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Parasitic infections during pregnancy and their consequences on child development in Benin

Amanda Garrison

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Amanda Garrison. Parasitic infections during pregnancy and their consequences on child development in Benin. Human health and pathology. Sorbonne Université, 2021. English. NNT : 2021SORUS194 . tel-04217667

HAL Id: tel-04217667

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Sorbonne Université

École doctorale 393 : Pierre Louis de santé publique

Laboratoire Centre de Recherche Épidémiologie et Statistiques Sorbonne Paris Cité (CRESS)

*/ INSERM UMR 1153 Équipe de recherche en épidémiologie obstétricale, périnatale et
pédiatrique (EPOPé)*

Infections parasitaires pendant la grossesse et ses conséquences sur le développement chez l'enfant au Bénin

Par Amanda Louise GARRISON

Thèse de doctorat d'Épidémiologie

Dirigée par Dr. Florence BODEAU-LIVINEC

Thèse préparée dans le cadre du Réseau doctoral en santé publique animé par l'EHESP

Présentée et soutenue publiquement le 24 septembre 2021

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For the women and children of Allada, Benin

For Dad & Mom

Acknowledgements

Research in any domain is a group collaboration, and I am immeasurably grateful for the support and collaboration I have received from so many individuals during these past three years of this thesis. The following are my thanks to all of those who walked with me along this journey. First and foremost, I need to thank my thesis director, Dr. Florence Bodeau-Livinec, who is one of the primary reasons I decided to complete a PhD thesis at this point in my life. After working with her for my M2 internship and thesis at Ecole des Hautes Etudes en Santé Publique (EHESP), I knew that she was a professor and researcher who was gifted in mentoring and helping young, aspiring students to reach their full potential. This work could not have been completed without her thoughtful guidance, her humble suggestions, and her gently pushing me to think critically at every stage of this work. She is truly my role model when it comes to conducting meaningful research and connecting with all those involved along the way. Thank you, Florence.

Thank you to Asia Maselko and Suzanne Maman from UNC Chapel Hill Gillings School of Global Public Health for their brilliant expertise related to the qualitative study mentioned within this thesis. It has been an honor to learn from both of them and expand my research capabilities under their guidance.

Thank you to ED 393, particularly Dominique Costagliola, Pierre-Yves Boëlle, and Magali Moulié, for their tireless advocacy for myself and for all of the doctoral students at this school. Working so closely with them gave me meaningful insight into just how much they do for us students so that we may receive the best education possible, and it is immensely appreciated.

Thank you to the members of the jury for this thesis, Jean-François Faucher, Jaqueline Wendland, Marie-Aline Charles, and Michael Ramharter, for taking the time to review and support this work.

Thank you to Pierre-Yves Ancel, who welcomed me with enthusiasm into the EPOPé research team and who inspires new researchers like myself to voice our opinions and participate in team efforts, no matter our career status. He is an exemplary leader and researcher.

Thank you to the EPOPé team, particularly those at Hopital Tenon who I had the honor of working alongside these past years: Valérie, Véronique V, Véronique P, Laetitia, Monique, Thomas, Marie-Jo, Alice, Helöise, Sandra, Sabrina, Mathilde, Shukrullah, Robert, Andrei, Sylvie, Marine, and countless others who passed through our lab. Professional endeavors are

made all the more enriching when surrounded by wonderful, loving colleagues, and I am grateful for the laughs, interesting discussions, and experiences we all shared together.

Thank you to all of the co-authors involved in the publications related to this thesis: Babak Khoshnood, David Courtin, Michel Cot, André Garcia, Jules Alao, Nadine Fievet, Roméo Zoumenou, Achille Massougbodji, Michael Boivin, Michael Mireku, Moudachirou Ibikounle, Asia Maselko, Marie-Josèphe Saurel-Cubizolles, Suzanne Maman, Jacqueline Milet, and Pierre Ayotte. For their contributions and guidance in teaching me how to conduct high-quality research and how to adequately communicate my work to others.

