0.005	12,1,2,3,11,14,18
		S. stercoralis		5.18	(1.45 - 18.46)	0.011	
		Eosinophil counts		3.28	(1.69 - 6.36)	0.001	
		Monocyte counts		0.02	(0.00 - 0.33)	0.007	
	Infants	Age (month)	55	0.51	(0.31 - 0.85)	0.009	12,1,2,3
	PSAC	Hookworm	297	11.53	(2.30 - 57.77)	0.003	12,1,2,3,10,19
		Platelet counts		1.01	(1.00 - 1.01)	0.008	
	SAC	Anthelmintic treatment	290	0.08	(0.01 - 0.48)	0.006	12,1,2,3,6,11,14
		S. stercoralis		6.63	(1.52 - 28.93)	0.012	
		Eosinophil counts		3.23	(1.73 - 6.02)	0.001	
***Plasmodium* parasitaemia**	**all age-groups**	Age (month)	952	1.03	(1.02 - 1.04)	<0.001	13,1,2,3,6,7,19,20
		Anthelmintic treatment		0.73	(0.49 - 1.10)	0.137	
		Haemoglobin		0.65	(0.56 - 0.76)	<0.001	
		Platelet counts		1.00	(0.99 - 1.00)	0.012	
	Infants	Gender	177	2.88	(0.79 - 10.55)	0.111	13,1,2,3,6,7,11,19
		Weight		1.39	(0.97 - 1.99)	0.071	
		S. stercoralis		5.75	(1.21 - 27.41)	0.028	
		Platelet counts		0.99	(0.99 - 1.00)	0.022	
	PSAC	Weight	230	1.27	(1.06 - 1.52)	0.010	13,1,2,3,7,18,20
		Haemoglobin		0.49	(0.34 - 0.73)	<0.001	
		Monocyte counts		13.88	(3.42 - 56.30)	<0.001	
	SAC	Age (month)	312	1.03	(1.01 - 1.05)	0.004	13,1,2,3,18,20
		Anthelmintic treatment		0.65	(0.36 - 1.19)	0.161	
		Haemoglobin		0.69	(0.53 - 0.90)	0.007	
		Monocyte counts		2.54	(0.77 - 8.37)	0.126	

***Plasmodium* parasitaemia**	**<2 years**	Light anaemia	625	1.84	(1.04 - 3.27)	0.037	13,1,2,3,21
		Moderate anaemia		5.36	(2.92 - 9.82)	<0.001	
		Severe anaemia		11.20	(4.20 - 29.86)	<0.001	
		Age (month)		1.04	(1.025 - 1.05)	<0.001	
		Anthelmintic treatment		0.72	(0.47 - 1.10)	0.129	
	>2 years	Light anaemia	537	1.57	(0.88 - 2.78)	0.123	13,1,2,3,21
		Moderate anaemia		3.07	(1.96 - 4.81)	<0.001	
		Severe anaemia		7.27	(2.04 - 25.96)	0.002	
		Gender		0.70	(0.47 - 1.04)	0.076	
		Age (month)		1.02	(1.00 - 1.03)	0.021	

* Explanatory variables with significant outcome in the univariable analysis and included in the original model of the multivariate analysis 1: Gender; 2: Age (month); 3: Anthelmintic treatment; 4: Temperature; 5: mid-upper arm circumference (MUAC); 6: Height; 7: Weight; 8: *A. lumbricoides* infection; 9: *E. vermicularis* infection; 10: Hookworm infection; 11: *S. stercoralis* infection; 12: *T. trichiura* infection; 13: Asymptomatic *Plasmodium* parasitaemia; 14: Eosinophil counts; 15: Basophil counts; 16: Neutrophil counts; 17: Lymphocyte counts; 18: Monocyte counts; 19: Platelet counts; 20: Haemoglobin; 21: Anaemia; 22: Malnutrition; 23: Stunting; 24: Thinness; 25: Underweight; 26: Wasting; 27: Fever.
The table shows the significant explanatory variables that remained in the final model with $p < 0.2$.

Discussion

Little is known about the epidemiology and public health importance of *S. stercoralis* and *E. vermicularis* infections in Sub-Saharan Africa. We found that strongyloidiasis and enterobiasis were predominant to other helminthiases in our study population. Noteworthy, already infants, an age-group mostly ignored in studies pertaining to soil-transmitted helminthiases, presented with *S. stercoralis* (5.8%) and *E. vermicularis* infections (4.2%). Prevalences increased with age, and 7.5% and 7.1% of PSAC and SAC, respectively, were infected with *S. stercoralis* and 16.7% and 26.3%, respectively, with *E. vermicularis*.

The prevalences for all helminth infections determined in our study population are relatively low compared to those reported from other settings in the United Republic of Tanzania (Knopp et al., 2010b, Tatala et al., 2008, Kinung'hi et al., 2014). The following considerations are offered for explanation: Firstly, over the past decade, Tanzania has successfully implemented a series of helminth control interventions (Nyhus Dhillon et al., 2013, Mwakitalu et al., 2014), which might have reduced the infections in the population. Indeed, our under five year old children might have received Mebendazole in the frame of the biannual interventions of the Expanded Programme on Immunisation (EPI), which provides vitamin A supplementation, measles vaccination and deworming in the Bagamoyo District, and the SAC might have been targeted by school-based deworming organized by the Ministry of Health. Secondly, we included infants, PSAC and SAC aged aged two months to ten years in our study. The age-prevalence-curve for the major soil-transmitted helminth infections and schistosomiasis increases with age and peaks in children between 8 and 15 years (Gryseels, 2012, Mwangi et al., 2006). Our children were hence not yet in the peak age for infection, but an increase in the prevalence of all investigated helminth species with age was indeed observed. Thirdly, the conventional parasitological diagnostic methods that we applied lack sensitivity, particularly when infection intensities are low and only a single faecal sample is examined (Knopp et al., 2008b, Knopp et al., 2014, Jeandron et al., 2010). While we used duplicate Kato-Katz thick smears and the FLOTAC dual technique on a single stool sample to detect soil-transmitted helminth and *S. mansoni* infections and duplicate urine filtrations from a single urine for *S. haematobium* diagnosis, the true helminth prevalences in our study were likely considerably higher.

S. stercoralis infections were associated with eosinophilia in our study population, a condition that is generally attributed to strongyloidiasis (Leder and Weller, 2000, Nuesch et al., 2005). Our study also confirmed results from the neighbouring Zanzibar island with regard to a positive association between *S. stercoralis* and *T. trichiura* infections (Knopp et al., 2010b). Novel is our finding that strongyloidiasis was associated with asymptomatic *Plasmodium* parasitaemia in infants. While the expected prevalence of this co-infection in infants was only 0.5%, the observed prevalence was 2.1%. However, due to the very low number of co-infected infants and multiple testing, these findings must be interpreted with care. If future studies with a larger number of co-infected children confirm our results, it might be reasonable to assume that this tissue invasive helminth species in particular is modulating immunological pathways related to the acquisition, replication and pathologic sequelae of *Plasmodium* infections. In the worst case, *S. stercoralis* infections in infants may be a contributing factor to the delayed acquisition of clinical immunity to malaria in this highly vulnerable age-group. More indepth-studies on the immunological interplay of strongyloidiasis and malaria are clearly needed.

To date, leading reviews on the global distribution and risk factors for *S. stercoralis* do not mention malaria, and publications summarizing the epidemiology and immunological interplay of helminth and malaria co-infection ignore *S. stercoralis*, respectively. The only study we found assessing *S. stercoralis* infections and malaria was on pregnant women from Uganda, and revealed no association (Hillier et al., 2008). Moreover, there are only very few publications mentioning *S. stercoralis* infections in infants and PSAC in sub-Saharan Africa, and there are no recent reports assessing strongyloidiasis in any age-group on mainland Tanzania (Schär et al., 2013b, Stothard et al., 2008, Becker et al., 2011, Joyce et al., 1996, Tanner et al., 1987). Our findings that *S. stercoralis* was infecting a considerable number of young children of our study population from the age of ten months onwards, and was associated with asymptomatic *Plasmodium* parasitaemia, underline the importance of diagnosing this disease. Inclusion of *S. stercoralis* into helminth control programs, as suggested by other research teams, needs to be considered (Khieu et al., 2013a, Krolewiecki et al., 2013, Bisoffi et al., 2013).

Enterobiasis was not associated with any specific haematological marker or helminth co-infection, but children of all age-groups with a reported anthelminthic treatment history over the past six months and SAC with an increased temperature had higher odds of an infection. This finding suggests that children with enterobiasis suffer, likely from anal itching and ill-being, and sought treatment for relief. The higher odds of infection might be a sign that either the treatment as administered did not cure the infection or that children got rapidly reinfected, likely due to transmission from additional infected family members (Cook, 1994). Similarly, infants with reported anthelminthic treatment history had higher odds of hookworm infection in our study. Also hookworm infection can considerably impact on a child's health and wellbeing (Becker et al., 2011) and therefore treatment might have been sought. However, infants with hookworm infection who had a reported treatment history might either not have been cured by the treatment or live in a particularly unhygienic environment that favours rapid reinfection. Cure might not have been achieved since, in case mebendazole was administered, cure rates for hookworm are very low (Keiser and Utzinger, 2008) or, in case no liquid formulation was available, crushed tablets were difficult to administer to very young children and might not have resulted in complete clearance of infection.

Neither *E. vermicularis* nor hookworm infection was associated with asymptomatic *Plasmodium* parasitaemia in our study. While there are hardly any cross-sectional surveys assessing *E. vermicularis* infections with appropriate diagnostic methods in sub-Saharan Africa, let alone its potential association with malaria, there are multiple studies investigating associations between hookworm and *Plasmodium* infections. In line with our findings, there are studies that did not find an association between hookworm and *Plasmodium* infections (Mazigo et al., 2010, Shapiro et al., 2005). Other studies, however, indicate significant associations between these infectious diseases (Righetti et al., 2012, Boel et al., 2010, Hillier et al., 2008). The heterogeneous results and potential consequences of co-infections are nicely summarized in recent reviews (Adegnika and Kremsner, 2012, Nacher, 2011).

Neither *S. stercoralis*, *E. vermicularis* nor any other investigated helminth infection was associated with wasting, underweight, thinness, stunting, or anaemia in our study population. While wasting, underweight, and thinness were rarely seen in the surveyed children and therefore no association could be determined, stunting occurred in 18.5% of SAC and anaemia affected more than half of the examined children. Previous studies conducted in the Kilombero district in Tanzania in the early 1980s had also

revealed no association between wasting or stunting and intestinal helminth infection or *Plasmodium* parasitaemia (Tanner et al., 1987). In that particular time and place the observed substantial differences in the nutritional status of children were related to the lean and post-harvest seasons (Tanner and Lukmanji, 1987, Tanner and de Savigny, 1987). The Bagamoyo district, however, is relatively rich in cash-crop production agricultural systems throughout the year, and was one of the four councils in Tanzania, that were not reported to have major food and nutrition insecurity problems in 2011/12 (MUCHALI, 2012). This might explain why no acute but only chronic signs of malnutrition like stunting were found in our study. Other studies indicated that particularly moderate-to-heavy intensity helminth infections were associated with reduced length-for-age z-score in young infected children from Peru (Gyorkos et al., 2011), with decreased weight-for-age z-scores in SAC from Honduras (Sanchez et al., 2013), and with stunting in SAC from China (Shang et al., 2010). The occurrence of mostly light helminth infection intensities in our study might hence explain why no association was found.

Anaemia was related with asymptomatic *Plasmodium* parasitaemia but not with helminthiases in infants, PSAC, and SAC in our study. It is widely acknowledged that *Plasmodium* infections, also if low-grade and asymptomatic as a result of semi-immunity, contribute to the development of anaemia (Kurtzhals et al., 1999). Also hookworm infections have been shown to contribute to anaemia, particularly in children and women of childbearing age, and when infection intensities were moderate-to-heavy (Mwangi et al., 2006, Smith and Brooker, 2010, Brooker et al., 2008, Dreyfuss et al., 2000, Stoltzfus et al., 2000). Also here, the reason for not finding any association of helminth infections with anaemia might be due to the mostly light infection intensities found in our study population. Clearly, the low prevalence of helminth infections and specifically of moderate and high infection intensities, resulting in a lack of statistical power to determine effects on nutritional aspects including anaemia and stunting, are a limitation of this study. Moreover, we did not assess additional reasons for malnutrition and anaemia, such as restricted access to micro-nutrients, agricultural and dietary practices, food security, or social, political, and economic determinants, which might have biased our results.

Elevated eosinophil counts were strongly associated with hookworm (OR: 16.6) and *S. stercoralis* (OR: 4.0) infections in infants and with *T. trichiura* (OR: 3.2), hookworm (OR: 2.7) and *S. stercoralis* (OR: 2.2) infections in SAC. Marked eosinophilia is considered a common marker for early hookworm and *S. stercoralis* infections, and is explained by the immune reaction to the worm larvae, which migrate through the body tissues to reach their destination in the lungs and in the gastrointestinal tract. (Leder and Weller, 2000). The eosinophilia caused by *T. trichiura* is reported to be mostly mild (Leder and Weller, 2000). The hematology values of our study population were only partially in line with reference values derived from a similar study population based in the Kilimanjaro district (Buchanan et al., 2010). Considerably higher neutrophil counts were found in almost a quarter and elevated monocyte, eosinophil, and basophil counts in more than a third of all participants of all age-groups in our study children. While higher eosinophil counts were associated with specific helminth species infections and monocyte counts were associated with asymptomatic *Plasmodium* parasitemia in some age-groups, the latter being a common sign of acute malaria (Antonelli et al., 2014, Halim et al., 2002), there might be additional underlying reasons such as viral or bacterial infections causing monocytosis or allergies and other diseases causing eosinophilia (Schulte et al., 2002, Leder and Weller, 2000). Elevated basophil counts were not

associated with helminth or asymptomatic *Plasmodium* infections in our study, confirming that it is not a useful clinical marker for the evaluation of suspected parasitic disease (Mitre and Nutman, 2003). Moreover, considering the unpublished reference values used in the IHI-BRTC laboratory, the normal range of basophils at the IHI-BRTC is 0.0-0.3 per 10^9/L and thus much higher than the Kilimanjaro values (Buchanan et al., 2010) and rather resembling the pediatric reference values reported for Uganda (Lugada et al., 2004). A limitation of our study is that no other infections or diseases that might have caused elevated or decreased blood cell counts were investigated and there is a clear need for the establishment of standardized hematological reference values for the Bagamoyo area.

Conclusion

The results of our cross-sectional study showed that *E. vermicularis* and *S. stercoralis* were moderately prevalent in young children from rural coastal Tanzania. A considerable number of infants were infected and prevalences increased with children's age. Our data can contribute to inform yet missing global burden of disease and prevalence estimates for strongyloidiasis and enterobiasis. The association between *S. stercoralis* and asymptomatic *Plasmodium* parasitaemia found in infants of our study population warrants further investigations.

Authors' contributions

SK analysed the data and drafted the manuscript. BG, SA, MT and CD initiated the study. NS, DK, JM and OL conducted fieldwork and were responsible for the anthropometric and haematological assessments. SK, TS, JR, and ASM implemented and conducted the parasitological examinations. UA cleaned the data. All authors contributed to the full conception and implementation of the study, revised the manuscript and approved its final version.

Acknowledgments

All researchers are grateful for the grant provided by the European Commission under the Health Cooperation Work Program of the 7th Framework Program to conduct part of the IDEA project "Dissecting the Immunological Interplay between Poverty Related Diseases and Helminth infections: An African-European Research Initiative" (http://ec.europa.eu/research/health/infectious-diseases/neglected-diseases/projects/014_en.html) at IHI-BRTC (Grant agreement n°241642). The funders played no role in in design, collection, analysis, and interpretation of data; in the writing of the manuscript; and in the decision to submit the manuscript for publication.

We are particularly obliged to Raymond Singo, Shabani Halfan, Rehaema Mangoli, and Tatu Nassor for their help in the field and laboratory work and the data unit of the IHI-BRTC for entering the large amount of data. Moreover, we acknowledge the Bagamoyo district officials and specifically the district immunization and vaccination officer Farah Mohammed for his great collaboration. Finally, we are grateful to all children and their parents who agreed to participate in this study and submitted blood, stool and urine samples.

Chapter 7: Distribution and risk factors for *Plasmodium* and helminth co-infections: a cross sectional survey among children in Bagamoyo district, coastal region of Tanzania

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The authors declare that they have no competing interests.

The article has been accepted in PLoS Neglected Tropical Diseases (PNTD), (Salim et al, 2015)

Abstract

Background: *Plasmodium* and soil transmitted helminth infections (STH) are a major public health problem particularly among children. There are conflicting findings on potential association between these two parasites. This study investigated the *Plasmodium* and helminth co-infections among children aged 2 months to 9 years living in Bagamoyo district, coastal region of Tanzania.

Methods: A community based cross sectional survey was conducted among 1033 children. Stool, urine and blood samples were examined using a broad set of quality controlled diagnostic methods for common STH (*Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Enterobius vermicularis*, and *Trichuris trichura*), schistosoma species and *Wuchereria bancrofti*. Blood slides and malaria rapid diagnostic tests (mRDTs) were utilized for *Plasmodium* diagnosis.

Results: Out of 992 children analyzed, the prevalence of *Plasmodium* infection was 13% (130/992), helminth 28.5% (283/992); 5% (50/992) had co-infection with *Plasmodium* and helminth. The prevalence rate of *Plasmodium*, specific STH and co-infections increased significantly with age ($p < 0.001$), with older children mostly affected except for *S. stercoralis* monoinfection and co-infections. Spatial variations of co-infection prevalence were observed between and within villages. There was a trend for STH infections to be associated with *Plasmodium* infection [OR adjusted for age group 1.4, 95% CI (1.0 - 2.1)], which was more marked for *S. stercoralis* (OR= 2.2, 95% CI (1.1 - 4.3). Age and not schooling were risk factors for *Plasmodium* and STH co-infection.

Conclusion: The findings suggest that STH and *Plasmodium* infections tend to occur in the same children, with increasing prevalence of co-infection with age. This calls for an integrated approach such as using mass chemotherapy with dual effect (e.g. ivermectin) coupled with improved housing, sanitation and hygiene for the control of both parasitic infections.

Author Summary

Parasitic infectious agents rarely occur in isolation and multiparasitism is a norm specifically in children living in endemic areas of Tanzania. We studied the pattern and predictors of *Plasmodium* and STH co-infections in rural Bagamoyo district, coastal region of Tanzania. Parents/guardians of healthy children aged 2 months to 9 years who were willing to participate into the study were invited from the community. Stool, urine and blood were examined for helminth and *Plasmodium* parasites. We found that children aged above five years and those who are not schooling had the greatest burden of co-infection with *Plasmodium* and helminth parasites. The risk of being co-infected with *Plasmodium* increased with age with all the common types of STH isolated (*E. vermicularis*, hookworm and *S. stercoralis*). Younger children had a significantly higher risk of having *Plasmodium* when co-infected with *S. stercoralis*. Integrated control approaches including health education, environmental sanitation and hygiene, novel chemoprophylaxis as well as long lasting Impregnated Nets (LLINs) distributions should be implemented considering the pattern and types of infections within the area in order to interrupt transmission of both parasites among young and school-aged children.