Thank you to EHESP, including Martine Belanger and Florence Bodeau-Livinec, for allowing me the opportunity to work as a teaching assistant for various Biostatistic and Epidemiology courses in the MPH, which has ignited in me a passion for teaching and working in tandem with public health students across disciplines.

Thank you to the Réseau Doctorale de Santé Publique (RDSP) of EHESP, particularly Karine Laboux and Emmanuelle Denieul, for the scientific meetings, formal trainings, and educational opportunities bestowed on me through this network of talented and dedicated public health actors. Being a part of this network reminded me how important interdisciplinary research is in tackling public health issues, once again reinforcing the fact that research is not a solitary endeavor, but a group effort.

Thank you to all of the researchers and field staff, those already mentioned here and those who I have not had the opportunity to work with directly, who were part of the MiPPAD, APEC, TOVI, TOLIMMUNPAL, and EXPLORE studies in Allada, Benin. I would like to particularly thank Roméo Zoumenou for his expertise and collaboration on the qualitative study mentioned in this thesis, his dedication and work towards helping others is extremely admirable.

Thank you to the women and their children who generously participated in these studies and furthered the epidemiological work needed towards eliminating the burden of parasitic infection in Benin.

Thank you for the funders of the work carried out for this thesis, Sorbonne Université and PRINCEPS. Without the funding and support of institutions such as these, scientific and public health research and their subsequent innovations would not be possible.

Lastly, thank you to my friends and family in France, Spain, the United States, and all around the world. They are the people who kept me sane, even in the insane moments of life, and were my biggest cheerleaders. I especially thank my Dad, my sisters Emily and Shadia, my sassy nephews and niece, my aunts Mish and Wee, my uncles, and my amazing cousins who are more like siblings.

Research unit and funding

Research unit:

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Research funding:

This thesis was funded by a grant awarded to Amanda Garrison from Sorbonne Université (doctoral contract n° 3228/2018). A grant was also received from the Programme de Recherche Interdisciplinaire sur les Crises et la Protection Sanitaires (PRINCEPS). Funding sources for the studies included in the work of this thesis are presented in the Materials and Methods.

List of publications and conferences

Accepted publications

1. **Garrison A**, Khoshnood B, Courtin D, Milet J, Garcia A, Massougbojji A, Ayotte P, Cot M, Bodeau-Livinec F. (2019) Blood lead level in infants and subsequent risk of malaria: A prospective cohort study in Benin, Sub-Saharan Africa. PLoS ONE 14(7): e0220023. <https://doi.org/10.1371/journal.pone.0220023>

2. **Garrison A**, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, Ibikounlé M, Massougbojji A, Cot M, Bodeau-Livinec F. (2021) Soil-transmitted helminth infection in pregnancy and long-term child neurocognitive and behavioral development: A prospective mother-child cohort in Benin. PLoS Neglected Tropical Diseases 15(3): e0009260.

Submitted publications

1. **Garrison A**, Maselko J, Courtin D, Zoumenou R, Boivin MJ, Massougbojji A, Alao JM, Cot M, Maman S, Bodeau-Livinec F. The impact of maternal depression and parent-child interactions on risk of parasitic infection in Beninese children.

Submitted August 2020 to *Maternal and Child Health Journal*

2. **Garrison A**, Boivin MJ, Fiévet N, Zoumenou R, Alao JM, Massougbojji A, Cot M, Bodeau-Livinec F. The effects of malaria in pregnancy on neurocognitive development in children at one and six years of age in Benin: a prospective mother-child cohort.

Submitted April 2021 to *Clinical Infectious Diseases*

Conference presentations

1. **Garrison A**, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, Ibikounlé M, Massougbojji A, Cot M, Bodeau-Livinec F. Infections par helminthes transmis par le sol pendant la grossesse et ses conséquences sur le développement de l'enfant. *Journée de Jeunes Chercheurs en Périnatalité : DHU Risque Et Grossesse*. July 2019, Paris, France. (Poster)

2. **Garrison A**, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, Ibikounlé M, Massougbojji A, Cot M, Bodeau-Livinec F. Soil-transmitted helminth infection in pregnancy

and long-term child neurocognitive and behavioral development. *Journée de l'ED 393*. October 2019, St. Malo, France. (Poster)