Background

Parasitic infections such as *Plasmodium* present a major public health problem among children in Africa (WHO, 2013, RBM, 2012) and its coexistence with Soil Transmitted Helminth (STH) infections is common (Brooker et al., 2012, Pullan et al., 2011). Multipasitism is a norm among children in developing countries including United Republic of Tanzania (Mazigo et al., 2010, Kinung'hi et al., 2014). It is defined as a concurrent infection in a single host with two or more species whereas monoinfection consists of only one infection from a single species (Steinmann et al., 2010). Variety of environmental and host related factors can influence the structure and dynamics of the parasite communities which make up these multiple infections (Petney and Andrews, 1998, Rothman and Greenland, 2005, Brooker and Clements, 2009). These conditions include poverty, environmental contamination with infected faeces containing helminth eggs, water bodies, lack of effective preventive measures (Booth, 2006) and immunity of the host. In addition, overlap of *Plasmodium* infection and other pathogens depends on the conditions that favour multiple parasitic species survival and transmission such as exposure related risk and within host interactions between co-infecting species (Booth, 2006, Brooker and Utzinger, 2007, Brooker et al., 2012). There is mounting evidence indicating that helminth infections increase susceptibility to *Plasmodium* infection (Druilhe et al., 2005, Nacher et al., 2002, Spiegel et al., 2003) . On the other hand, some studies showed that specific STH like *Ascaris lumbricoides* are protective against *Plasmodium* disease and its severe manifestations (Nacher, 2011). Previous epidemiological studies have shown coexistence of *Plasmodium* and helminth infections with spatial heterogeneity in their distribution (Pullan et al., 2011, Mboera et al., 2011, Brooker et al., 2012, Kinung'hi et al., 2014). In Tanzania, cross sectional surveys conducted among school and preschool children showed that multiple parasitic infections are common (Mboera et al., 2011, Mazigo et al., 2010, Kinung'hi et al., 2014). Study by Kinung'hi et al showed that the prevalence of malaria parasites tended to increase with increasing number of co-infecting helminth species as compared to helminth free children although the difference was not statistically significant (Kinung'hi et al., 2014).

In Tanzania, global strategies to control malaria are being conducted by the National malaria control program (NMCP) via Long Lasting Insecticide Impregnated Nets (LLINs), Intermittent Preventive Treatment in Pregnancy (IPTp) and prompt treatment with artemether/lumefantrine. The helminth control is done via mass drug administration (MDA), chemotherapy based morbidity control campaigns. The planning for prevention and control program are designed to focus on a single infection approach despite occurrence of co-infections (Mazigo and Ambrose-Mazigo, 2012) . There is an underestimation of the burden of infection and lack of understanding how these parasitic infections interact (Mazigo and Ambrose-Mazigo, 2012). This underlines the importance of investigating the epidemiology of co-infections in different geographical locations where different pattern of infections are expected. In the present study we aimed to determine the relation between *Plasmodium* and STH co-infections among children aged 2 months to 9 years living in Bagamoyo district, coastal region of Tanzania. Knowledge of the magnitude and on the common risk factors for co-infections should guide the development of focused integrated control programs targeting multiple infections endemic in each country.

Materials and methods

Reporting of the study follows STROBE checklist (Strengthening the Reporting of Observational studies in Epidemiology) (Vandenbroucke et al., 2007).

Ethics statement

The study was conducted under the IDEA study protocol which was approved by the institutional review boards of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland) and the Ifakara Health Institute (IHI; Dar es Salaam, United Republic of Tanzania). The ethical approval for the conduct of the study was granted by the Ethikkomission beider Basel (EKBB; Basel, Switzerland; reference number: 257/08) and the National Institute for Medical Research of Tanzania (NIMR; Dar es Salaam, United Republic of Tanzania; reference number: NIMR/HQ/R.8a/Vol. IX/1098).

The local district, community, school teachers and health authorities were informed during sensitization meetings about the purpose, procedures, risk and benefits of the study prior to the start. Written informed consent was obtained from the parents/guardians of children prior to study procedures after explaining them to the group. Illiterate parents/ guardians were asked to bring witness who participated within the discussion prior to obtaining their thumbprints and witness signature. Participants infected with helminth and/or malaria or other medical conditions received appropriate treatment/referral according to the national treatment guidelines of Tanzania.

Study area

Bagamoyo is a district in the coastal region of Tanzania where Ifakara Health Institute (IHI) through its branch, Bagamoyo Research and Training Centre (BRTC) works in close collaboration with the Bagamoyo District hospital (BDH) officials to ensure quality health care delivery using its research platforms. The BRTC study area covers about 1160 square kilometres. The eastern border of the study area is formed by the Indian Ocean, with the Ruvu River forming part of the western and northern borders. The area extends for approximately 7 km on either side of a road running westwards for 62 kilometres. To the south is an uninhabited forest reserve. According to the 2012 Tanzania National Census, the population of the Bagamoyo District was 311,740 which can be reached by dirt road throughout the year; all are within an hour drive (NBS, 2012).

The main rainy season is from March to May, with a second period from November to December, although occasional rain occurs at all times of the year. Average rainfall is 1200 to 2100 mm per year. There is year round grassland vegetation or subsistence agriculture throughout the study area. According to meteorological statistics the average temperature for the region is about 28^0C. Majority of the people are either subsistence farmers who cultivate rice, maize and cassava, or fish from the sea or the Ruvu River and its tributaries. Agriculture employs 76% of the population (NBS, 2012).

The survey was conducted in the western rural area including hamlets within the villages of Kiwangwa, Msata, Mkange and Magomeni. The settlements are located about 20 to 60 km from Bagamoyo town. The inhabitants of the villages are mainly smallholder farmers engaged in food crop production such as pineapples, cassava, maize, vegetables and in fishing and salt mining. The prevalence of malaria within

the western study area is still high compared to Bagamoyo town with seasonal variations secondary to malaria interventions through research and Tanzania National Malaria Control program (NMCP) (Williams et al., CSS report-ihi, 2013). Researches on malaria drugs and vaccine have been conducted in Bagamoyo town since 2005 through IHI and its collaborative partners. The rural water supply is mostly from dams and ponds (Kusiluka et al., 2004) highly contaminated with fecal coliform bacteria (Kusiluka et al., 2005). Eighty four percent of the communities have soil based latrines (Kusiluka et al., 2004). The latter resemble to simple pit latrines but without floor nor hygiene cover slab, nor lid covering the hole.

Study design

The study is part of the IDEA project, an African-European Research initiative, funded by European community, with the aim of dissecting the immunological interplay between poverty related diseases (malaria, TB and HIV) and helminth infections (Knopp et al., 2014). The present community cross-sectional survey was conducted at the start of the IDEA malaria project to provide baseline data to inform further prospective immune-epidemiological studies of malaria infected individuals.

Participant recruitment and sample collection

Study population included a random sample of healthy children as regarded by their parents/guardians, aged 2 months to 9 years inclusively, whose parents/guardians where informed about the study through Village Health Care Workers (VHCW) and agreed to come for screening at the meeting points. The villages were purposely selected based on the environmental conditions favouring both malaria and helminth survival and transmission. In the malaria arm, a sample size of 100 children with asymptomatic *Plasmodium* parasitemia was required. The malaria prevalence being 10% within the study area (Tanzania HIV/AIDS and Malaria Indicator Survey, 2012), we enrolled about ~1000 children from the community survey (Salim et al., 2014). Standardized questionnaires were used to collect information on demographics, vital and clinical signs and symptoms to ensure that they were free of common diseases at that point in time. Parents or guardians where asked about interventions implemented within the Tanzanian National program, namely the use of long-lasting impregnated bednets (LLINs) and prior anti-helminth treatment. Participant recruitment and data collection was done between July 2011 and November 2012 covering an entire year and thus including seasonal variation (Salim et al., 2014).

All children had a finger prick to obtain about 1ml of blood which was collected in an Ethyl Diamine Tetra acetic Acid (EDTA) tube for malaria slide and full blood count which were performed at the main BRTC laboratory. Malaria rapid diagnostic test (SD BIOLINE, SD standard diagnostics, inc.Korea) and hemoglobin level using HemoCue hemoglobinometer (EKF diagnostic GmbH, Germany) were done in the field for inclusion/exclusion criteria and immediate management of the children with malaria and severe anemia.

Additionally, each participant was provided with i) two clean containers (100mls) for stool and urine samples ii) a plastic pocket with an adhesive tape (50 x 20mm) and a glass slide. All labelled with participant identification number. Parents/guardians were instructed on how to apply the adhesive tape and advised to collect sufficient amount of fresh stool and urine. The filled containers and adhesive tape slide were collected by the VHCW at a predefined meeting point in the village centre, the next day

before noon and submitted to the Helminth Unit (HU) of the BRTC where all stool and urine samples were examined by experienced technicians.

Diagnosis of *Plasmodium* infection

Thick and thin blood films were prepared, air dried and Giemsa stained for detection and quantification of malaria parasites according to the IHI laboratory Standard Operating Procedures (SOP). To detect malaria parasites, 200 fields were examined. Parasite density expressed per µl of blood was calculated by multiplying a factor of 40 to the number of parasites counted, assuming 8,000 leucocytes per µl of blood (WHO, 2009a). All slides were read by two independent qualified technicians. In case of discrepancy between two readers, a third reader was requested. The final result was the geometric mean of the two geometrically closest readings out of the three. For cases of positive/negative discrepancy the majority decision was adopted. If the test results were positive, the final one was then taken as the geometrical mean of the two positive results.

Diagnosis of helminth infection

Duplicate Kato-Katz thick smear slides using a 41.7 mg template, adhesive tape slides, Baermann and FLOTAC methods were used to diagnose intestinal helminth (Knopp et al., 2014). Microhaematuria was examined using a dipstick (Hemastix; Siemens Healthcare Diagnostics, Eschborn, Germany) and for *S. haematobium* eggs by urine filtration (hydrophilic polycarbonate membrane filter; pore size 20 micron, diameter 13mm; Sterlitech, Kent, WA, United States of America). Binax NOW Filariasis rapid immunochromatic test (ICT) card (inverness medical professional diagnostics; ME; United States of America) was performed at the HU to detect *W. bancrofti* antigen using whole blood All Kato-Katz thick smear, adhesive tape and urine filtration slides were stored in boxes and 10% of slides re-examined for quality control by the senior experienced personnel after 3-6 months (Knopp et al., 2014).

Data Management and Statistical Analysis

The helminth species specific results derived by each method were entered into an electronic data base using Microsoft ACCESS 2010. Double entry of the clinical and laboratory data was done using the DMSys software (FDA approved for ICH/GCP clinical trials). The two datasets were transferred into STATA format merged and cleaned. Data analysis was performed using STATA version 11.0 software (Stata Corp LP; College Station, Texas, USA). For duplicate Kato-Katz methods, the average of the two slides multiplied by a factor 24 was done to obtain egg per gram of stool (EPG). *S. stercoralis* and *E. vermicularis* intensity expressed as larvae counts and number of eggs counted respectively. Helminth infection intensity was categorized according to WHO criteria (WHO, 2011b).

To investigate the relationship between *Plasmodium* and helminth, only the children who had both *Plasmodium* and helminth results were included. Table 9 defines the terms used in the analysis for easiness of interpretation. Geographical information system (arcgis 10) was used to map the distribution of *Plasmodium* and helminth monoinfections/co-infections among children in the coastal region of Bagamoyo. Harmonization of the identified hamlets within the studied villages was achieved using the known registered villages and hamlets/streets names from the Tanzania shape file

(http://openmicrodata.wordpress.com/2010/12/16/tanzania-shapefiles-for-eas-villages-districts-and-regions/). Hamlets with low numbers (less than 10) were systematically merged to the nearest hamlet within the same village. Baseline characteristics were presented within age groups (children less than 3 years, preschool children aged 3-5 years and school-aged children from 6-9 years). The categorization was chosen to explore the age dependency variability considering the ongoing malaria and mass drug administration helminth programs with different approaches based on age, mainly focusing on under five and above five years of age.

Table 9. Definitions of the Terms

Single helminth infection	Helminth monoinfection, one species only
Mixed helminth infection	More than one species of helminth
All helminth infections	Single, mixed helminth infection and co-infection
Plasmodium monoinfection*	*Plasmodium* species infection only
All *Plasmodium* infections	*Plasmodium* monoinfection and co-infection
Co-infection	*Plasmodium* and helminth co-infection
	Plasmodium and single helminth
	Plasmodium and Mixed helminth

*The term single *Plasmodium* was not used because only *P. falciparum* species is considered as the main cause of infection during this analysis (RBM, 2012)

Crude odds ratios (ORs) including 95% confidence interval and p-values were calculated for variables potentially associated with infections/co-infections. To investigate risk factors, different models were explored with all *Plasmodium, Plasmodium* monoinfection*,* helminth mono- and mixed infections, and *Plasmodium* + helminth co-infections. The association between helminth species and *Plasmodium* infection was explored to further study co-infection patterns. Variables that were associated in bivariate analysis with a p-value level of < 0.05 were considered in multiple logistic regression models. The association between *Plasmodium* and helminth co-infections was subsequently investigated using negative binomial regression estimation after comparing the conditional means and variances of variables, all variances greater than means signifying over dispersion of data. To further investigate the age dependency relationship, Mantel – Haenszel stratified odds ratios (ORs) were conducted.

A more detailed description and analysis of the helminth distribution is provided in the published paper (Salim et al., 2014).The present one focuses on the relationship of soil transmitted helminth (STH) and *Plasmodium* as prevalence of other helminth like *W. bancrofti* and *S. hematobium* were low among the studied children.

Results

Baseline characteristics of the study participants

A total of 1033 children were recruited. Of these, 41 (3.9%) did not submit stool samples which left 992 children as study analysis population (Figure 15). Demographic characteristics and intervention coverage are described in Table 10. Overall median age was 4.7 years with 25th of 2.3 and 75th quartiles of 6.5; 459 (46%) were represented by children above five years of age. Among children aged five years and above

185 (40.3%) were not schooling at the time of the survey. 494 (49.8%) were males. Eight hundred and twenty (82.7%) of the study population slept under a long lasting insecticide impregnated net (LLIN) the night before the survey. The parents/guardians reported use of albendazole and mebendazole past six months in 411 (41.4%) and 188 (18.9%) of their children respectively.

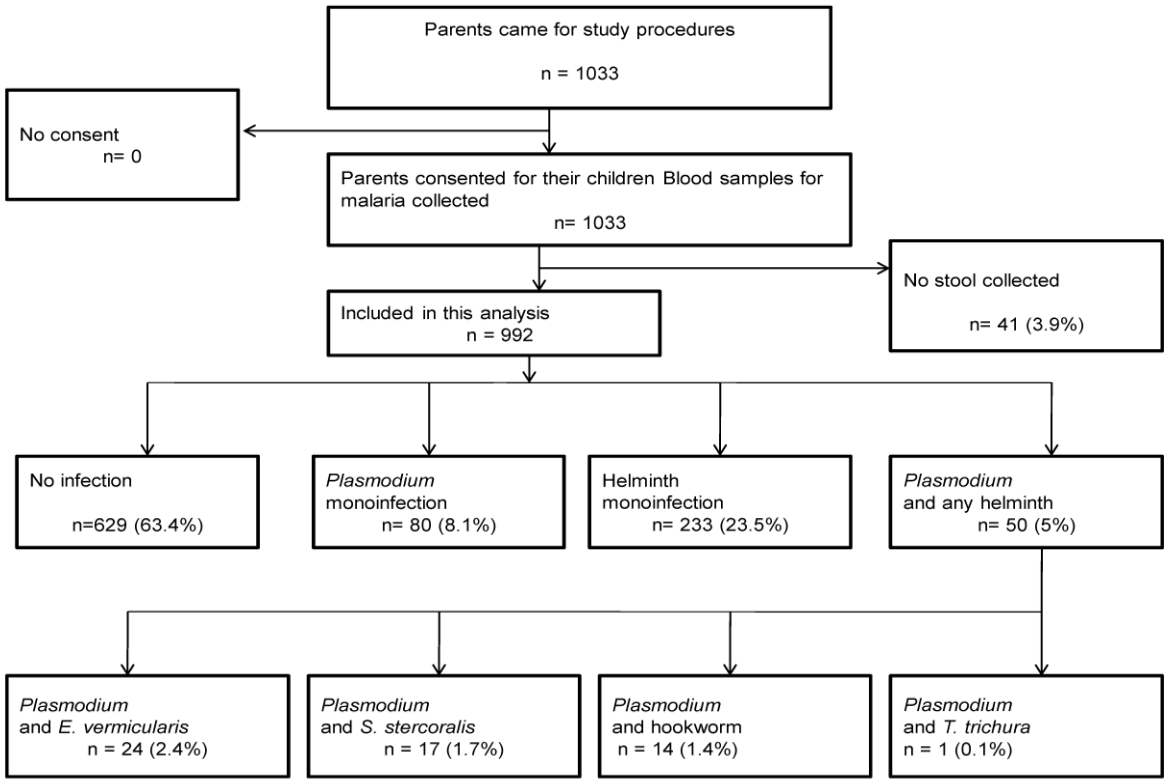

Figure 15. Flow of study participants and prevalence of *Plasmodium* and heminth infections

Distribution of infections

Prevalence of *Plasmodium*, helminth and co-infections are shown in Figure 15 and Table 11. Out of the 992 children included in the analysis, 130 (13.1%) were infected with *Plasmodium* species, 283 (28.5%) had helminth infection and 50 (5%) harbored both infections (co-infected). The prevalence of *Plasmodium* and helminth monoinfection were 8.1% (80/992) and 23.5% (233/992) respectively. *E. vermicularis* was the most prevalent single helminth infection 116 (11.7%) followed by hookworm 60 (6.1%) and *S. stercoralis* 42 (4.2%).

Table 10. Demographic characteristics and intervention coverage of study participants by age group

Characteristics	< 3 years (n = 297)	3 - 5 years (n = 236)	> 5 years (n = 459)	Total (%) N = 992
Median age (25^{th} – 75^{th} Quartile)	1.5 (0.8 - 2.2)	4.1 (3.7 - 4.5)	6.7 (5.8 - 7.7)	4.7 (2.3 – 6.5)
Gender				
Male	162 (54.5)	118 (50.0)	214 (46.6)	494 (49.8)
Female	135 (45.5)	118 (50.0)	245 (53.4)	498 (50.2)
Education level				
Too young	290 (97.7)	181 (76.7)	61 (13.3)	532 (53.6)
Preschool	0 (0.0)	25 (10.6)	112 (24.4)	137 (13.8)
Primary school	0 (0.0)	0 (0.0)	145 (31.6)	147 (14.8)
Age to go but doesn't go	0 (0.0)	21(8.9)	124 (27.0)	145 (14.6)
Missed information	7 (2.3)	9 (3.8)	17 (3.7)	31 (3.1)
Bednet information **				
Reported to have a bednet	261 (87.9)	197 (83.5)	383 (83.4)	841 (84.8)
Slept under a bednet last night	261 (87.9)	196 (83.1)	373 (81.3)	830 (83.7)
used treated bednet (LLIN)	259 (87.2)	191 (80.9)	370 (80.6)	820 (82.7)
Bednet with holes	69 (23.2)	54 (22.9)	84 (18.3)	207 (20.9)
Reported dewormed past 6 months **				
Albendazole	90 (30.3)	116 (49.2)	205 (44.7)	411 (41.4)
Mebendazole	43 (14.5)	42 (17.8)	103 (22.4)	188 (18.9)
Don't know	2 (0.7)	24 (10.2)	29 (6.3)	55 (5.5)

Note: Data are number (%) of participants or infection, unless otherwise indicated.
LLIN=Long Lasting Insecticide impregnated Net. **The column total doesn't add up to the specified total age group as the information were collected dependently.

The prevalence of *Plasmodium*, STH and co-infections increased with age, older children were mostly affected (Figure 16 & Figure 17A and Table 11). This was especially true for the most prevalent infections, namely *E. vermicuralis* and hookworm infections, co-infected or not with *Plasmodium* (Figure 17B and C). The only exception was the prevalence of *S. stercoralis* monoinfection which was slightly higher in children below five years of age (Table 11 and Figure 17D). Co-infection with *Plasmodium* and *S. hematobium* was found in two children (0.2%) and co-infection with *Plasmodium* and *T. trichura* in only one child (0.1%), all above five years of age. Two children (0.2%) had positive ICT for *W. bancrofti* infection.

Figure 18 shows administrative map of Tanzania locating Bagamoyo district within coastal region and the spatial distribution of monoinfection/co-infections prevalence in the four villages studied namely Kiwangwa, Mkange, Msata and Magomeni. Spatial heterogeneity of infection prevalence was observed between and within villages. Figure 19 shows the distribution of monoinfection and co-infections among the hamlets of the four studied villages. There were significantly different prevalences of helminth ranging from 44.7% in Mkange to 26.3% in Kiwangwa. The prevalence of *Plasmodium* infection ranged from 15.4% in Kiwangwa to zero in Magomeni village, with co-infection prevalence being higher in the hamlet of Kiwangwa Kiwangwa, Kiwangwa Msinune ($p = 0.028$), Kiwangwa Bago and Mkange Matipwili (Table 12 and Figure 18 and Figure 19).

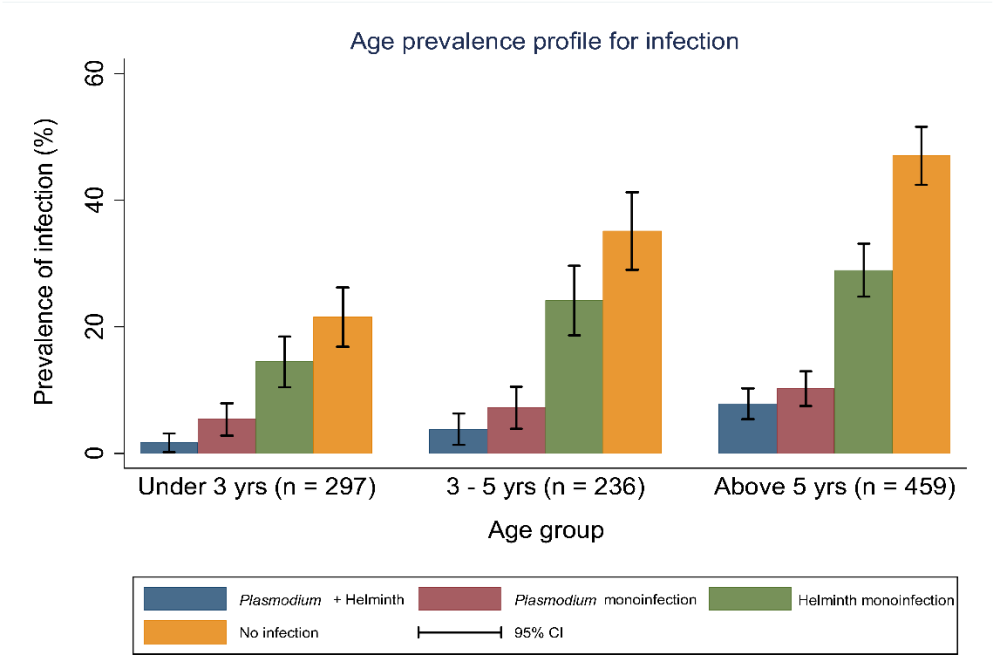

Figure 16. Age prevalence profile for infection (*Plasmodium* and helminth monoinfections and co-infections) within each age group

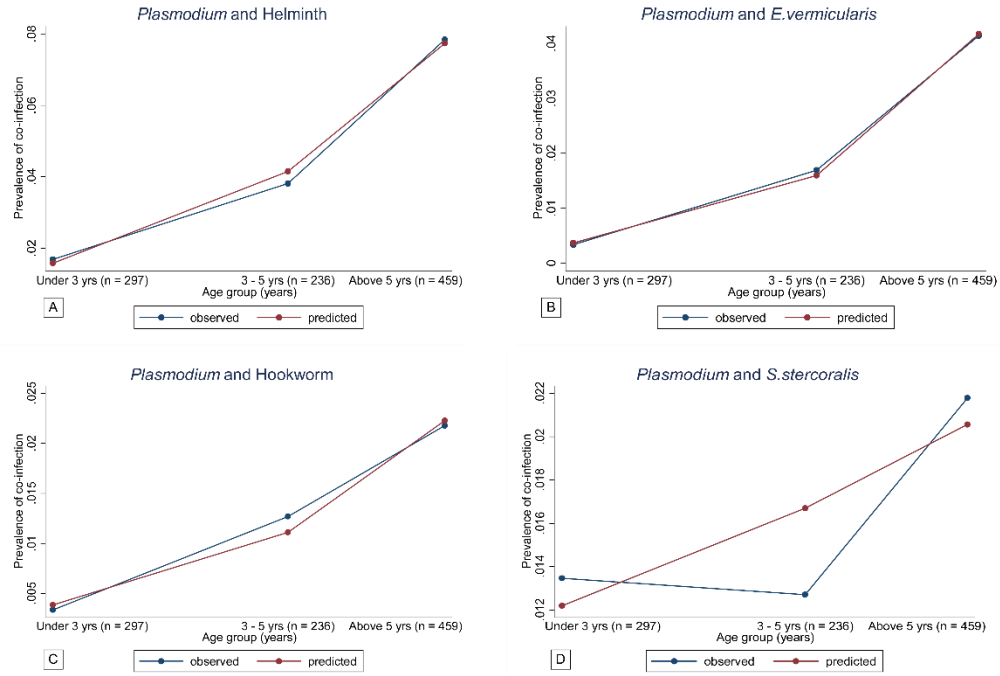

Figure 17. A – D: Age prevalence profile of co-infection as predicted from a logistic regression model (Predicted Vs Observed prevalence) Figure 17A shows *Plasmodium* and helminth co-infection; 17B *Plasmodium* and *E. vermicularis* co-infection; 17C *Plasmodium* and hookworm co-infection; 17D *Plasmodium* and *S. stercoralis* co-infection

Relationship between *Plasmodium* and STH infections and predictors of co-infections

There was a pattern for *Plasmodium* to be associated with helminth infection [OR= 1.7, 95% CI (1.1 – 2.5)], which was marked for *S. stercoralis* monoinfection [OR= 2.5, 95% CI (1.2 – 5.2), p = 0.0146] as shown in Table 12. The effect was statistically significant in a multivariate negative binomial regression model when other factors were considered [IRR= 2.0, 95% CI (1.0 – 4.00), p = 0.034] as shown in Table 13.

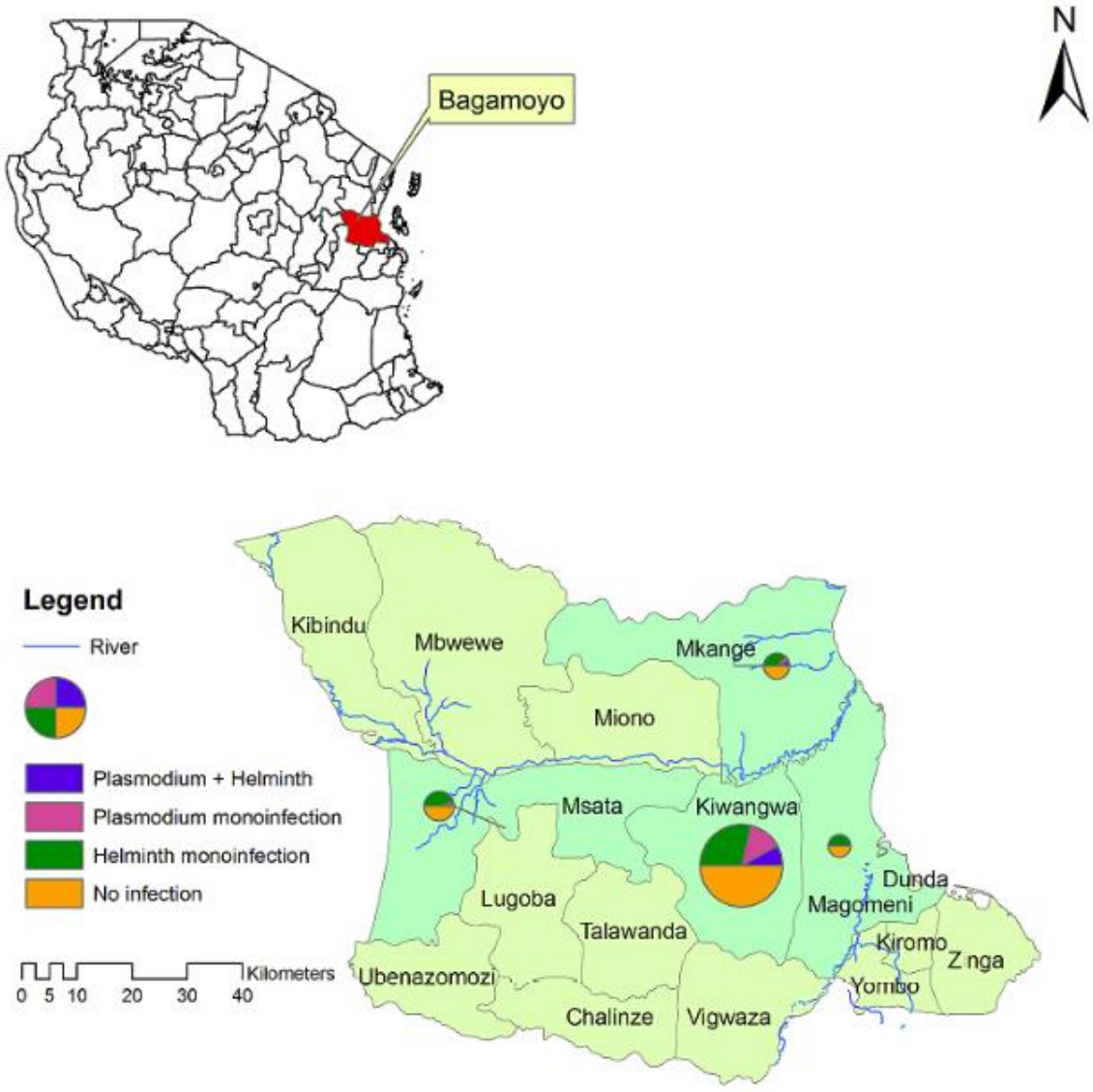

Figure 18. Admistrative map of Bagamoyo district, coastal region of Tanzania and the spatial distribution of monoinfection and co-infections within four villages namely Magomeni, Kiwangwa, Msata and Mkange.

The size of the pie is proportional to the sample size contributed by each village/hamlet.

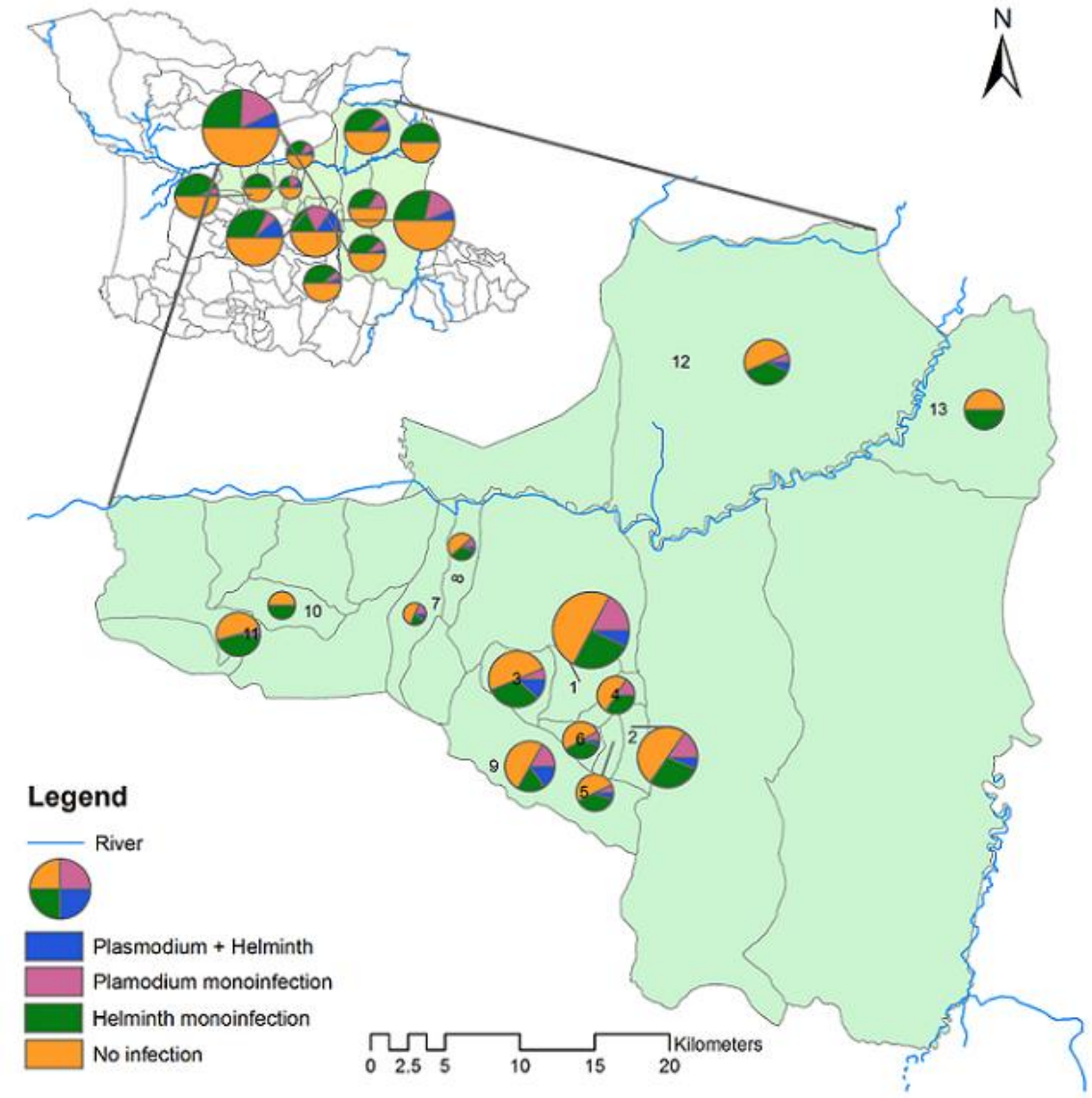

Figure 19. Spatial distribution of monoinfection and co-infection status among hamlets of the four villages within Bagamoyo, coastal region of Tanzania.

1= Kiwangwa kiwangwa 2= Kiwangwa Mwavi 3= Kiwangwa Bago 4= Kiwangwa Kibaoni 5= Kiwangwa Kwambwela 6= Kiwangwa Pipani 7= Kiwangwa Masuguru 8= Kiwangwa Mwetemo 9= Kiwangwa Msinune 10= Msata Kihangaiko 11= Msata Msata 12= Mkange Matipwili 13= Magomeni (Makurunge – Kitame). The size of the pie is proportional to the sample size contributed by each village/hamlet.

Table 11. Prevalence of *Plasmodium* and helminth infections of study participants by age group

Characteristics	< 3 years (n = 297)	3 - 5 years (n = 236)	> 5 years (n = 459)	Total (%) N = 992
All *Plasmodium* infection				
Plasmodium (+ve)	21 (7.1)	26 (11.0)	83 (18.1)	130 (13.1)
Plasmodium (-ve)	276 (92.9)	210 (89.0)	376 (81.9)	862 (86.9)
Geometric mean parasite count	1993	1896	979	1227
(25^{th} - 75^{th} Quartile)	(1200 – 6740)	(1260 – 2680)	(480 – 1600)	(560 – 2200)
Plasmodium monoinfection	16 (5.4)	17 (7.2)	47 (10.2)	80 (8.1)
All helminth infection				
Helminth (+ve)	48 (16.2)	66 (28.0)	169 (36.8)	283 (28.5)
Helminth (-ve)	249 (83.8)	170 (72.0)	290 (63.2)	709 (71.5)
Single helminth infection				
All single infection	41 (13.8)	52 (22.0)	140 (30.5)	233 (23.5)
E. vermicularis	17 (5.7)	25 (10.6)	74 (16.1)	116 (11.7)
Hookworm	8 (2.7)	14 (5.9)	38 (8.3)	60 (6.1)
S. stercoralis	13 (4.4)	11 (4.7)	18 (3.9)	42 (4.2)
T. trichura	2 (0.7)	2 (0.8)	7 (1.5)	11 (1.1)
S. haematobium	0 (0.0)	0 (0.0)	2 (0.4)	2 (0.2)
W. bancrofti	1 (0.3)	0 (0.0)	1 (0.2)	2 (0.2)
Mixed helminth infection				
Double helminth species	7 (2.4)	12 (5.1)	24 (5.2)	43 (4.3)
> 2 helminth species	0 (0.0)	2 (0.8)	5 (1.1)	7 (0.7)
***Plasmodium* and helminth co-infection**				
All *Plasmodium* + helminth co-infection	5 (1.7)	9 (3.8)	36 (7.8)	50 (5.0)***
Plasmodium + *E. vermicularis*	1 (0.3)	4 (1.7)	19 (4.1)	24 (2.4)
Plasmodium + hookworm	1 (0.3)	3 (1.3)	10 (2.2)	14 (1.4)
Plasmodium + *S. stercoralis*	4 (1.4)	3 (1.3)	10 (2.2)	17 (1.7)
Plasmodium + *T. trichura*	0 (0.0)	0 (0.0)	1 (0.2)	1 (0.1)
Plasmodium + *S. hematobium*	0 (0.0)	0 (0.0)	2 (0.4)	2 (0.2)

*** The total below is more than 5.0% as some specific *Plasmodium* helminth co-infection have more than one helminth species.