3. **Garrison A**, Maselko J, Maman S, Bodeau-Livinec F. The impact of maternal depression and parent-child interactions on risk of parasitic infection in Beninese children. *68th Annual Meeting of the American Society of Tropical Medicine and Hygiene*. November 2019, National Harbor, MD, USA. (Poster)

4. **Garrison A**, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, Ibikounlé M, Massougbodji A, Cot M, Bodeau-Livinec F. Soil-transmitted helminth infection in pregnancy and long-term child neurocognitive and behavioral development. *11^{ème} Rencontres Scientifiques du Réseau Doctorale de l'EHESP*. March 2020, Marseille, France. (Poster)

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List of Abbreviations and Acronyms

ADHD	Attention-deficit hyperactivity disorder
ANV	Antenatal care visit
APEC	<i>Anemia in Pregnancy: Etiologies and Consequences</i>
BOT-2	Bruininks-Oseretsky Test of Motor Proficiency Edition 2
CDC	Centers for Disease Control
CRP	C-reactive protein
CI	Confidence interval
DAG	Direct Acyclic Graph
DALY	Disability-adjusted life years
EPDS	Edinburgh Postnatal Depression Scale
EXPLORE	<i>EXposition au PLomb et au manganèse et Risques pour l'Enfant</i>
GHA	Global Health Assessment
HIV	Human immunodeficiency virus
HOME	Home Observation for Measurement of the Environment
IDA	Iron deficiency anemia
IPTp	Intermittent preventive treatment in pregnancy
IRS	Indoor residual spraying
ITN	Insecticide-treated bed nets
IRD	Institut de Recherche pour le Developpement
KABC-II	Kaufman Assessment Battery in Children 2 nd Edition
LLITN	Long-lasting insecticide-treated bed nets
MIP	Malaria in pregnancy
MiPPAD	<i>Malaria in Pregnancy Preventive Alternative Drugs</i>
MPI	Mental Processing Index
MSEL	Mullen Scales of Early Learning
MQ	Mefloquine
NVI	Non-Verbal Index
qPCR	Quantitative polymerase chain reaction
rDNA	Ribosomal deoxyribonucleic acid
RDT	Rapid diagnostic test
SDQ	Strengths and Difficulties Questionnaire
SHIP	Soil-transmitted helminth infection in pregnancy

SP	Sulfadoxine-pyrimethamine
STH	Soil-transmitted helminth
TOLIMMUNPAL	<i>Tolérance Immunitaire et Paludisme</i>
TOVA	Test of Variables of Attention
WHO	World Health Organization

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Abstract

Soil-transmitted helminth and malaria infection, two parasitic infections found primarily in low- and middle-income populations, impact up to 50% of pregnant women in Sub-Saharan Africa. Parasitic infection in pregnant women is known to have negative consequences on maternal and infant health, such as increased risk of maternal anemia, pre-term birth, low birth weight, and childhood parasitic infection. However, its impacts on child neurocognitive and behavioral development remain widely unknown. Therefore, this thesis sought to study the impacts of soil-transmitted helminth infection in pregnancy (SHIP) and *Plasmodium falciparum* malaria in pregnancy (MIP) on child neurocognitive and behavioral development in a mother-child cohort from Benin, as well as investigate additional risk factors for parasitic infection that could impact associations. Our mother-child cohort included pregnant women enrolled in a clinical trial in their second trimester comparing the efficacy of two intermittent preventive treatments in pregnancy against malaria, sulfadoxine-pyrimethamine and mefloquine, in Allada, Benin. Blood and stool samples were collected from women at three antenatal care visits (ANVs); SHIP was diagnosed using the Kato-Katz technique and MIP was diagnosed by thick blood smear tests at ANVs and quantitative polymerase chain reaction (qPCR) methods at delivery. Women received treatment for infection according to existing national guidelines, as well as the standard antenatal care package for Benin at the first ANV. Surviving offspring were assessed by trained investigators for neurocognitive and motor development at one year using the Mullen Scales of Early Learning and again at six years using the Kaufman Assessment Battery in Children 2nd Edition (KABC-II), the Bruininks-Oseretsky Test of Motor Proficiency Edition 2, the Test of Variables of Attention, and the Strengths and Difficulties Questionnaire (SDQ). A sub-cohort of mothers and children were included in independent analyses to test the role of child blood lead level and maternal depression on subsequent risk of parasitic infection. Prevalence of SHIP was 13% at the 1st ANV, 9% at the 2nd ANV, and 1% at delivery. According to thick blood smear tests, 122 (25%) women were infected with malaria at least once during pregnancy and 121 (31%) were infected with placental malaria diagnosed by qPCR at delivery. Within the sub-cohort, median blood lead level among children measured at 12 months was 56.50 (4.81–578) µg/L and 39/302 (13%) mothers had depressive symptoms 12-months post-partum according to the Edinburgh Postnatal Depression Scale. According to multiple linear regression analyses, SHIP was not associated with low neurocognitive scores in children at six years. Higher SDQ internalizing scores, indicating increased emotional impairments in children, were associated with helminth

infection at the 2nd ANV/delivery [1.07 Confidence Interval (CI) (0.15, 2.00)] and at least once during pregnancy [0.79 CI (0.12, 1.46)] in adjusted models. Beta coefficients showed that impaired gross motor scores at one year were associated with MIP at least once [-2.55 CI (-5.15, 0.05)], placental malaria by qPCR [-4.95 CI (-7.65, -2.24)], and high parasite density at delivery [-1.92 CI (-3.86, 0.02)]. Malaria and high parasite density at the 2nd ANV were associated with lower KABC-II Non-Verbal Index scores at six years [-2.57 CI (-4.86, -0.28)] and [-1.91 CI (-3.51, -0.32)], respectively. Neither blood lead level nor maternal depression were found to impact subsequent risk of parasitic infection in children according to regression analyses, before and after adjustment of relevant factors. This thesis provides evidence of long-term behavioral and neurocognitive consequences of parasitic infection in pregnancy in a mother-child cohort with extensive, prospective biological and neurocognitive assessments in West Africa. A better understanding of the mechanisms behind these associations is necessary in order to add to existing evidence that sufficient and early diagnosis and treatment of these infections is imperative and of international public health concern.

Keywords: soil-transmitted helminth infection; malaria; pregnancy; child neurocognitive development; Sub-Saharan Africa

Résumé court

Les infections par helminthes transmis par le sol et le paludisme, deux infections parasitaires que l'on trouve principalement dans les populations à faible et moyen revenu, touchent jusqu'à 50 % des femmes enceintes en Afrique subsaharienne. Les infections parasitaires chez la femme enceinte ont des conséquences négatives sur la santé de la mère et du nourrisson, comme un risque accru d'anémie maternelle, de naissance prématurée, de faible poids de naissance et d'infection parasitaire infantile. Cependant, leur impact sur le développement neurocognitif et comportemental de l'enfant reste largement inconnu. Par conséquent, cette thèse visait à étudier l'impact de l'infection par helminthes transmis par le sol pendant la grossesse (SHIP) et du paludisme à *Plasmodium falciparum* pendant la grossesse (MIP) sur le développement neurocognitif et comportemental de l'enfant dans une cohorte mère-enfant du Bénin, ainsi qu'à rechercher des facteurs de risque supplémentaires d'infection parasitaire qui pourraient avoir un impact sur ces associations. Notre cohorte mère-enfant comprenait 493 femmes enceintes inscrites à un essai clinique au deuxième trimestre comparant l'efficacité de deux traitements préventifs intermittents contre le paludisme pendant la grossesse, la sulfadoxine-pyriméthamine et la méfloquine, à Allada, Bénin. Des échantillons de sang et de selles ont été prélevés chez les femmes lors de trois consultations prénatales (CPNs) ; le SHIP a été diagnostiqué en utilisant la technique de Kato-Katz et le MIP a été diagnostiqué par des tests gouttes épaisses lors des CPNs et par des méthodes de réaction en chaîne par polymérase quantitative (qPCR) lors de l'accouchement. Les femmes ont reçu des traitements de prévention contre les infections parasitaires conformément aux directives nationales existantes, ainsi qu'un paquet de soins prénatals standard pour le Bénin au premier CPN. Les enfants survivants ont été évalués par des enquêteurs qualifiés sur le plan du développement neurocognitif et moteur à un an avec le Mullen Scales of Early Learning, puis à six ans avec le Kaufman Assessment Battery in Children 2nd Edition (KABC-II), le Bruininks-Oseretsky Test of Motor Proficiency Edition 2 (BOT-2), le Test of Variables of Attention (TOVA), et le Strengths and Difficulties Questionnaire (SDQ). Une sous-cohorte de mères et d'enfants a été incluse dans des analyses indépendantes pour tester le rôle de la plombémie et de la dépression maternelle dans le risque ultérieur d'infection parasitaire. La prévalence du SHIP était de 13% au 1er CPN, 9% au 2ème CPN, et 1% à l'accouchement. D'après les gouttes épaisses, 122 (25%) femmes étaient infectées par le paludisme au moins une fois pendant la grossesse et 121 (31%) étaient infectées par le paludisme placentaire diagnostiqué par qPCR à l'accouchement. Au sein de la sous-cohorte, la plombémie médiane des enfants mesurée à 12 mois était de 56,50 (4,81-