The risk of *Plasmodium*, STH and *Plasmodium* STH co-infections increased with age, with older children (>5 years) being more affected compared to younger children (<3 years and 3-5 years). The differences were statistically significant by bivariate and multivariate negative binomial regression analysis (Table 12 and Table 13). Overall there was an age pattern for *Plasmodium* to be associated with STH [multivariate negative binomial regression [IRR= 2.9, 95% CI (1.7 - 5.1)], which was more marked for *S. stercoralis* [IRR= 3.9, 95% CI (1.2 - 13.1)], and especially in young children. Mantel – Haenszel stratified ORs (Table 14) indicated that the risk of *Plasmodium* infection with *S. stercoralis* was higher among younger children aged below 3 years (stratum specific OR= 9.2, 95% CI (0.8 – 105.5) when exploring for confounding effect of age groups (M-H adjusted OR= 2.2, 95% CI (1.1 – 4.3), p = 0.0266; homogeneity of ORs, p = 0.3774). Compared to the results with other type of STH, *S. stercoralis* infection showed increased risk of *Plasmodium* among younger age group but the homogeneity test suggests no difference in the odds between age groups.

Table 12. Variables associated with *Plasmodium*, STH and *Plasmodium* + STH co-infection using bivariate analysis

	***Plasmodium* infection**		**STH infection**		***Plasmodium* + STH co-infection**	
Risk factors	OR (95% CI)	p- value	OR (95% CI)	p-value	OR	p-value
Gender (Female (Ref))						
Male sex	0.9 (0.6 - 1.2)	0.423	0.9 (0.7 - 1.2)	0.476	0.9 (0.6 - 1.7)	0.9767
Age						
Age in years	1.2 (1.1 - 1.3)	< 0.001	1.2 (1.1 - 1.3)	<0.001	1.2 (1.1 -1.4)	0.0001
Age group (<3 years (Ref))						
3-5 years	1.6 (0.9 - 3.0)	0.113	2.0 (1.3 - 3.1)	0.001	2.3 (0.8 - 7.0)	0.137
> 5years	2.9 (1.7 - 4.8)	< 0.001	3.0 (2.1 - 4.3)	<0.001	5.0 (1.9 -12.8)	0.001
Education level (Too young (Ref))						
Preschool	0.9 (0.5 - 1.8)	0.868	1.6 (1.1 - 2.4)	0.024	1.4 (0.6 - 3.5)	0.409
Primary	2.0 (1.2- 3.4)	0.006	1.8 (1.2 - 2.6)	0.004	2.0 (0.9 - 4.3)	0.092
Age to go but doesn't	3.0 (1.9 - 4.8)	< 0.001	1.8 (1.2 - 2.6)	0.005	2.9 (1.4 - 5.9)	0.004
Villages (Hamlets), (Kiwangwa Kiwangwa (Ref))						
Kiwangwa Mwavi	1.0 (0.5 - 1.8)	0.990	1.3 (0.8 - 2.1)	0.358	1.0 (0.3 - 2.7)	0.959
Kiwangwa Bago	0.9 (0.5 - 1.7)	0.770	2.1 (1.3 - 3.4)	0.004	2.1 (0.9 - 5.1)	0.094
Kiwangwa Kibaoni	0.5 (0.2 - 1.5)	0.248	1.0 (0.5 - 2.0)	0.931	Omitted	-
Kiwangwa Kwambwela	0.6 (0.2 - 1.6)	0.285	2.2 (1.1 - 4.2)	0.019	0.8 (0.2 - 4.0)	0.826
Kiwangwa Pipani	0.3 (0.1 – 1.2)	0.085	0.9 (0.4 – 2.0)	0.839	0.4 (0.1 – 3.4)	0.414
Kiwangwa (Mwetemo + Masuguru)	1.3 (0.6 - 2.9)	0.526	1.6 (0.8 - 3.3)	0.183	1.4 (0.4 - 5.4)	0.593
Kiwangwa Msinune	1.7 (0.9 – 3.1)	0.070	1.3 (0.7 – 2.2)	0.413	2.7 (1.1 – 6.7)	0.028
Msata (Msata + Kihangaiko)	0.2 (0.1 - 0.6)	0.004	1.4 (0.8 - 2.4)	0.244	0.2 (0.0 - 1.7)	0.140
Mkange Matipwili	0.8 (0.4 - 1.7)	0.589	3.0 (1.7 - 5.2)	< 0.001	1.4 (0.5 - 4.4)	0.510
Magomeni (Makurunge – Kitame)	Omitted	-	2.3 (1.2 - 4.3)	0.008	Omitted	-
Did not Slept under bednet last night (Ref)						
Slept under bednet last night	0.5 (0.3 - 0.9)	0.0193	0.7 (0.4 - 1.2)	0.1951	1.2 (0.4 - 4.1)	0.7206
Did not Use antihelminth past 6 months (Ref)						
Albendazole	1.0 (0.7 - 1.5)	0.8397	1.5 (1.1 - 2.0)	0.004	1.7 (0.9 - 3.0)	0.0679
Mebendazole	0.9 (0.5 - 1.4)	0.6066	1.3 (0.9 - 1.8)	0.1617	0.7 (0.3 - 1.5)	0.3444
Helminth negative (Ref)						
Any helminth	1.7 (1.1 – 2.5)	0.0072				
E. vermicularis monoinfection	0.8 (0.4 – 1.5)	0.4300				
Hookworm monoinfection	0.8 (0.4 – 1.7)	0.5423				
S. stercoralis monoifection	2.5 (1.2 – 5.2)	0.0146				
T. trichuris monoinfection	-	0.1177				
***Plasmodium* density**			1.0 (0.9 - 1.0)	0.6971	1.0 (1.0 - 1.0)	<0.001
Low density parasitemia			0.5 (0.1 - 2.2)	0.3844	3.1 (0.6 - 16.0)	0.147

Note: Density of parasitemia was defined as: Low density parasite <5000/µL and high density ≥5000/µL. CI, confidence interval; OR, odds ratio

In addition, children who were not attending school although they should have according to their age had increased risk of *Plasmodium* and co-infections by bivariate analysis although the statistical association was lost in a multivariate analysis. Male and female children were equally affected (Table 12).

Table 13. Association between *Plasmodium* and STH infection by multivariate analysis (Negative binomial regression)

	All helminth[a]	*E. vermicularis*[b]	Hookworm[c]	*S. stercoralis*[d]	*T. trichura*[e]
Independent variables	Adjusted IRR (95% CI)	Adjusted IRR (95% CI)	Adjusted IRR (95% CI)	Adjusted IRR (95% CI)	Adjusted IRR (95% CI)
Age group[f]	1.3 (0.9 - 1.9)	1.0 (0.5 - 1.8)	0.6 (0.3 - 1.2)	1.8 (1.0 - 3.4)*	0.3 (0.0 - 1.9)
< 3 years (Ref)					
3 - 5 years	1.8 (0.9 - 3.4)	2.3 (0.6 - 8.8)	2.4 (0.6 - 9.2)	2.5 (0.6 - 9.5)	2.5 (0.6 - 9.3)
> 5 years	3.2 (1.6 - 6.6)*	4.3 (1.1 - 16.9)*	4.5 (1.1 - 17.9) *	4.7 (1.2 - 18.8)*	4.5 (1.0 - 17.9)*
Education level [g]					
Too young (Ref)					
Preschool	0.4 (0.2 – 0.9)*	0.5 (0.2 – 1.6)	0.5 (0.2 – 1.5)	0.5 (0.2 – 1.5)	0.5 (0.2 - 1.5)
Primary	0.9 (0.5 – 1.7)	0.6 (0.2 – 1.7)	0.6 (0.2 – 1.6)	0.6 (0.2 – 1.7)	0.6 (0.2 - 1.7)
Age to go but doesn't	1.3 (0.7 -2.3)	1.1 (0.4 – 2.9)	1.1 (0.4 – 2.8)	1.2 (0.4 – 3.1)	1.0 (0.4 - 2.8)
Village (Hamlets) [h]	0.9 (0.9 – 1.0)	0.9 (0.9 – 1.0)	0.9 (0.9 – 1.0)	1.0 (0.9 – 1.0)	1.0 (0.9 – 1.1)
Slept under bednet last night [i]	0.7 (0.4 - 1.3)	2.1 (0.6 - 7.0)	1.9 (0.6 - 6.5)	2.1 (0.6 - 6.8)	2.0 (0.6 - 6.7)
Use of antihelminth past 6 months [j]					
Albendazole	1.0 (0.8 - 1.3)	1.0 (0.5 - 2.0)	1.0 (0.5 - 2.0)	1.0 (0.5 - 2.0)	0.9 (0.5 - 1.8)
Mebendazole	0.8 (0.6 - 1.1)	0.5 (0.2 - 1.3)	0.4 (0.2 - 1.2)	0.5 (0.2 - 1.3)	0.5 (0.2 - 1.2)

IRR is the incidence rate ratio

All the models a, b, c, d and e were adjusted for f, g, h, i and j.

[a]Reference group was helminth negative

[b, c, d, e] Reference group were other worm positive species; Variable village (Hamlets) – Report on hamlets categorization was removed as were insignificant when the model was run.

*p values were significant

The geometric mean *Plasmodium* parasite count decreased with age as shown in Table 11. Most of the studied children had low intensity helminth infections. Moderate and heavy STH infections intensity were noted among older children. Generally, there was no significant correlation between *Plasmodium* and STH densities. There was a trend for a negative correlation between *Plasmodium* parasite density and *S. stercoralis* larvae count (r = -0.0786, p = 0.8082) and a positive correlation with a hookworm (r = 0.2123, p = 0.5560) and *E. vermicularis* (r = 0.0418, p = 0.7095) infections.

Table 14. Association between *Plasmodium* and STH infection by Mantel-Haenszel analysis using age group as justification

	All helminth	*E. Vermicularis*	Hookworm	*S. stercoralis*	*T. trichura*
Age group (years)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Under 3 years	1.7 (0.6 - 4.9)	0.3 (0.0 - 3.2)	0.5 (0.0 - 5.2)	9.2 (0.8 - 105.5)	0.0
	p = 0.3241	p = 0.3042	p = 0.5704	p = 0.0293	p = 0.6259
3 - 5 years	1.4 (0.6 - 3.4)	0.8 (0.2 - 3.2)	0.9 (0.2 - 3.8)	1.5 (0.3 - 7.1)	0.0
	p = 0.4242	p = 0.7219	p = 0.8401	p = 0.5789	p = 0.2698
Above 5 years	1.4 (0.9 - 2.3)	0.9 (0.4 - 1.8)	0.7 (0.3 - 1.5)	1.8 (0.8 - 4.4)	0.3 (0.0 - 2.1)
	p = 0.1718	p = 0.6995	p = 0.3129	p = 0.1604	p = 0.178
Mixed helminth infection					
Crude OR (95% CI)	1.7 (1.1 - 2.5)	0.8 (0.4 - 1.5)	0.7 (0.3 - 1.3)	1.9 (1.0 - 3.7)#	0.2 (0.0 - 1.5)
	p = 0.0072	p = 0.5392	p = 0.2784	p = 0.0588	p = 0.0811
Helminth monoinfection					
Crude OR (95% CI)	-	0.8 (0.4 - 1.5)	0.8 (0.4 - 1.7)	2.5 (1.2 - 5.2)#	0.0
	-	p = 0.43	p = 0.5423	p = 0.0146	p = 0.1177
M-H adjusted for age group[a]					
OR (95% CI)	1.4 (1.0 - 2.1)	0.8 (0.4 - 1.4)	0.7 (0.3 - 1.3)	2.2 (1.1 - 4.3)	0.2 (0.0 - 1.5)
	p = 0.0684	p = 0.4125	p = 0.2641	p = 0.0266	p = 0.0739
Homogeneity of ORs[b]	p = 0.9491	p = 0.7055	p = 0.9272	p = 0.3774	p = 0.8257

# Denotes crude ORs. To assess for confounding, crude and adjusted ORs are compared in terms of the difference in relation to magnitude

[a] The test assess whether the exposure is significant after adjusting for the age groups

[b] The test compares whether there is significant difference between age group specific ORs (homogeneity of the stratum ORs), hence whether the overall adjusted OR is valid.

Discussion

The current study provides baseline epidemiological data of *Plasmodium* and STH infections in the whole population of children, including the young ones who are rarely surveyed for the latter. To increase sensitivity, different diagnostic methods for helminth infection were used including adhesive tape slides for *E. vermicularis* and Baermann technique for *S. stercoralis*. This enabled us to have full range of helminth pattern within the area where malaria transmission occurs throughout the year and National malaria and helminth control programs are undertaken.

Results show that infection with *Plasmodium*, STH and co-infections are common among children aged below five and above five years living in Bagamoyo district, Tanzania. Despite high coverage of LLINs (above 80%), pockets of *Plasmodium* infection remain in west side areas like Kiwangwa compared to Magomeni village which is closer to Bagamoyo town where malaria prevalence was documented to be low (Williams et al., CSS report-ihi, 2013). This spatial variation could be explained by behavioral factors such as outdoor activities, coverage and effective use of LLINs in the Magomeni village and easy access to health care and hence effective treatment. The geometric mean *Plasmodium* parasite count

decreased with advancing age as expected in most of the malaria endemic countries in relation to development of antimalarial specific immunity (Doolan et al., 2009, Woolhouse, 1998) but the malaria infection prevalence increased with age. These findings may be an indication of a shift of *Plasmodium* infection towards older age group as observed in Muheza district, Tanzania (Winskill et al., 2011) and other parts of Africa (O'Meara et al., 2008, Carneiro et al., 2010, Mawili-Mboumba et al., 2013) where malaria transmission tends to decline and acquisition of immunity is thus delayed. Prevention strategies need thus to take into account the older age group too in the momentum of malaria elimination (Nankabirwa et al., 2014).

The association of STH and *Plasmodium* infections highlights the extent of the burden of parasites in older age groups. Our results show a definite increase of parasite prevalence, especially STH, with age, as do most of the previous studies (Kinung'hi et al., 2014, Mboera et al., 2011, Brooker et al., 2007, Becker et al., 2011, Mazigo et al., 2010). The prevalence and pattern of co-infections observed in Bagamoyo differ from those reported in Magu (Kinung'hi et al., 2014) and Mvomero districts (Mboera et al., 2011), Tanzania. Our results show lower prevalence of helminth and *Plasmodium* helminth co-infections with predominance of *E. vermicularis*, hookworm and *S. stercoralis*. The method of detection might be the reason for these differences. Indeed, we used adhesive tape slides for *E. vermicularis* and Baermann technique for *S. stercoralis* together with Kato-Katz technique, which detected these specific worms, mostly missed in other surveys. Factors such as exposure and intervention coverage may also explain the types of helminth infection isolated and high prevalence in Magu and Mvomero districts. In Tanzania, published reports on mass drug administration (MDA), mostly under National Lymphatic Filariasis Elimination Program (NLFEP), have shown coverage to fluctuate (Parker and Allen, 2013) and its effectiveness to vary depending on the chemoprophylaxis used and duration between cycles (Simonsen et al., 2013, Simonsen et al., 2010). The uptake variations could results into persistence of parasites in certain areas and subgroups. In this study, an increased risk of STH, *Plasmodium* and co-infections was observed among the school-aged children who were not schooling. These may have missed the opportunity to be dewormed, either at school level or within the under-fives program. These children may also be exposed to other risk factors in the environment, or behave differently in terms of sanitation and hygiene. The latter have not been assessed in the present study, which represents a definite limitation. Such a burden in this age group is not only deleterious for them but also acts as a reservoir of infection within the population (Campbell et al., 2014).

Co-infection patterns increased with age as predicted from the age specific prevalence rates by the simple probability model except for *S. stercoralis* co-infection. The exposure of *S. stercoralis and Plasmodium* co-infections was significantly different from the other species of helminth. The results show that children with *S. stercoralis* had twice the risk of *Plasmodium* infection, with even higher odds among children below 3 years of age, as compared to those with other species of STH. This observation requires further exploration as reports on the age profile for *S. stercoralis* infection are rare and still conflicting (Steinmann et al., 2007, Egido et al., 2001, Lindo et al., 1995, Glinz et al., 2010a). A study done in Côte d'Ivoire showed that hookworm and *S. stercoralis* had an almost parallel shape of age prevalence pattern (Becker et al., 2011), but it did not stratify the <5 children into smaller age categories. This could be the reason for the masked trend compared to what was observed in the

present study where a negative correlation between *S. stercoralis* larvae and *Plasmodium* parasitemia was shown compared to positive correlation with hookworm and *E. vermicularis*, although not significant. The observed relation between *Plasmodium* and *S. stercoralis* infection may arise due to biological associations, whereby *S. stercoralis* being an early life and chronic infection, starts in the first years and persists through older aged groups promoting establishment and/or survival of *Plasmodium* infection potentially through Th2 lymphocytes immune modulation (Concha et al., 2005). It has been repeatedly shown that hookworm tends to exaggerate *Plasmodium* infection but knowledge on the immunomodulation with *S. stecoralis* co-infection is still scarce. Depending on the pro and anti-inflammatory responses mounted within the host, immune response could either promote or inhibit *Plasmodium* infection (Knowles, 2011, Nacher, 2011, Concha et al., 2005). The equilibrium with *S. stercoralis* could have been maintained through its intrinsic characteristic to persist for many years in asymptomatic immunocompetent host surviving through low grade autoinfection cycles (Concha et al., 2005). In this study, children were not tested for HIV infection which is among the risk factor for *S. stercoralis* hyperinfection syndrome (Keiser and Nutman, 2004, Marcos et al., 2008, Olsen et al., 2009). The immaturity and predominance of Th2 response among younger children could also explain the increased risk of *Plasmodium* co-infection with *S. stercoralis* (PrabhuDas et al., 2011).

Heterogeneity of infection prevalence within and between villages indicates that other factors apart from biological association determine the co-infection patterns. Behavioural such as outdoor activities and walking barefooted, sanitation, hygiene and socio-economic factors could explain the variations (Becker et al., 2011). Considering the type of STH species isolated and their modes of transmission, exposure factors conducive to both parasites are suspected to be main contributors of *Plasmodium* and STH co-infections within the studied population (Booth, 2006). Previous studies done within Bagamoyo district in 2004 suggested that availability of safe water is a serious problem with public health consequences (Kusiluka et al., 2004). Up to 40% of people reporting water to be not easily accessible (Kusiluka et al., 2004) and 70.8% of the water sources were contaminated with fecal coliforms (Kusiluka et al., 2005). The situation has not much changed, at least in the rural areas, just few kilometers from the Bagamoyo town. Recent studies conducted within the area showed high rates of water contamination (Mattioli et al., 2014, Mattioli et al., 2012). Both hookworm and *S. stercoralis* are transmitted via skin penetration in poorly maintained latrines and sites of promiscuous defecation. As of *E. vermicularis* direct transfer of eggs into mouth, inhalation and retroinfection are possible in areas of poor hygiene and scarcity of water (Knight, 1982, Cook, 1994). All these could contribute to high reinfection rates (Brooker et al., 2004) and persistence of chronic infection post treatment.