578) $\mu\text{g/L}$ et 39/302 mères (12,9%) présentaient des symptômes dépressifs 12 mois après l'accouchement selon l'échelle de dépression postnatale d'Édimbourg. Selon les analyses de régression linéaire multiple, le SHIP n'était pas associé à de faibles scores neurocognitifs chez les enfants à six ans. Des scores d'intériorisation SDQ plus élevés, indiquant des déficiences émotionnelles accrues chez les enfants, étaient associés à une infection helminthique lors de la 2e CPN/accouchement [1,07 Intervalle de Confiance (IC) (0,15, 2,00)] et au moins une fois pendant la grossesse [0,79 IC (0,12, 1,46)] dans les modèles ajustés. Les coefficients bêta ont montré que l'altération des scores de motricité globale à un an était associée à une MIP au moins une fois [-2,55 IC (-5,15, 0,05)], au paludisme placentaire par qPCR [-4,95 IC (-7,65, -2,24)] et à une densité parasitaire élevée à l'accouchement [-1,92 IC (-3,86, 0,02)]. Le paludisme et une densité parasitaire élevée lors de la 2e CPN étaient associés à des scores inférieurs à l'indice non verbal KABC-II à six ans [-2,57 IC (-4,86, -0,28)] et [-1,91 IC (-3,51, -0,32)], respectivement. Ni la plombémie ni la dépression maternelle ne se sont avérées avoir un impact sur le risque ultérieur d'infection parasitaire chez les enfants selon les analyses de régression, avant et après ajustement des facteurs pertinents. Cette thèse fournit des informations des conséquences comportementales et neurocognitives à long terme de l'infection parasitaire pendant la grossesse dans une cohorte mère-enfant avec des évaluations biologiques et neurocognitives exhaustives et prospectives en Afrique de l'Ouest. Une meilleure compréhension des mécanismes à l'origine de ces associations est nécessaire afin d'ajouter aux preuves existantes qu'un diagnostic et un traitement suffisants et précoces de ces infections sont impératifs et constituent une préoccupation de santé publique internationale.

Mots-clés: infection aux helminthes transmis par le sol ; paludisme ; grossesse ; développement neurocognitif de l'enfant ; Afrique subsaharienne