Overall, the results of this study demonstrate that both *Plasmodium* and STH exhibit marked age dependency in infection patterns. In main land Tanzania, control program against helminth has been implemented through expanded program of immunization (EPI) using mebendazole or albendazole among <5 years children and community based Global Program to Eliminate Lymphatic Filariasis (GPELF) using ivermectin plus albendazole among school-aged children and adult population. Under five years children have been targeted by interventions against malaria via antenatal and later postnatal programs where LLINs are distributed. Generally, school-aged children have been rather neglected and therefore not well covered by both control programs. In the current study, the heaviest load of helminth infection

was detected among children aged above five years underlying the importance of deworming program to be focused on this age group as suggested by the WHO in order to reduce morbidity and transmission of helminth (WHO, 2011b). Integrated control approaches emphasizing on health education, improvement of environmental sanitation and hygiene coupled with improved housing and access to water, chemoprophylaxis (Campbell et al., 2014, WHO, 2011b) and LLINs distributions are required considering the pattern and types of infections within the area to interrupt transmission of both STH and *Plasmodium* among both the school-aged children but also the under-fives. Frequent and effective antihelminth administrations at least twice a year with a drug like ivermectin which has shown to reduce both helminth and malaria transmission could be prioritized to reduce the burden of co-infection in school-aged children (Slater et al., 2014). Potential safety and additional impact of ivermectin to reduce malaria requires further exploration considering the risk of co-infection early in childhood with *S. stercoralis* (Slater et al., 2014). The risk of *Plasmodium* with *S. stercoralis* infection among young children requires more investigation to better understand this singular interaction.

Conclusion

The findings suggest that STH and *Plasmodium* infections tend to occur in the same children, with increasing prevalence of co-infection with age. This calls for an integrated approach such as using mass chemotherapy with dual effect (e.g. ivermectin) coupled with improved housing, sanitation and hygiene for the control of both parasitic infections.

Acknowledgments

The author's acknowledge all staff of the IDEA project and all children and their parents who agreed to participate in this study and submitted blood, stool and urine samples to achieve and accomplish what we have in the study. We are particularly obliged to Raymond Singo, Shabani Halfan, Rehema Mangoli, and Tatu Nassor for their help in the field and laboratory work, the data unit of the BRTC for entering the large amount of data and providing ample support. In addition, we acknowledge the Bagamoyo district officials, specifically the district immunization and vaccination officer (DIVO) Farah Mohammed for his great collaboration.

Chapter 8: The impact of soil transmitted helminth on malaria clinical presentation and treatment outcome: A case control study among children in Bagamoyo district, coastal region of Tanzania

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Funding: This study received financial support from the European Commission (Contract/Grant agreement number: 241642) in the frame of the IDEA project "Dissecting the Immunological Interplay between Poverty Related Diseases and Helminth infections: An African-European Research Initiative". The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared no competing interests.

Working Paper

Abstract

Background: Parasitic infectious agents rarely occur in isolation. Epidemiological evidence is mostly lacking and little is known and invested on how the two common parasites *Plasmodium* and soil transmitted helminth interact. There are contradictory findings in different studies. Synergism, antagonism and neutral effect have been documented between *Plasmodium* and soil transmitted helminth. This study investigated the impact of soil transmitted helminth (STH) on clinical malaria presentation and treatment outcome.

Methods: A matched case control study with a semi longitudinal follow up according to WHO antimalarial surveillance guideline was done among children aged 2 months to 9 years inclusively living in western rural areas of Bagamoyo, coastal region of Tanzania. Cases were children with uncomplicated and severe malaria enrolled from the health facilities while controls were children with asymptomatic *Plasmodium* parasitemia enrolled from the same community.

Results: In simple conditional regression analysis there was a tendency for a protective effect of helminth on the development of clinical malaria [OR=0.6, 95% CI of 0.3 – 1.3] which was more marked for *E. vermicularis* species [OR=0.2, 95% CI of 0.0 – 0.9]. On the contrary, hookworm species tended to be associated with clinical malaria [OR= 3.0, 95% CI of 0.9 – 9.5]. In multiple conditional regression analysis, the overall protective effect was lower for all helminth infection [OR= 0.8, 95% CI of 0.3 – 1.9] but remained significantly protective for *E. vermicularis* species [OR= 0.1, 95% CI of 0.0 – 1.0] and borderline significant for hookworm species [OR= 3.6, 95% CI of 0.9 – 14.3]. Using ordinal logistic regression which better reflects the progression of asymptomatic *Plasmodium* parasitemia to severe malaria, there was a 50% protective effect with overall helminth [OR= 0.5, 95% CI of 0.3 – 0.9]. On the contrary, hookworm species was highly predictive of uncomplicated and severe malaria [OR= 7.8, 95% (CI of 1.8 – 33.9) and 49.7 (95% CI of 1.9 – 1298.9) respectively]. Generally, children infected with STH had higher geometric mean time to first clearance of parasitemia.

Conclusion: The findings of a protective effect of *E. vermicularis* and an enhancing effect of hookworms may explain the contradictory results found in the literature about impact of helminths on clinical malaria. More insight should be gained on possible mechanisms for these opposite effects. These results should not deter at this stage deworming programs but rather foster implementation of integrated control program for these two common parasites to speed up the momentum of moving from morbidity and transmission control to elimination.

Background

Parasitic infections such as *Plasmodium* and soil transmitted helminth (STH) are highly prevalent in the tropical regions and their paradoxical association has been debated in the scientific world (Druilhe et al., 2005, Mwangi et al., 2006, Nacher, 2004, Nacher, 2011, Adegnika and Kremsner, 2012, Wiria et al., 2012) . The practical implication of this co-infection is potentially huge, especially in children (Wammes et al., 2014, Webb et al., 2011). Children usually suffer from higher prevalence and heavy parasitic loads of both *Plasmodium* and STH (WHO, 2013, WHO, 2011b). Deworming program has been advocated to prevent helminth related morbidity (WHO, 2011b). The question of how the two parasitic infections interact and if deworming should continue is hard to answer due to conflicting research findings. The co-infecting parasites can interact through different mechanism including resource competition, direct interference and immune mediated response (Knowles, 2011). Immune response induced by chronic helminth infection may modify immune response to a *Plasmodia* and alter infection and disease risk and vise versa (Roussilhon et al., 2010, Knowles, 2011, Nacher, 2011, Druilhe et al., 2005). Synergism as well as antagonism have been documented between *Plasmodium* and different species of STH (Nacher, 2011, Adegnika and Kremsner, 2012).

Two reviews have been conducted on epidemiology and interaction between helminth and malaria in humans (Adegnika and Kremsner, 2012, Nacher, 2011) and validity of previous studies on association between helminth and the incidence of malaria have been critically discussed by Fernández et al (Fernández et al., 2008). In general it has been shown that helminth infection increases the risk of *Plasmodium* infection and protects against severe manifestations. Both reviews suggested that hookworms are associated with malaria incidence while *Ascaris lumbricoides* shows a protective effect (Nacher, 2011, Adegnika and Kremsner, 2012). Most of the co-infection studies have been conducted among older children and utilized Kato – Katz technique to isolate helminth eggs. A cross sectional study conducted at Alaba Kulito health centre in south Ethiopia found that STH had very little contribution to malaria severity and had no significant impact on clearance rate of *Plasmodium* infection (Degarege et al., 2009). A case control study among older children and adults (aged 5 – 60 years) in Tierralta, Colombia found a positive association of malaria and hookworm and a protective effect with *A. lumbricoides* (Fernandez-Nino et al., 2012). In Kabale district, south west Uganda, an area of low malaria transmission, results suggest no evidence of association between helminth and risk of malaria (Shapiro et al., 2005). A recent published cohort study conducted among the Entebbe Mother and Baby study (EMaBS) along the northern shore of lake Victoria where malaria is endemic showed an increased burden of childhood malaria morbidy associated with hookworm helminth infection during pregnancy (Ndibazza et al., 2013). In northwestern Tanzania, co-infection of hookworms and *P. falciparum* have been reported in school-aged children (Mazigo et al., 2010). In the island of Zanzibar, United Republic of Tanzania, early helminth infection has been documented to negatively associate with malaria among the 6 – 23 month old children (Kung'u et al., 2009).

Several factors such as population age, geographical location, type and intensity of helminth and of *Plasmodium* infection, immune status and malaria clinical state may interplay to cause effect variations. Most of the published studies were conducted in areas where the prevalence of STH is generally high (above 50%) and not much has been investigated on *Strongyloides stercoralis* and *Enterobius*

vermicularis species. To our knowledge, this is the first case-control study designed to investigate the impact of STH on malaria clinical presentation, response to treatment and outcome and first report on the effect of *E. vermicularis* and *S. stercoralis* on malaria burden. It is also the first time that the whole childhood range (2months to 9 years) is covered. The results will guide improved implementation of existing programs and stimulate a tailored approach for intergrated control programs, specifically in Tanzania.

Materials and methods

Reporting of the study follows STROBE checklist (Strengthening the Reporting of Observational studies in Epidemiology) (Vandenbroucke et al., 2007).

Ethics statement

The study was conducted under the IDEA study protocol which was approved by the institutional review boards of the Swiss Tropical and Public Health Institute (Swiss TPH; Basel, Switzerland) and the Ifakara Health Institute (IHI; Dar es Salaam, United Republic of Tanzania). The ethical approval for the conduct of the study was granted by the Ethikkomission beider Basel (EKBB; Basel, Switzerland; reference number: 257/08) and the National Institute for Medical Research of Tanzania (NIMR; Dar es Salaam, United Republic of Tanzania; reference number: NIMR/HQ/R.8a/Vol. IX/1098).

Sensitization meetings were conducted with the local district, community, school teachers and health authorities to inform about the purpose, procedures, risk and benefits associated with the study. Study related procedures were implemented once the appropriate and adequate informed consent process has taken place and the informed consent form has been signed off by the parents or his/her legally authorized representative as explained previously (Knopp et al., 2014, Salim et al., 2014).

Participants infected with helminth and/or malaria or other medical conditions received appropriate treatment/referral according to the national treatment guidelines of Tanzania. Children with STH were treated with albendazole (400mg single oral dose) and those with asymptomatic *Plasmodium* parasitemia and uncomplicated malaria received artemether lumefantrine (ALU). Children with severe malaria received quinine injections until clinically stable and able to take ALU before seven days of completing quinine treatment. In order to investigate the impact of STH on clinical presentation and treatment outcome, children diagnosed with STH received a delayed antihelminth treatment at the end of study follow up (day 42). To prevent unnecessary complication, children were closely followed up for safety and those with heavy helminth load and severe disease received treatment prior to day 42. To ensure protocol adherence, parents were explained the purpose of the study and advised not to give antihelminth treatment to their children during the course of follow up. Parents were advised to bring their children to the nearest dispensary in case of clinical emergency or call the study clinician in case they have further questions on the study procedures.

Study area

The study was conducted in the western rural area of Bagamoyo district, coastal region of Tanzania about 20 to 60 kilometres from Bagamoyo town as described previously (Salim et al., 2014). The study area is covered by a clinical surveillance system (CSS), part of the Ifakara Health Institute (IHI), Bagamoyo Research and Training Centre (BRTC) which works in close collaboration with the Bagamoyo District hospital (BDH) officials to ensure quality health care delivery using its research platforms (Mwangoka et al., 2009). In total, there are 5 health centres and 59 dispensaries within the district where all cases are assessed and treated. Severe cases are referred to the district referral hospital, BDH (Leshabari et al., 2012). Generally, the prevalence of malaria around Bagamoyo town is low as compared to the rural areas in the west (Williams et al., CSS report-ihi, 2013). Prior to the start of the study, deworming campaign took place among under five years and school aged children. Two mass long lasting insecticidal nets (LLIN) "catch-up" campaigns were implemented in Tanzania between 2009 and 2011. The first "catch-up" campaign was launched in 2009 and 8.7 million LLIN were distributed to children under five years (Bonner et al., 2011, Koenker et al., 2013). The 2010 universal coverage campaign targeted all sleeping spaces not protected through the previous catch-up campaign and the Tanzania national voucher scheme (TNVS) (Renggli et al., 2013).

Study design

The study was part of the IDEA project, an African-European Research initiative, funded by European community, with the aim of dissecting the immunological interplay between poverty related diseases (malaria, tuberculosis (TB) and Human Immunodeficiency Virus (HIV)) and helminth infections (Willyard, 2009). A case-control component with a semi longitudinal follow-up was developed within the malaria arm. Cases were recruited in two different groups, namely children with severe malaria, and cases with uncomplicated malaria. Controls were asymptomatic children *Plasmodium* parasitemia. The outcome was clinical presentation and exposure presence or absence of a co-infection with at least one of the helminth species investigated. The longitudinal short term observational part of the study consisted of the assessment of response to anti-malaria treatment in the three groups according to World Health Organization (WHO) procedure (day 0, 1, 2, 3, 7, 14, 28, 42) (WHO, 2009b)

Participant recruitment and follow up visits

Inclusion criteria were age ≥ 2months to < 10 years, written informed consent as obtained from the parents/legally accepted representative, resident and willing to stay within the study area for at least two months of the study follow up. A case of severe malaria was defined according to WHO malaria case definitions. A case of uncomplicated malaria was defined as temperature ≥37.5 associated with positive blood slide for *Plasmodium* or positive malaria rapid diagnostic test (mRDT) and no criteria for severe malaria. Asymptomatic *Plasmodium* parasitemia was defined as no symptoms of malaria from day 0 to 7 (assessed by history and physical examination at day 0 and 7) and associated with positive blood slide for *Plasmodium* (or positive mRDT) at day 0 and 7. A community cross sectional study was planned to recruit 100 asymptomatic *Plasmodium* parasitemia children (controls). Since the prevalence of *Plasmodium* parasitemia was known to be around 10% within the study area, 1,000 children from the

community were to be screened as previously explained (Salim et al., 2014). Included children were assessed at each visit (recruitment and follow up visits) by a qualified, trained study clinician for signs and symptoms of malaria and other common diseases using a structured questionnaire designed for the study. Recruitment of uncomplicated malaria cases were conducted in the dispensaries, west of the study area. Suspected severe malaria cases recruited from the Bagamoyo district hospital (BDH) received additional investigation in the ward to exclude other medical conditions mimicking malaria syndrome. All clinical follow up visits were conducted at the nearest dispensary where children were recruited.

Sample collection, diagnosis of *Plasmodium* and helminth infection

Sample collection and diagnostic procedures have been explained previously (Salim et al., 2014, Knopp et al., 2014). Briefly stool, urine and blood samples were collected and examined using a broad set of quality controlled diagnostic methods for common STH (*Ascaris lumbricoides*, hookworm, *Strongyloides stercoralis*, *Enterobius vermicularis*, and *Trichuris trichiura*), schistosoma species and *Wuchereria bancrofti*. Blood slides and malaria rapid tests (mRDTs) were utilized for *Plasmodium* diagnosis. Thin and thick blood films were performed, air dried and Giemsa stained for detection and quantification of malaria parasites according to the IHI laboratory standard operation procedures (SOP) as adopted from WHO (Greenwood and Armstrong, 1991). Polymerase chain reaction (PCR) technique was not performed on the positive follow up slides, all were then considered as recrudescence.

Data Management and Statistical Analysis

Clinical and laboratory data were double entered using the DMSys software (FDA approved for ICH/GCP clinical trials). The helminth species specific results derived by each method were entered into an electronic data base using Microsoft ACCESS 2010. The two datasets were transferred into STATA that was used for data analysis (version 13.0 software, Stata Corp LP; College Station, Texas, USA). Data management, conversion and classification for the helminth species and *Plasmodium* parasitemia were done as explained previously following WHO criteria (Knopp et al., 2014).

Baseline data (demographic and helminth distribution) were summarized according to malaria clinical status. In the report investigating distribution and risk factors for *Plasmodium* and helminth co-infections (Salim et al, *Plos Neglected Tropical Disease Journal*, in press), age, location (village) and education level (not schooling) were among the significant predictors for co-infection. These three variables were thus used for matching the cases and controls. Age was categorized as (children less than 3 years, preschool children aged 3-5 years and school-aged children from 6-9 years inclusively). The categorization was chosen to explore the age dependency variability considering the ongoing malaria and mass drug administration helminth programs with different approaches based on age, mainly focusing on children under five and above five years of age.

A matching program was developed with support from the STATA conference and users group (http://www.stata.com/meeting/). The program reduces to the following syntax, whose arguments are explained below: radmatch varlist [id( ) mpair( ) case( ) k( ) rad( ) seed( ). Briefly the program works as

follows: i) first it creates a dataset of controls and cases separately from the variable that defines them (varlist) ii) then it randomly orders the cases using the unique identification id (varname) iii) a variable is then defined that linked a given control to its matched case, mpair( ) iv) using a loop over the observations in case and control datasets, matches are established wherever the cases and controls shares the same values, hence ending up with a dataset with all possible matches called (match) v) for each pair labelled 0 for a control and 1 for a case) vi) k( ) defines the k in the 1: k matching, the default is k = = 1 vii) rad( ) these are the respective radius corresponding to the variables specified in the varlist, viii) seed( ) this is the seed to replicate results.

Simple and multiple conditional logistic regression models were used to investigate the strength of association between helminth infection and malaria clinical status (from asymptomatic *Plasmodium* parasitemia to severe malaria). Odds ratios (OR) including 95% confidence interval and p-values were calculated. Most of other explanatory variables (gender, nutritional status and interventions used) were not significant in the simple regression analysis but were still included in the multiple conditional regressions as all of them are somehow expected to have an effect on the outcome. No interaction effect was observed with the matched variables used. The relationship between STH infection species and malaria clinical status was further explored using ordinal logistic regression model to better reflect the risk with increasing severity of malaria clinical status.

Treatment response was analyzed using the definitions of adequate clinical and parasitological response as advised by WHO (WHO, 2009b). Geometric mean time (in hours) to first parasite clearance according to helminth infection was estimated using a regression model adjusted for age group. The parasite counts were converted into log scale for analysis. The occurrence of diseases other than malaria was then summarized according to the helminth infection status. We could not analyze the correlation between STH species intensity and *Plasmodium* parasite density as most of the children presented with light intensity STH infection and insufficient numbers within the malaria infection clinical status.