Résumé extensif

Introduction

Deux des infections parasitaires les plus fréquentes en Afrique subsaharienne sont les infections par helminthes transmises par le sol, qui affecte 1,5 milliard d'individus chaque année (1), et le paludisme, l'infection parasitaire la plus courante en Afrique d'où proviennent plus de 94 % des 229 millions de cas diagnostiqués en 2019; les autres 6 % des cas provenant d'Asie et d'Amérique du Sud. (2). Le terme "helminthes transmis par le sol" désigne plusieurs espèces de vers parasites, dont les plus courants sont les ascaris (*Ascaris lumbricoides*), les trichures (*Trichuris trichiura*) et les ankylostomes (*Ancylostoma duodenale* et *Necator americanus*) (3). Les femmes enceintes et les enfants de moins de cinq ans sont particulièrement vulnérables à ces infections parasitaires et à leurs conséquences sur la santé telles que l'anémie maternelle, l'insuffisance pondérale à la naissance, la naissance prématurée, le retard de croissance fœtale et la malnutrition (4–7). L'Organisation mondiale de la santé (OMS) recommande actuellement le traitement prophylactique de l'infection par les helminthes et du paludisme lors des visites de routine des soins prénatals dans les pays d'Afrique subsaharienne où les taux de morbidité et de mortalité dus aux infections parasitaires sont élevés. Au Bénin, une petite nation francophone d'Afrique de l'Ouest, les femmes enceintes reçoivent une dose unique de mébendazole (500 mg) ou d'albendazole (400 mg) en prophylaxie pour prévenir les infections par helminthes pendant la grossesse et trois doses de traitement préventif intermittent pendant la grossesse par sulfadoxine-pyriméthamine contre le paludisme (2,8). Même en présence de soins prénatals de routine et de prévention de ces infections, pour lesquels l'adhésion peut souvent être faible (9), la morbidité chez les femmes enceintes et leur enfants survivants continue d'être une grande préoccupation de santé publique internationale (10,11).

Les conséquences de ces infections sur le développement neurocognitif du fœtus restent largement inconnues. Une étude précédente a révélé que les infections par helminthes pendant la grossesse, en particulier les ankylostomes, altère négativement le développement de la motricité globale de la progéniture un an après l'accouchement dans une cohorte d'enfants béninois (12). En ce qui concerne le paludisme pendant la grossesse, une étude en laboratoire a trouvé une corrélation entre la progéniture exposée au paludisme expérimental et les déficits neurologiques (13). En outre, les femmes enceintes et les enfants des pays à faibles revenus ou à revenus intermédiaires sont exposés à un large éventail de facteurs de risque d'infections parasitaires. Des études récentes ont montré des résultats inédits liant l'exposition environnementale au plomb (Pb) à une diminution du risque d'infection par le paludisme (14),

et la dépression maternelle à une augmentation du risque d'infection du nourrisson (15). Cependant, des recherches supplémentaires sur ces sujets sont nécessaires afin de mieux comprendre les facteurs de risque d'infection parasitaire dans les zones de forte transmission. Compte tenu de ces lacunes dans la littérature et dans la compréhension épidémiologique des infections parasitaires pendant la grossesse dans les régions du monde où ces infections sont fréquentes, nous avons cherché à étudier ces questions dans le cadre de cette thèse. Cette thèse a donc cherché à étudier l'impact des infections par helminthes pendant la grossesse (SHIP) et du paludisme à *Plasmodium falciparum* pendant la grossesse (MIP) sur le développement neurocognitif et comportemental de l'enfant dans une cohorte mère-enfant du Bénin suivie du deuxième trimestre de la grossesse jusqu'à six ans après l'accouchement. Les objectifs principaux de cette thèse étaient d'étudier l'impact de deux infections parasitaires pendant la grossesse, l'infection par helminthes et le paludisme, sur le développement neurocognitif et comportemental de l'enfant à court et à long terme dans une cohorte mère-enfant du district d'Allada, au Bénin. Les objectifs secondaires de cette thèse visaient à étudier les facteurs sociaux et environnementaux potentiels liés au risque d'infection parasitaire qui auraient pu avoir un impact sur les associations entre l'infection parasitaire pendant la grossesse et le développement neurocognitif de l'enfant dans notre population.

Objectifs principaux

1. Étudier l'impact des infections par helminthes pendant la grossesse sur le développement neurocognitif et comportemental de l'enfant à l'âge de six ans.
2. Étudier l'impact de l'infection palustre à *P. falciparum* pendant la grossesse sur le développement neurocognitif de l'enfant à un et six ans.