Results

The study was conducted between July 2011 and November 2012. Among 1,130 children whose parents gave consent and enrolled, 94 had asymptomatic *Plasmodium* parasitemia, 124 had uncomplicated malaria and 19 had severe malaria (Figure 20). The prevalence of helminth infection among children according to malaria clinical status before matching is described in Table 15. There was a reduced prevalence of helminth with increasing clinical malaria severity. Hookworm single helminth species co-infection significantly increased with increasing clinical malaria status severity while *E. vermicularis* co-infection showed a declining pattern (Table 15 and Figure 21)

Distribution of cases and controls

There were 143 possible cases and 94 possible controls available for matching as shown in Figure 20.

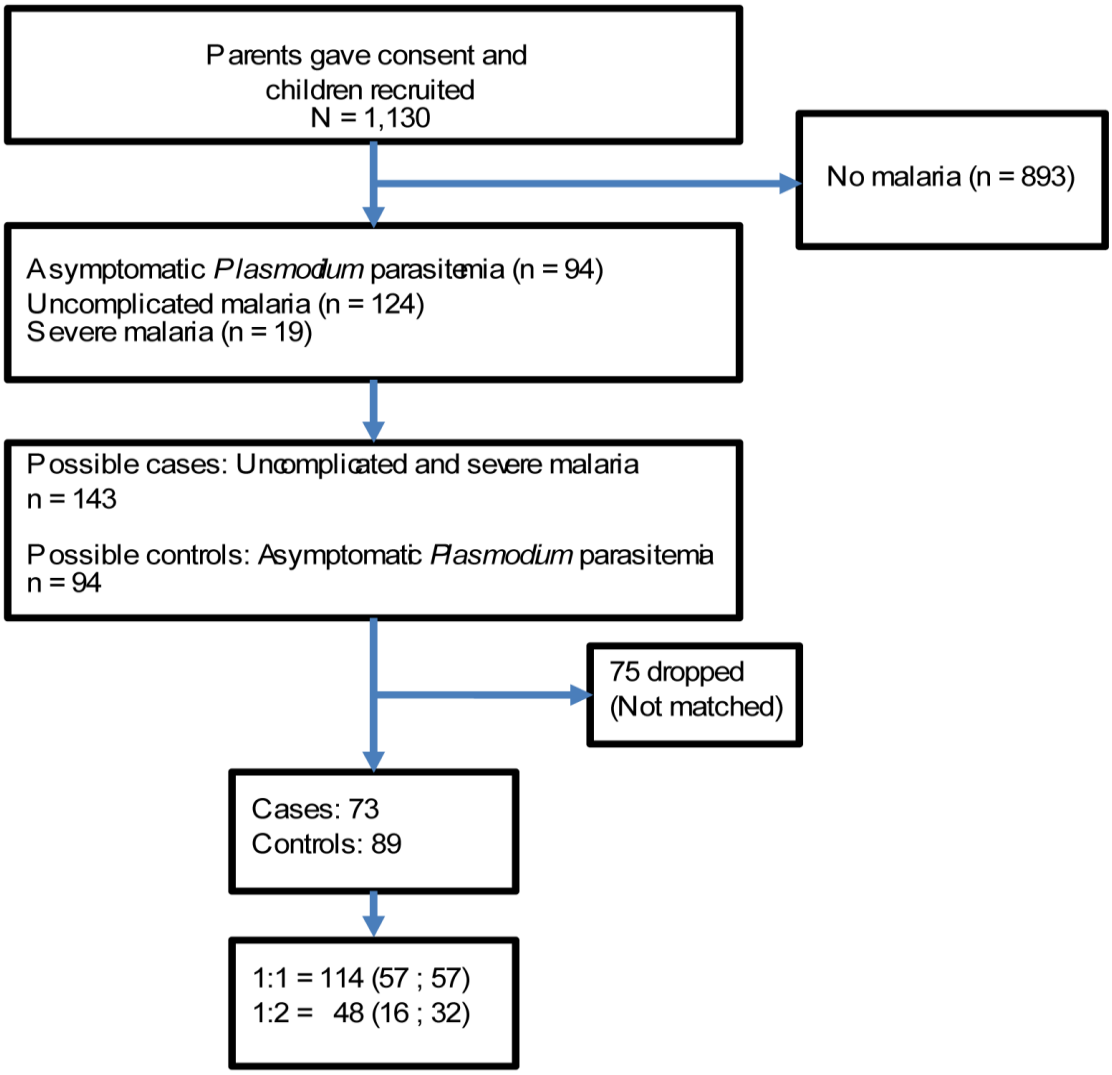

Figure 20. Flow diagram of the participants and matching procedures

Table 15. Prevalence of helminth infection among children according to malaria clinical status (before matching)

Variables	Malaria infection status			
	Total N = 228	Asymptomatic n = 92	Uncomplicated n = 118	Severe n = 18
All helminth				
Helminth (+ve)	65 (28.5)	34 (37.0)	27 (22.9)	4 (22.2)
Helminth (-ve)	163 (71.5)	58 (63.0)	91 (77.1)	14 (77.8)
Helminth infection				
Single species	56 (24.6)	28 (30.4)	24 (20.3)	4 (22.2)
Double species	9 (3.9)	6 (6.5)	3 (2.5)	0 (0.0)
> 2 species	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Single helminth infection				
E. vermicularis	20 (8.8)	13 (14.1)	7 (5.9)	0 (0.0)
Hookworm *	21 (9.2)	6 (6.5)	12 (10.2)	3 (16.7)
S. stercoralis	13 (5.7)	7 (7.6)	5 (4.2)	1 (5.6)
T. trichiura	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Helminth intensity				
*E. vermicularis****				
light	10 (4.4)	7 (7.6)	3 (2.5)	0 (0.0)
Moderate	5 (2.2)	2 (2.2)	3 (2.5)	0 (0.0)
Heavy	5 (2.2)	4 (4.3)	1 (0.8)	0 (0.0)
Hookworm***				
light	14 (6.1)	5 (5.4)	8 (6.8)	1 (5.5)
Moderate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Heavy	3 (1.3)	0 (0.0)	2 (1.7)	1 (5.5)
*T. trichiura****				
light	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Moderate	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Nine children excluded because of no stool collected (2 in asymptomatic *Plasmodium* parasitemia, 6 in uncomplicated and 1 in severe malaria). *p values was significant. *** The total number differ from that of total single helminth species as some species were isolated using other methods apart from Kato-Katz and hence couldn't be quantified.

After running the program, 73 cases and 89 controls were selected in the ratios of 1:1 (57:57) and 1:2 (16:32) as shown in Figure 20. The baseline characteristics of the selected cases and controls including the matching variables are described in Table 16. There were no significant differences in gender, nutritional status, bednets and antihelminth use among cases and controls.

Table 16. Baseline characteristics among cases and controls

Characteristics	Malaria disease status **Cases** N = 73 n (%)	 **Controls** N = 89 n (%)
Matching variables		
Age group		
< 3 years	15 (20.6)	15 (16.9)
3 – 5 years	13 (17.8)	14 (15.7)
> 5 years	45 (61.6)	60 (67.4)
Location (village)		
Kiwangwa	71 (97.2)	86 (96.6)
Msata	1 (1.4)	1 (1.1)
Magomeni	1 (1.4)	2 (2.3)
Education level		
Too young	15 (20.6)	15 (16.8)
Not schooling	38 (52.0)	45 (50.6)
Preprimary	11 (15.1)	11 (12.4)
Primary	9 (12.3)	18 (20.2)
Other demographics		
Gender		
Male	39 (53.4)	45 (50.6)
Female	34 (46.6)	44 (49.4)
Nutritional status		
Normal	62 (84.9)	73 (82.0)
Underweight	11 (15.1)	16 (18.0)
Normal	56 (76.7)	63 (70.8)
Stunted	17 (23.3)	26 (29.2)
Normal	73 (100.0)	86 (96.6)
Wasted	0 (0.0)	3 (3.4)
Intervention coverage		
Bednets		
Slept under a bednet last night	61 (88.4)	74 (88.1)
Antihelminth		
Used albendazole	25 (39.1)	40 (44.9)
Used Mebendazole	15 (23.4)	16 (18.0)

Malaria disease status: Cases are children who had uncomplicated and severe malaria (disease) and controls are children with asymptomatic *Plasmodium* parasitemia infection.

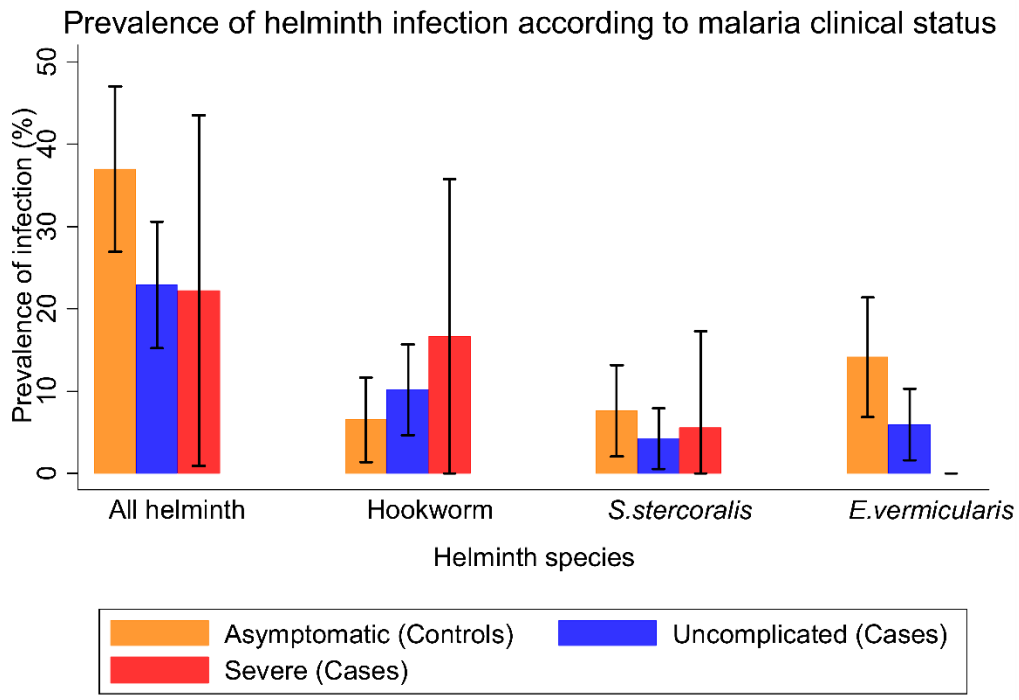

Figure 21. Prevalence of helminth infection according to malaria clinical status using all possible cases and controls before matching
(143 and 94 respectivey)

Effect of soil transmitted helminth on malaria clinical status

In simple conditional logistic regression analysis, there was a tendency for a protective effect of helminth on the development of clinical malaria [OR= 0.6, 95% CI of 0.3 – 1.3] which was more marked with *E. vermicularis* species [OR= 0.2, 95% CI of 0.0 – 0.9]. On the contrary, there was a tendency of hookworm species to be associated with clinical malaria [OR= 3.0, 95% CI of 0.9 – 9.5], Table 17.

Table 17. Strength of association between malaria disease and helminth infection using simple conditional logistic model

Variables	Cases **N = 73 n (%)**	Controls **N = 89 n (%)**	**OR (95% CI)**	**p - value**
Helmith#				
Helminth (+ve)	19 (27.5)	34 (39.1)	0.6 (0.3 – 1.3)	0.241
Helminth (-ve)	50 (72.5)	53 (60.9)		
Single helminth species (+ve)				
Hookworm	11 (15.1)	6 (6.7)	3.0 (0.9 – 9.5)	0.065
S. stercoralis	4 (5.5)	7 (7.9)	0.6 (0.1 – 2.5)	0.501
E. vermicularis	2 (2.7)	13 (14.6)	0.2 (0.0 – 0.9)	0.037

Total number of cases was 69 and controls were 87.

In multiple conditional regression analysis, the overall protective effect was lower with all helminth infection [OR= 0.8, 95% CI of 0.3 – 1.9] but remained significantly protective with *E. vermicularis* species [OR= 0.1, 95% CI of 0.0 – 1.0] and borderline significant with hookworm species [OR= 3.6, 95% CI of 0.9 – 14.3], Table 18.

Table 18. Adjusted odds ratios using multiple conditional logistic model

Variables	Malaria disease*	
	OR (95% CI)	p - value
All helminth	0.8 (0.3 - 1.9)	0.558
Hookworm	3.6 (0.9 - 14.3)	0.063
S. stercoralis	1.0 (0.2 - 4.9)	0.984
E. vermicularis	0.1 (0.0 - 1.0)	0.049

*All models were adjusted for gender, nutritional status, bed net use and use of antihelminth (Albendazole and mendazole).

Using ordinal logistic regression which better reflect the progression of asymptomatic *Plasmodium* parasitemia to severe malaria, there was a 50% protective effect with overall helminth [OR= 0.5, 95% CI of 0.3 – 0.9]. On the contrary, hookworm species was highly predictive of malaria disease [OR= 7.8, 95% CI of 1.8 – 33.9 for uncomplicated malaria] and [OR= 49.7 (1.9 – 1298.9) for severe malaria] (Table 19). Figure 22 shows how the prevalence of STH species varies with clinical malaria status in a proportional way among cases and controls using matched data.

Table 19. Strength of association between malaria disease and helminth infection using ordinal logistic regression model

Explanatory variables	Malaria disease*	
	OR (95% CI)	p- value
Overall		
All helminth	0.5 (0.3 - 0.9)	0.026
No malaria		
Asymptomatic	1	
Uncomplicated	0.5 (0.3 - 1.0)	0.065
Severe	0.6 (0.2 - 2.1)	0.410
Hookworm		
No malaria		
Asymptomatic	1	
Uncomplicated	7.8 (1.8 - 33.9)	0.006
Severe	49.7 (1.9 - 1298.9)	0.019
S. stercoralis		
No malaria		
Asymptomatic	1	
Uncomplicated	0.3 (0.1 - 2.1)	0.247
Severe	0.1 (0.0 - 2.3)	0.155
E. vermicularis		
No malaria		
Asymptomatic	1	
Uncomplicated	0.6 (0.2 - 2.0)	0.381
Severe	-	0.996

Model for malaria disease: compares children with malaria disease (uncomplicated and severe malaria) as cases and those with asymptomatic *Plasmodium* parasitemia infection as controls.
*The model was adjusted for gender, age group, location and education level.

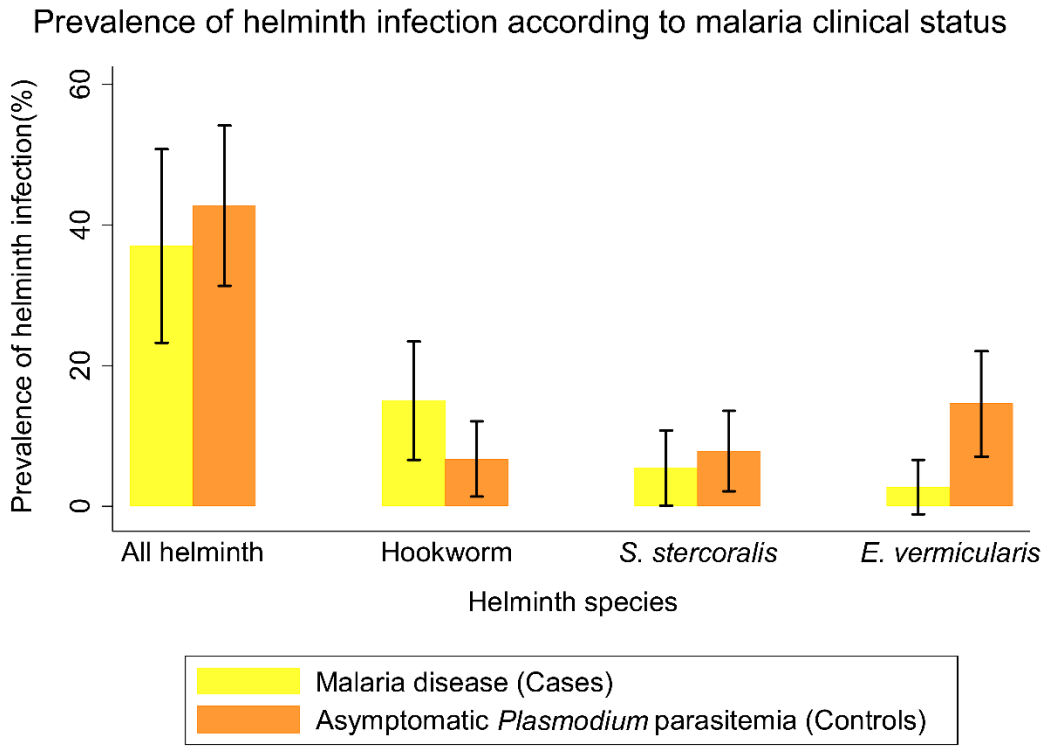

Figure 22. Prevalence of helminth infection according to malaria clinical status (after matching)
(n= 73 cases and 89 controls)

Effect of soil transmitted helminth infection on response to treatment

Adequate clinical and parasitological response (ACPR)

The crude ACPRs were 62/65 (95.4%) and 156/163 (95.7%) for STH positive and helminth negative children respectively. Most of the recurrent infections were late clinical failure (LCF). Out of a total 10 recurrent infections, 3 (4.6%) were among the STH positive and 7 (4.3%) among the helminth negative children. All were LCF except for 3 late parasitological failures (LPF) in the helminth negative children.

Generally, children infected with STH had higher geometric mean time to first clearance of parasitemia. The time to first clearance was significantly longer for children who presented with severe malaria and co-infected with *E. vermicularis* and hookworm. The geometric mean time was also longer with *S. stercoralis* co-infection, although not statistically significant (Table 20).

Table 20. Geometric mean time (in hours) to first clearance of malaria parasitemia according to helminth species

Variables	All helmith**		*E. vermicularis***		Hookworm**		*S. stercoralis***	
	Coefficient (95% CI)	βco.	Coefficient (95% CI)	βco.	Coefficient (95% CI)	βco.	Coefficient (95% CI)	βco.
Overall	0.04 (-0.17 - 0.25)	1.04	0.31 (- 0.17 - 0.79)	1.36	- 0.18 (- 0.61 - 0.25)	0.83	0.49 (- 0.09 - 1.07)	1.63
Asymptomatic (Ref)								
Uncomplicated	0.04 (- 0.17 - 0.25)	1.04	0.06 (- 0.29 - 0.4)	1.06	0.22 (- 0.16 - 0.60)	1.24	0.06 (- 0.27 - 0.39)	1.06
Severe	1.17* (0.77 - 1.57)	3.21	1.87* (0.85 - 2.89)	6.5	1.90* (0.86 - 2.93)	6.67	1.42 (0.28 - 2.56)	4.12

βco. denotes beta coefficient (mean time in hours). Note: **all the four models were adjusted for age group. * p values were significant

Occurrence of other diseases than malaria

Respiratory infections [pneumonia and upper respiratory tract infections (URTI)] were among the common disease. Malaria clinical status significantly influenced the occurrence of other diseases (p = 0.038).

Table 21. Occurrence of other diseases stratified by helminth status

	Helminth status		
Diagnosis	Positive	Negative	OR
Pneumonia	4 (18.2)	21 (35.6)	0.4
URTI	7 (31.8)	19 (32.2)	1.0
Gastroenteritis	2 (9.1)	7 (11.9)	0.7
Ill-defined illness	3 (13.6)	4 (6.8)	2.2
Others	6 (27.3)	8 (13.6)	2.4
Total	22 (33.8)	59 (36.2)	

Note: URTI means upper respiratory tract infections.

p values were not significant

Discussion

To our knowledge, this is the first case control study to investigate the effect of soil transmitted helminth (STH), and specifically *E. vermicularis* and *S. stercoralis* on clinical malaria status, with the whole spectrum from asymptomatic *Plasmodium* parasitemia to uncomplicated and severe malaria.

The results of this study showed an overall tendency of a protective effect with any helminth but opposite results with *E. vermicularis* and hookworm species which partly explain the contradictory results and controversy in the literature about the association of STH and malaria. Our study shows that co-infection with hookworm is associated with clinical malaria and even more so with severe malaria while *E. vermicularis* protects against development of clinical and severe malaria. *S. stercoralis* species tends to be protective against clinical malaria although not statistically.

The overall protective effect with any helminth is in line with the previous findings as summarized in the recent systematic review by Adegnika et al (Adegnika and Kremsner, 2012). In addition, our results are consistent with the findings from previous studies that show hookworm to be a risk factor for uncomplicated and severe malaria (Pullan et al., 2011, Humphries et al., 2011, Fernandez-Nino et al., 2012, Ndibazza et al., 2013, Kinung'hi et al., 2014). Previous studies have documented the protective effect of *A. lumbricoides* (Murray et al., 1977, Murray et al., 1978, Nacher et al., 2000, Valencia et al., 2010, Fernandez-Nino et al., 2012) but none had yet documented such an effect of *E. vermicularis*. We were able to show a protective effect of this particular species because of the absence of *A. lumbricoides* species in our area, and thanks to the use of performant methods to identify *E. vermicularis*.

Immunological analysis of the same children included in this case-control study confirmed the pro-inflammatory effect of hookworm and *S. stercoralis* and the anti-inflammatory effect of *E. vermicularis* (Lenz et al, in preparation). Furthermore, epigenetic analysis showed that *E. vermicularis* acts through the gut microbiota to influence the immune system. Indeed, where the ratio of firmicutes: bacteroidetes is a biomarker of gut inflammation, *E. vermicularis* co-infected children had a much higher ratio compared to children co-infected with any helminth and those who had no infection (controls) (Lenz et al, in preparation).

The different pathogenesis mechanisms of hookworm and *E. vermicularis* could explain the observed opposite effect of the two species. While hookworm is an invasive blood sucking, migratory species associated with negative effect on health (Mwangi et al., 2006), *E. vermicularis* is rarely invasive, mostly asymptomatic and symbiotic commensal within the human body (Gale, 2002). This could indicate the adaptive mechanism in terms of degree of immune stimulation in relation to impact of a co-existing species such as *Plasmodium* parasite within the same host. *E. vermicularis* infection is most likely associated with the induction of a T helper 2 (Th2) / regulatory T cells (Treg) hypo-response with an increase release of immunoglobulin E (IgE) complexes that activate high affinity Fc receptors (CD23) and the anti-inflammatory interleukin-10 (IL-10) which activate the nitric oxide synthase releasing nitric oxide leading to reduced sequestration of parasitized red blood cells (Nacher, 2002). On the other hand, hookworm induce the Th2/Treg response which lead to suppression of Th1 and pro-inflammatory responses which are key for *Plasmodium* parasite clearance within the human body (Nacher, 2002).

Additionally, hookworm could alter antibody dependent cellular inhibition (ADCI) with the predominance of non- cytophilic IgG2, IgG4 and IgM while decreasing the cytophilic antibodies IgG1 and IgG3 leading to increased severity of malaria (Druilhe et al., 2005, Roussilhon et al., 2010). An association between total IgG and hookworm infection in children have been reported in Ghana (Humphries et al., 2011).

The overall protective or enhancing effect of helminths on clinical malaria is obviously driven by the most common STH species in the region studied. We observed that the proportion of hookworm increased as severity of malaria increased while that of *E. vermicularis* showed the opposite trend. In the present study, the trend of *S. stercoralis* was less clear but its effect was more marked on asymptomatic *Plasmodium* parasitemia as previously reported (Salim et al, *Plos Neglected Tropical Disease Journal*, in press). The low numbers limited our analysis and hence could be obscuring the effect of *S. stercoralis* on clinical malaria.

Helminth infected children had a higher geometric mean time to first clearance of *Plasmodium* parasitemia, with almost the same adequate clinical and parasitological response to helminth uninfected children. The findings are in line with those of Degarege et al in Ethiopia (Degarege et al., 2009) that STH don't have significant impact on clearance rate of *Plasmodium* parasite. In our study, severe malaria disease associated with the co-infection influenced the clearance time.

One limitation of this study was the low number of severe malaria and the low infection intensity which reduced the power of our study to investigate morbidity trends. These could be explained by the efforts of the ongoing malaria and STH interventions through the National malaria control program (NMCP) and the Neglected tropical disease control program (NTDCP) in Tanzania.

Conclusion

Overall, these results demonstrate a protective role of *E. vermicularis* against clinical malaria and confirm the enhancing effect of hookworm on malaria morbidity. The protective effect questions of course the relevance of deworming programs. However, dropping anti-helminth programs would leave all children infected, and would thus contribute to transmission. On the long-term, this would be counterproductive and go against the momentum of elimination. It would of course lead to perpetuating hookworm infection and increased attributable malaria morbidity. Considering the burden of diseases, access and cost, deworming program should thus continue. The most affected children live in impoverish societies. The infections cause high morbidity and quantification of the beneficial effect of deworming on economic, school performance, school attendance, cognitive function and overall health status is difficult. The present findings should foster the implementation of an integrated control program for these two common parasites coupled with screening and then treating the affected children to speed the process to elimination. The protective effect of *E. vermicularis* highlights the importance of diagnosing this infection and entail further studies to understand better its impact in low and high intensity areas including all ages and at risk groups to better advice our control programs.

Acknowledgments

The author's acknowledge all staff of the IDEA project and all children and their parents who agreed to participate in this study. We are particularly obliged to Raymond Singo, Shabani Halfan, Rehema Mangoli, and Tatu Nassor for their help in the field and laboratory work, the data unit of the BRTC for entering the large amount of data and providing ample support. In addition, we acknowledge the Bagamoyo district officials, specifically the district immunization and vaccination officer (DIVO) Farah Mohammed for his great collaboration.

Author contributions

Conceived and designed the experiments: NS, SK, SA, FA, MT, CD, BG. Performed the experiments: NS, SK, OL, TS, JR, JM, DK, ASM. Analysed the data: NS, UA, AM. Wrote the paper: NS, BG. All authors read and approved the manuscript.

Chapter 9: GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

9.1 General discussion

This PhD study was embedded in an already approved IDEA project, global research initiative funded by the European Union designed to intensively study the immunological interplay between helminth infections and the neglected tropical diseases (NTDs) namely malaria, TB, and HIV-AIDS (Willyard, 2009). The main goal of the thesis was to explore the interaction between *Plasmodium* and soil transmitted helminth (STH) infections in an area where both clinically important parasitic diseases are prevalent and large scale National control programs to prevent these infections are ongoing. A community cross sectional survey and a case control study were conducted among children aged 2 month to 9 years inclusively living in western rural areas of Bagamoyo district, coastal region of Tanzania. Four main villages were included, Magomeni, Mkange, Kiwangwa and Msata. Different diagnostic methods for helminth infection detection were used including adhesive tape slides for *E. vermicularis* and Baermann technique for *S. stercoralis*. This provided a full range of helminth profile to investigate the effect of each common STH species on *Plasmodium* infection. This chapter discusses mainly the principle findings from the epidemiological study but also considering the elucidated immunological and molecular mechanisms highlighted in chapter 8.

To put our findings into perspective, a review was performed to compare the observations made in this PhD with what has been shown in the literature, Table 22. This review was aimed at strengthening the conclusions and potential implications for future public health policies and identifies gaps to be addressed in future researches.

Table 22. Previous studies on the association between *Plasmodium* infection, clinical malaria and Soil transmitted helminth infections

Previous studies on the association between clinical malaria and Soil transmitted helminth infections

Author-year	Place	Malaria transmission	Helminth intensity	Study design	Age (years)	Helminth detection methods	Controlled for location	Effect size (95%CI)	Effect direction
Murray, 1977	Comoro Islands	High Vs moderate	High	Ecological study	< 14	Stoll, 1926	No	SED; Areas with high Ascariasis Vs malaria prevalence 93% Vs 1.7% 24% Vs 23%	Decreased risk of clinical malaria associated with *A. lumbricoides* infection
Murray, 1978	Comoro Islands	High Vs moderate	High	Intervention with Piperazine	2 -14	Stoll, 1926	No	Malaria attack in 54% of those treated compared to zero in placebo group	Protective effect for *A. lumbricoides* infection to clinical malaria
Nacher, 2000	Bangkok, Thailand	-	-	Unmatched case control study	15-62	-	No	*A. lumbricoides* [OR=0.6 (0.32 -1.03)]	Protective effect for A. *lumbricoides* infection to severe malaria
Nacher, 2002	5 hamlets from Thailand	Low	Low	Prospective cohort	All age	Kato-Katz	No	All helminth [ARR=2.2 (1.4 -3.6)]	Increased risk of clinical malaria associated with all helminth infections
Spiegel, 2003	Senegal	High	-	Surveillance and cross sectional survey of helminth	1 - 14	-	No	All helminth RR of 1.5 among the helminth infected compared to helminth non- infected	Increased risk for all helminth infection to clinical malaria
Le Hesran, 2004	Rural zone of Senegal	-	High	Prospective case control study	Mean age 6.6 (+/-3)	Direct microscopy And concentration method	No	*A. lumbricoides* [OR=9.9 (3.0 – 32.7)]	Increased risk for *A. lumbricoides* infection to severe malaria
Shapiro, 2005	4 villages in Kabale district, South west Uganda	Low, unstable with peaks	Moderate to high	Active surveillance (weekly)	All age	Duplicate Kato-Katz	No but household clustering reported	All helminth [OR=1.1 (0.6 – 1.9)] *A. lumbricoides* [OR=1.1 (0.6 – 2.2)] *T. trichiura* [OR=1.2 (0.4 – 4.2)] Hookworm [OR=1.0 (0.6 – 1.9)]	Neural (No association) For all helminth, *A. lumbricoides* and hookworm infections to clinical malaria

SED = Standard error of differences of percentages. OR = Odds ratio. ARR = Adjusted risk ratio. AHR = Adjusted hazard ratio. Not clearly stated (-)

Previous studies on the association between clinical malaria and Soil transmitted helminth infections

Author-year	Place	Malaria transmission	Helminth intensity	Study design	Age (years)	Helminth detection methods	Controlled for location	Effect size (95%CI)	Effect direction
Degarege, 2009	Southern Ethiopia	Unstable, with peaks	Low	Hospital based-cross sectional study	All age	Kato-Katz	No	All helminth [OR=0.3 (0.3 – 0.9)]	Protective effect for all helminth infections to severe malaria
Fernández-Niño J.A, 2012	Tierralta ,Cordoba-Colombia	High	-	Matched Case control study	5-60	Direct examination and Ritchie-Frick modified concentration technique	Yes	*A. lumbricoides* [OR=0.4 (0.2 – 1.0)] Hookworm OR=4.4 [(1.7– 11.3)]	Protective effect for *A. lumbricoides* infection on uncomplicated malaria Increased risk for hookworm infection on uncomplicated malaria
Degarege, 2012	Southern Ethiopia	Unstable, with peaks	Low	Hospital based, cross sectional	All age	Kato-Katz	No	All helminth [OR=2.9 (1.8 – 4.7)] *A. lumbricoides* [OR=2.6 (1.4 – 4.6)] *T.trichiura* [OR=2.8 (1.1 – 6.7)]	Increased risk for all helminth, *A.lumbricoides* and *T. trichiura* infections on clinical malaria
Mulu, 2013	Southern Ethiopia	Endemic	High	Hospital based- Cross sectional study	All age	Direct microscopy and formal ether concentration method Kato-Katz	No	*T. trichiura* [OR=1.4 (1.1 - 1.7)]	Increased risk for *T. trichiura* infection on clinical malaria
Ndibazza J, 2013	North shores of lake Victoria - Uganda	High	-	Mother baby cohort, 5 years follow up	<5years	Duplicate Kato-Katz	Yes	Hookworm AHR=1.2 (1.1 – 1.4)	Increased risk for hookworm infection on clinical malaria

SED = Standard error of differences of percentages. OR = Odds ratio. ARR = Adjusted risk ratio. AHR = Adjusted hazard ratio. Not clearly stated (-)

Previous studies on the association between *Plasmodium* infection and Soil transmitted helminth infections

Author-year	Place	Malaria transmission	Helminth intensity	Study design	Age (years)	Helminth detection methods	Controlled for location	Effect size (95%CI)	Effect direction
Brutus, 2006 and Brutus, 2007	Rural Madagascar	Mesoendemic	Low	RCT with anti-helminthic levamisole	All age >6 months	Merthiolate Iodine formaline	N/A	<4 years *A. lumbricoides* [RR=2.7 (1.9 – 3.4)] ≥5 years [RR=0.5 (0.2 – 0.8)]	Increased risk for *A. lumbricoides* infection among <4 years And a protective effect among the ≥5 years in relation to *P. falciparum* density
Kungu, 2009	Wete district, Pemba	High	Endemic	House - house community cencus	6-23months	Duplicate Kato-Katz and sedimentation method	No	All helminth [OR=0.6 (0.5 – 0.8)] *A. lumbricoides* [OR=0.6 (0.4 - 0.9)] *T. trichiura* [OR=0.7 (0.5 - 0.9)]	Protective effect for all helminth infections to *Plasmodium* infection
Valencia CA, 2010	Columbia	-	Moderate and high	Ecological study on National health survey	All age	Ritchie-Frick modified concentration technique	No	Correlation coefficient High prevalent area (>30%) was 0.219 No significant correlation with hookworm and *T.trichiura*	Positive correlation with *A. lumbricoides* to *Plasmodium* infection. No effect with hookworm and *T.trichiura* infections
Kirwan, 2010	Semi urban villages-Nigeria	Intense	Low	Double blind placebo control RCT with albendazole	1-5	Formal ether concentration	N/A	Increased risk in the treatment group but significantly slower than the placebo group	Increased risk of *Plasmodium* infection for all helminth and *A. lumbricoides* infections
Pullan, 2011	Rural Tororo district-Uganda	Stable	Low	Population based-cross sectional study	0 -88	Kato-Katz	Yes	Hookworm Pre-school aged [OR=2.4 (1.3 – 4.3)] Adults [OR=2.1 (1.3 – 3.2)]	Increased risk for hookworm infection on *Plasmodium* infection
Humphries, 2011	Kintapo, Ghana	Endemic	Low	Cross sectional survey	1-80	Kato-Katz	No	Hookworm School aged [OR= 2.8 (1.1-7.3)]	Increased risk for hookworm infection on *Plasmodium* infection
Kinung'i, 2014	Magu district-Mwanza, Tanzania	Hyper-holoendemic	Endemic	Cross sectional study	3-13	Kato-Katz	No	Hookworm OR=1.32, p=0.064	Increased risk for hookworm infection on *Plasmodium* infection

SED = Standard error of differences of percentages. OR = Odds ratio. ARR = Adjusted risk ratio. AHR = Adjusted hazard ratio. Not clearly stated (-)

9.1.1 *Plasmodium* and soil transmitted helminth co-infection among children

Evolutionary, humans have been infected with parasites. Considering the shared geographical overlap of soil transmitted helminth and *Plasmodium*, co-infection is not uncommon. Environmental factors play an important role in determining the infection and reinfection of the child (host). Once the child is infected, the pathogenesis and outcome of the infection and/or disease depend on the interaction of the co-infecting species within the child. The exposure and/or acquisition of the infection influence the immunity and determine the age pattern of the specific infection. The age group with the greatest burden of co-infection may not be the one with greatest level of co-morbidities. Actually those with greatest burden of co-infections could be protected to develop diseases and hence severe morbidities associated with high mortality. This is true for the case of *Plasmodium* and STH co-infection. In chapter 6 and 7 of this thesis we found that school-aged children (above 5 years) bear the dual burden of *Plasmodium* and STH and young children are the most affected with severe malaria as previously reported (Nankabirwa et al., 2014). Generally, STH infections protect against clinical malaria as observed in the present study and previous ones. If this is true, we would expect less clinical malaria in areas where STH are prevalent and vice versa. It could be that STH infections protect the school-aged children to develop clinical malaria once the two parasites coexist in the same child but we think that it was more of environmental factors which exposed children to both parasites including variations in intervention coverages and children's behaviors as discussed in chapter 7. We found heterogeneity of infections between and within the villages studied with no specific pattern to justify the distribution and explain the interaction of the two parasites. In areas where transmission is still high for both parasitic infections, environmental measures should be undertaken.

In this study, *Plasmodium* infection and not STH was found to be associated with anemia in all age groups. The prevalence and intensity of malaria within the study areas could explain this finding as most of STH infections were of light intensity. Previous co-infection studies showed that malaria is a predictor of anemia in endemic areas (Nkuo-Akenji et al., 2006, Kung'u et al., 2009, Humphries et al., 2011, Kinung'hi et al., 2014). A positive correlation was observed between malaria parasite density and anemia among children aged 9 months to 14 years in Cameroon when investigating effect of malaria and helminth co-infection (Nkuo-Akenji et al., 2006). It is unclear if the immune manipulation caused by the STH contributes to this finding. Light hookworm infection has been documented to have less effect on anemia among children (Righetti et al., 2012) and here postulated as less inflammation. Most likely, children in malaria endemic areas experience more inflammation secondary to *Plasmodium* infection causing sequestration of parasited red blood cells. In our study, *E. vermicularis* was the STH shown to have a significant protective effect on clinical and severe malaria. Children with asymptomatic *Plasmodium* parasitemia had higher prevalence of *E. vermicularis*. We would be tempted to hypothesize that the immune hypo-responsive and anti-inflammatory effect of *E. vermicularis* through gut microbiota could be interfering with the parasitized red blood cells sequestration via an endotoxemia effect caused by *P. falciparum* infection (Olupot-Olupot et al., 2013) and thus protecting children to develop anemia. This ratifies the burden of *Plasmodium* infection in areas where prevalence and intensity are still high.