Objectifs secondaires

- a. Étudier l'association entre la dépression maternelle et les interactions parent-enfant et le risque d'infection par helminthes et le paludisme.
- b. Étudier l'association entre la plombémie de l'enfant et le risque d'infection palustre.

Ces études préliminaires ont permis d'étudier les rôles potentiels de confusion de la plombémie, de la dépression maternelle et des interactions parent-enfant dans la relation entre les infections par helminthes et le paludisme et le développement de l'enfant.

Méthodes

Les femmes enceintes de cette cohorte prospective mère-enfant ont été recrutées dans l'essai clinique *Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD)* (NCT00811421) au cours du deuxième trimestre de grossesse. L'objectif de l'essai était de comparer l'efficacité de deux traitements préventifs intermittents du paludisme pendant la grossesse (TPIp), la sulfadoxine-pyriméthamine (SP) et la méfloquine, dans trois centres de santé du district d'Allada au Bénin (Sékou, Allada et Attogon). Les informations sur les critères d'inclusion et d'exclusion sont détaillées dans une publication précédente (16). Brièvement, les critères d'inclusion des femmes étaient les suivants : résidence permanente dans la zone d'étude, âge gestationnel ≤ 28 semaines, séronégativité au moment du recrutement, absence d'allergies connues aux sulfamides ou à la méfloquine, absence de traitement du paludisme par ces médicaments dans les quatre semaines précédant le recrutement, et absence d'antécédents de maladie rénale, hépatique, psychiatrique ou neurologique grave. Les femmes enceintes incluses dans la population d'étude de cette thèse ont été également suivies dans le cadre du projet *Anemia in Pregnancy : Etiologies and Consequences* (APEC) afin d'être suivies pour l'infection parasitaire pendant la grossesse.

Dans le cadre de l'essai clinique, 1,005 femmes enceintes ont été vues à deux consultations prénatales (CPNs) dans l'une des trois maternités à au moins un mois d'intervalle, ainsi qu'à l'accouchement, pour un total de trois CPNs programmées. Dans le cadre des directives de soins prénatals standard au Bénin, les femmes ont reçu des moustiquaires imprégnées et 600 mg de mébendazole (100 mg à prendre deux fois par jour pendant trois jours) pour la prévention des infections helminthiques au moment du recrutement, ainsi que des suppléments de sulfate ferreux et d'acide folique à chaque CPN pour la prévention de l'anémie. Les épisodes de paludisme clinique ont été traités par quinine par voie orale au premier trimestre ou par artéméther-luméfantrine aux trimestres suivants pour le paludisme non compliqué ; le paludisme grave a été traité par quinine parentale (17). Lors de la première CPN, des informations sociodémographiques et anthropométriques ont été recueillies, notamment l'âge maternel, la gravidité, l'indice de masse corporelle (IMC) avant la grossesse, l'état civil, la profession et le niveau d'éducation de la mère et du père, la zone de résidence, et des informations relatives à l'utilisation des moustiquaires imprégnées la nuit précédente. Dans chaque CPN, des gouttes épaisses d'échantillons de selles ont été préparés et évalués à l'aide de la technique de Kato-Katz pour obtenir une estimation quantitative de l'intensité de l'infection par les helminthes selon les normes de l'OMS (18). Les œufs d'helminthes des espèces *Ascaris lumbricoides*, *Trichuris trichiura*, *Ancylostoma duodenale* et *Necator americanus* ont été identifiés. L'infection palustre par *Plasmodium*