Soil transmitted helminth are generally most prevalent and intense among school aged children but slight variations occur with different species. For example, the burden of *Ascaris lumbricoides* starts early between 1 – 5 years (Knight, 1982) and peaks at school age, 5 - 15 years (Bethony et al., 2006) while hookworm species increase with age, usually with the heaviest load in adults especially males (Knight, 1982). *E. vermicularis* species mostly affect school-aged children, institutionalized persons such as prisoners and household members of persons infected with *E. vermicularis*. The age pattern of *S. stercoralis* was not uniform. The occurrence of *S. stercoralis* early in life and its association with asymptomatic *Plasmodium* parasitemia requires further investigation. In this study, children were not tested for HIV. HIV could confound the multiplication and progression of *S. stercoralis* as it accelerates autoinfection cycles from intestinal strongyloidiasis (presence of adult parasite) to hyper-infection or even disseminated strongyloidiasis. This could partly explain the rise of prevalence later in life among the school-aged children. Not much is known whether HIV can increase the risk of acquiring *S. stercoralis* infection. The link between HIV and *S. stercoralis* progression is still not solid and not many cases are found (Keiser and Nutman, 2004, Marcos et al., 2008, Olsen et al., 2009). Alternatively, it could also mean that *S. stercoralis* remain untreated among the young children as albendazole have a cure rate of only 45% (Marti et al., 1996) and ivermectin is not used in younger children, and thus infection peaks up later in school aged children.

In our findings, helminth infection was shown to be associated with an increased risk of *Plasmodium* infection but a protective effect to develop clinical malaria. However, when looking at the specific effect of different helminth species, *E. vermicularis* was indeed protective against the development of clinical malaria but hookworm increased the risk of clinical and severe malaria. We speculate that the particular effect of one STH species tends to be obscured when analysis is carried out with all helminth aggregated. The opposite effects on malaria of all helminth reported in previous studies could be due to the individual effect of the most prevalent helminth species within the studied population.

Hookworm was found to increase the risk of *Plasmodium* infection (Pullan et al., 2011, Humphries et al., 2011, Kinung'hi et al., 2014) and clinical malaria (Fernandez-Nino et al., 2012, Ndibazza et al., 2013) in areas where transmission is still high whereas no association/neutral effect was observed in low malaria transmission area (Shapiro et al., 2005). These findings are consistent with the hypothesis that i) hookworm increases the risk of *Plasmodium* infection and clinical malaria ii) the effect observed is dependent on the prevalence of the two parasitic infections iii) highlight the pathogenesis of hookworm in coexistence with *Plasmodium* infection where both parasites causes inflammation, destruction of red blood cells and compete for nutrients and survival within the same host. An immunological explanation would be suppression of Th1 and pro-inflammatory responses and a decrease in cytophilic IgG1 and IgG3 which are key for *Plasmodium* parasite clearance. With this phenomenon we would expect a positive correlation in terms of *Plasmodium* parasite density and egg per gram counts of hookworm as described in chapter 7.

Furthermore, *E. vermicularis* protects the development of severe malaria but does not prevent *Plasmodium* infection. The prevalence of *E. vermicularis* was higher among children with asymptomatic *Plasmodium* parasitemia. On one hand, it protects against severe malaria but on the other hand it keeps

a reservoir allowing continuing transmission of these infections in environments conducive for both parasites. This could be an adaptation mechanism of the two parasites for a co-survival within the same host. *E. vermicularis* has been shown to live in a symbiotic way within man for many years (Gale, 2002). Naturally, *E. vermicularis* is less invasive and maintains itself through its unique life cycle with tiny eggs which can easily contaminate the surrounding and spread to the whole family, schools or institutionalized persons. Immunologically, it causes anti-inflammatory response through the gut microbiota where it lives and prevents serious complications of malaria and permits optimal ecological balance for co-survival of the host and *Plasmodium* parasites. To date, we do not know if the same trend of protective effect of *E. vermicularis* is observed elsewhere or in adult populations who suffer less severe form of malaria in endemic areas. If this is so, we could expect to observe more severe malaria among adult population who bear high intensity of hookworm in endemic areas. Most likely, it is not the case as co-infecting parasite species usually adapt to maintain themselves in the host. Both interference and exploitation competition can occur depending on the co-infecting species virulence, abundance, over-dispersion within the host and sequence in which the host acquired the infections (Fakae et al., 1994, May and Nowak, 1995, Petney and Andrews, 1998).

Overall, environmental factors conducive for both parasites play a big role in determining occurrence of *Plasmodium*, STH and co-infections among children. Each parasite considered separately contributes to poor health of exposed population, especially in children. Co-infections in the same child, whether with positive or negative interactions between parasites, have consequences. A typical example is the use of piperazine in the Comoro Island which was associated with a marked reduction of parotid enlargement and edema as a consequence of ascariasis but increased malaria attacks (Murray et al., 1978). The same was observed with the clinical trial with albendazole (Kirwan et al., 2010) in area with intense malaria transmission. Concerted efforts to fight both parasites at once are urgently needed. Whether STH have an influence on the development of clinical malaria must not cast doubt on controlling these parasites, since determinants of its clinical course and of malaria mortality other than STH are well known (Fernández et al., 2008).

9.1.2 Novel contribution of the thesis

The contributions of different chapters of this thesis have been summarized in Table 23 below. The limitations of the study have been discussed in respective chapters of the thesis.

Table 23. Contribution of different chapters of the PhD thesis

Chapter	Title	Innovation	Finding/Validation	Implication/Application
5	Diagnostic accuracy of Kato-Katz, FLOTAC, Baermann and PCR methods for the detection of light intensity hookworm and *S. stercoralis* infections in Tanzania	For the first time, the diagnostic accuracy of FLOTAC and PCR methods were compared for hookworm and *S. stercoralis* infection	The high sensitivity of FLOTAC for hookworm detection was confirmed as compared to PCR and Kato-Katz.	The performance of Kato-Katz, FLOTAC and PCR were assessed in areas of low infection intensity. FLOTAC allow to performing more accurate assessment of the different STH species burden
6	Enterobiasis and strongyloidiasis and associated co-infections and morbidity markers in infants, preschool and school-aged children from rural coastal Tanzania: a cross sectional study	First community cross sectional survey to assess the burden of *E. vermicularis* and *S. stercoralis* infections	*E. vermicularis* and *S. stercoralis* infections are common in infants and children living in endemic areas.	All STH species should be searched for in epidemiological studies including *E. vermicularis* and *S. stercoralis*
7	Distribution and risk factors for *Plasmodium* and helminth co-infections: A cross sectional survey among children in Bagamoyo district, coastal region of Tanzania	Baseline epidemiological data of *Plasmodium* and helminth co-infections in the population of children, including infants who are rarely surveyed in endemic areas	The high burden of *Plasmodium*, STH and *Plasmodium* helminth co-infections among school aged and children who were not schooling. *S. stercoralis* species and its associated high risk of *Plasmodium* infection early in infants.	Epidemiology of STH, including *S. stercoralis,* should be investigated in all age groups, including infants and older children, also in relation to *Plasmodium*, so that appropriate control programs can be designed for both malaria and helminth
8	The impact of soil transmitted helminth on malaria clinical presentation and treatment outcome: A case control study among children in Bagamoyo district, coastal region of Tanzania	First time report on the impact of *E. vermicularis* and *S. stercoralis* on malaria disease	Finding of a protective effect of *E. vermicularis* species on clinical malaria. Confirmation of the association between hookworm infection and clinical malaria	The need to use adhesive tapes in epidemiological studies in order to detect *E. vermicularis*. Accurate assessment of the respective burden of all STH species is needed to properly assess effect of STH infections on clinical malaria and tailor control programs, in particiluar for the use of the most appropriate antiparasitic medication that would optimize the public health benefit of deworming and antimalarial programs.

9.1.3 Challenges and opportunities for integrated control program in Tanzania

Tanzania has already adopted an integrated control approach for the seven common neglected tropical diseases (NTDs). These include schistosomiasis, soil transmitted diseases (hookworm, ascarisis, trichuriasis), trachoma, lymphatic filariasis and onchocerciasis. Through the neglected tropical disease control program (NTDCP), monitoring and evaluation system and national NTDs data base have been established. The program has integrated data collection for lymphatic filariasis, STH and schistososmiasis. This is the most cost effective way to monitor the impact of the NTDs control program. Tanzania is using a phased approach to scale up the NTDs treatment and has reached 68% (17/25) of the regions in the county.

Scale up delivery of preventive chemotherapy (PC) are used either in a single dose or combination therapy depending on the targeted disease. Administration of PC consists of population based diagnosis (which is not done in Tanzania), population based treatment and implementation at regular intervals. PC can be delivered as a universal chemotherapy (where the entire population of an area is targeted), targeted chemotherapy (where a high risk group for example school-aged children is targeted) or selective chemotherapy (where screened individuals found or suspected to be infected are targeted) (Gabrielli et al., 2011). Epidemiological data are important in planning, strategizing cost effective integrated interventions in endemic areas. An experience from Korea emphasized on the program to be accompanied by rigorous scientific efforts to monitor the progress so that the results are deployed for ongoing assessment and public health awareness campaigns (Kim et al., 2014). Epidemiologic overlap among the affected population generate significant program integration opportunities. In this PhD thesis we found that on top of hookworm, *E. vermicularis* and *S. stercoralis* also contribute to the burden of STH. Additionally, *A. lumbricoides* was not found and schistosomiasis was uncommon in Bagamoyo western rural areas. Furthermore, the school-aged children not schooling were more at risk of both *Plasmodium* and STH infections (chapter 7). Considering the life cycle of the highlighted STH species, the sanitation conditions and access of water within the studied villages, implementation of preventive chemotherapy alone will take time to eliminate infections, especially so because of the high reinfection rate. In Tanzania, the overall use of improved sanitation facilities is >50 -75% in urban areas and <25% in rural areas and access to clean water is <50% in rural Bagamoyo (GAHI, 2015). Fortunately, school-based program can be easily implemented and achieve good coverage. Based on epidemiological data, the department of preventive services at the ministry of health in collaboration with other partners including research partners need to provide feedback to the communities through the district commission and primary health care system to coordinate activities, identify integration opportunities for the program and specify time periods for the planned activities. A model example based on the epidemiological findings of this thesis would be an integrated intervention targeting school-aged children including children who are not schooling with the use of ivermectin and albendazole under the Lymphatic filariasis control program combined with distribution of LLINs and health education emphasizing on the transmission and prevention of the two parasites (Bacon et al., 2012, Bieri et al., 2013). The community participation coupled with health education should empower affected populations to run the program, share the tasks and finding their own solutions leading to good utilization and high coverage. Flow of information in both directions, community to authorities and back, is crucial. Failing to synthesize and analyze health information and provide feedback to the community, even when mobile technologies are available to

village health care workers to facilitate control of NTDs have been reported (Madon et al., 2014). The potential impact of adding ivermectin in mass treatment intervention to reduce malaria transmission should also be monitored through the program (Chaccour et al., 2013, Alout et al., 2014, Slater et al., 2014, Kobylinski et al., 2014). It has also been documented that the efficacy of albendazole is increased when combined with ivermectin (Knopp et al., 2010a). To achieve substantial changes at regional and national level, it is essential to have strong collaboration between ministry of health and social welfare and other related ministries such as ministry of water and irrigation, ministry of community development, gender and children, ministry of land, housing and human settlement development and ministry of education and vocational training. Collaborative work with other stakeholders including research groups, non governmental organizations, international agencies and the community is necessary to properly coordinate, systematically collect data for monitoring and designing new intergrated strategic interventions within our country.

9.2 Conclusions

- Multiple diagnostic techniques should be performed including adhesive tapes and Baermann methods when evaluating the burden of helminth infections in children.
- PCR faces a diagnostic challenges in accurately diagnosing hookworm eggs and *S. stercoralis* larvae as compared to FLOTAC and Kato-Katz methods in areas of low intensity helminth infections.
- Multiparasitism is common among children, with school-aged children bearing the dual burden of both *Plasmodium* and STH infections.
- The prevalent STH infections among children were *E. vermicularis*, hookworm and *S. stercoralis*, mostly of light intensity.
- *S. stercoralis* was associated with increased risk of *Plasmodium* infection early in life.
- Co-infection with hookworm was associated with uncomplicated and even more so severe malaria.
- Co-infection with *E. vermicularis* protected against clinical malaria.

9.3 Recommendations

9.3.1 What can be directly translated into public health policy

- STH and malaria control programs should be based on regional and national epidemiological data so that cost effective campaigns with most appropriate measures, especially medication, can be implemented. These two programs should be integrated since both parasites affect the same population and environmental measures as well as medication can be common.
- Monitoring and evaluation of use of ivermectin and albendazole among school-aged children within the NTDCP in Tanzania on the impact of the *Plasmodium* and STH co-infection should be carried out.
- The neglected tropical disease control program (NTDCP) should emphasize and effectively implement health education and utilize community led total sanitation (CLTS) approach to increase coverage, and hence effectiveness of the program among the school-aged children including those who are not schooling.

9.3.2 Research needed in future

- Novel technologies with diagnostic assays that can be performed in a high throughput system on large number of population samples to detect all relevant parasite species in low intensity areas would be desirable.
- Epidemiological studies on *Plasmodium* and STH co-infections should be harmonized and conducted using a broad range of standardized diagnostic techniques to monitor the burden of disease in different regions. Similarly, impact studies should be done to assess the effectiveness of different control programs in different settings and with different prevalence of STH species.
- A well design research to investigate the effect of *E. vermicularis* and *S. stercoralis* on malaria infection and diseases in low and high intensity areas including all ages and at risk groups to better understand the interaction between *Plasmodium* and STH interaction.
- Potential safety and additional impact of ivermectin on malaria transmission requires further exploration considering the risk of *S. stercoralis* and *Plasmodium* co-infection early in life.

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Work experience

2013 – To date	**Lecturer,** Muhimbili University of Health and Allied Sciences (MUHAS), Department of Pediatrics and Child Health.
2007 – To date	**Research Scientist,** Ifakara Health Institute, Bagamoyo branch
2004 – 2007	**Resident,** Department of Pediatrics and Child Health, Ministry of Health and Social Welfare (MOHSW), Tanzania.
2003 – 2004	**Causality officer,** Emergency Department, Muhimbili National Hospital (MNH)
2002 – 2003	**Study Physician,** Vitamin study among HIV pregnant women, MUHAS HAVARD collaborative research works, Tanzania
2001 – 2002	**Intern doctor,** Internship program, Muhimbili National Hospital (MNH)

Research experience

2007 – 2010	**Safety physician, Pediatrician** supporting Phase II RTS, S malaria vaccine studies (**MAL 040** and **MAL 050**), Bagamoyo branch, Tanzania. Sponsored by PATH-MVI and GSK.
2009 – 2014	**Lead safety physician, Project leader and co-principle investigator** for a Phase III, RTS, S/AS01E multi-center malaria vaccine trial in Africa (**MAL 055**), at Bagamoyo site, Tanzania. Sponsored by PATH-MVI and GSK.
2010 – 2014	**Principle investigator,** for **TB Child** evaluation of new and emerging diagnostics for childhood diagnostics in high burden countries. Sponsored by EDCTP.
2011 – To date	**Project Leader, co-principle investigator** for **IDEA project**, Dissecting immunological interplay between poverty related diseases (Malaria, Tuberculosis and HIV) and helminth infections. An African European research initiative sponsored by European Union.
2011 - 2012	**Collaboration work** with Baystate medical centre in Springfield, Massachusetts for a project on measuring cough using vocalization analysis software. Sponsored by GATES foundation.
2011 – 2014	**Project leader, co-principle investigator** for **malaria parasite isolation (MPI project),** Human monoclonal antibodies to aid antigen discovery and antigen prioritization for malaria vaccine development. Sponsored by International center for genetic engineering and biotechnology, Cape Town, South Africa.

Publications

- **Nahya salim,** Tobius Schindler, Ummi Abdul, Julian Rothen, Blaise Genton, Omary Lweno, Alisa S Mohammed, John Masimba, Denis Kwaba, Salim Abdulla, Marcel Tanner, Claudia Deubenberger, Stefanie Knopp. Enterobiasis and strongyloidiasis and associated co-infections and morbidity markers in infants, preschool, school aged children from rural coastal Tanzania: a cross sectional study. *BMC infectious disease* 2014, 14:644.
- **Steffi Knopp, Nahya salim,** Tobius Schindler, Dimitrios A Karagiannis Voules, Julian Rothen, Omary Lweno, Alisa S Mohammed, Raymond Singo, Myrna Benninghoff, Antony A Nsojo, Blaise Genton and Claudia Deubenberger. Diagnostic accuracy of Kato Katz, FLOTAC, Baermann, and PCR methods for detection of light intensity hookworm and *Strongyloides stercoralis* infections in Tanzania. *Am J Trop Med Hyg* 2014, Vol 90 no. 3, 535-545.
- **Nahya salim,** Stefanie Knopp, Omary Lweno, Ummi Abdul, Ali Mohamed, Tobius Schindler, Julian Rothen, John Masimba, Denis Kwaba, Alisa S Mohammed, Fabrice Althaus, Salim Abdulla, Marcel Tanner, Claudia Deubenberger, Blaise Genton. Distribution and risk factors for *Plasmodium* and helminth co-infections: A cross sectional survey among children in Bagamoyo

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- Agnandji ST, Lell B, Soulanoudjingar SS, Fernandes JF, Abossolo BP, Conzelmann C, Methogo BG, Doucka Y, Flamen A, Mordmuller B *et al*: First results of phase 3 trial of RTS,S/AS01 malaria vaccine in African children. *The New England journal of medicine* 2011, **365**(20):1863-1875.
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- **Masoud NS**: Factors related to severity and outcome of pneumonia among children aged 2-59 months, in Dar es salaam, Tanzania. Muhimbili University of Health and Allied Sciences; 2007.

Submitted manuscript

- Co-author with the RTSS team. The effect of immunization schedule with the malaria vaccine candidate RTS, S/AS01E on protective efficacy and anti-circumsporozoite protein antibody avidity in African infants. **Submitted to Malaria Journal, MS # 1796081721145418**
- **Nahya salim et al,** The impact of soil transmitted helminth on malaria acquisition, clinical presentation and disease outcome. A case control study among children in Bagamoyo district, coastal region of Tanzania. **Working paper.**
- RTS, S clinical Trial Partnership. Final results from a phase 3, individually randomised, controlled trial of the RTS, S/AS01 malaria vaccine in African infants and children, including an evaluation of the efficacy of a booster dose. **Submitted to the Lancet journal**

Membership

- Member: Association of Clinical Research Professionals (ACRP), East African chapter
- Council member of Pediatric Association of Tanzania (PAT)
- Member: Management committee, Bagamoyo Research and Training centre (BRTC).
- Board member: H-M foundation, Bagamoyo
- Reviewer: Tanzania Journal of Health Research through African Journal on line; www.ajol.info

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