falciparum a été déterminée à chaque CPN, y compris lors de l'accouchement, par des tests de gouttes épaisses et fins à partir d'échantillons de sang collectés en utilisant la technique de Lambaréné. Lors de l'accouchement, le sang périphérique maternel et le sang placentaire ont été déposés sur des papiers filtres pour une évaluation parasitologique. Les papiers filtres ont été testés pour la présence et la densité de *P. falciparum* par un test quantitatif en temps réel de réaction en chaîne par polymérase (qPCR) ciblant l'ADN ribosomal 18S (19). Une sous-cohorte de 400 enfants a été choisie pour participer à deux études auxiliaires, APEC et *Tolérance Immunitaire et Paludisme* (TOLIMMUNPAL), qui ont suivi les enfants de la naissance jusqu'à l'âge de 24 mois pour l'infection parasitaire et les facteurs hématologiques et environnementaux associés. A chaque visite entre la naissance et 12 mois, 8 ml de sang veineux étaient prélevés chez les nourrissons et la technique de Lambaréné était utilisée pour diagnostiquer le paludisme sur des gouttes épaisses. De 12 à 24 mois, l'infection palustre a été diagnostiquée au cours du suivi de 12 mois en utilisant à la fois des tests gouttes épaisses et des tests de diagnostic rapide (TDR) lors des visites systématiques mensuelles et bihebdomadaires, ainsi que lors des visites d'urgence.

Les 863 singletons survivants à l'âge d'un an et nés de femmes enceintes de l'essai MiPPAD étaient éligibles pour participer à l'étude TOVI (*enfant* en Fon). Le développement neurocognitif et moteur du nourrisson a été mesuré par une infirmière de recherche qualifiée lors d'une visite programmée un an après l'accouchement dans une maternité locale, à l'aide de l'édition de l'American Guidance Service des Mullen Scales of Early Learning (MSEL) dont la traduction française réalisée lors de l'étude a été approuvée par Pearson assessment (20). Les scores bruts de chacune des cinq sous-échelles ont été calculés puis convertis en scores t normatifs ; les scores t pour les échelles de motricité fine, de langage réceptif, de langage expressif et de perception visuelle ont été convertis en score composite d'apprentissage précoce (ELC), indicatif du fonctionnement cognitif global des enfants. La plombémie des enfants a été évaluée en analysant des échantillons de sang veineux par spectrométrie de masse à plasma à couplage inductif. Des informations sur l'environnement d'apprentissage à domicile, mesurées par l'inventaire HOME (Home Observation for Measurement of the Environment), ont été recueillies après son adaptation pour être utilisées dans cette population. Les mères ont également été évaluées pour les symptômes de dépression du post-partum au moyen de la version française validée de l'EPDS (Edinburgh Postnatal Depression Scale).

À l'âge de six ans, 578 enfants ont été suivis dans le cadre de l'étude EXPLORE (*EXposition au PLomb et au manganèse et Risques pour l'Enfant*) pour des évaluations

neurocognitives supplémentaires. Des échantillons de sang et de selles ont été prélevés chez les enfants pour évaluer l'infection par les helminthes et le paludisme au moment des évaluations neurocognitives, et les enfants infectés ont été traités conformément aux directives nationales. La batterie d'évaluation Kaufman pour enfants, 2e édition (KABC-II), a mesuré les processus simultanés et séquentiels, ainsi que les capacités de planification et d'apprentissage des enfants. L'indice des processus mentaux (IPM), une mesure de la capacité cognitive globale, et l'indice non verbal (INV), une évaluation non verbale du traitement et des capacités cognitives de l'enfant, tous deux générés par les scores KABC-II, ont été utilisés dans les analyses. La Bruininks-Oseretsky Test of Motor Proficiency Edition 2 (BOT-2) a été utilisée pour l'évaluation des habiletés motrices dans cette population ; les scores composites finaux pour la motricité fine et la motricité globale ont été normalisés selon l'âge et le sexe et utilisés aux fins de cette thèse. Le Test of Variables of Attention (TOVA), un test de performance non basé sur le langage, a été administré pour identifier le déficit d'attention et l'hyperactivité chez les enfants. Enfin, les mères ont répondu au Strengths and Difficulties Questionnaire (SDQ), un bref outil de dépistage de 25 questions visant à évaluer le développement comportemental des enfants en fonction de cinq sous-échelles : problèmes émotionnels, problèmes de comportement, hyperactivité, problèmes avec les pairs et comportements pro-sociaux ; les quatre premières sous-échelles étant utilisées pour générer un score global de difficultés.

Les caractéristiques démographiques de la population incluse dans les analyses ont d'abord été décrites et comparées à la population perdue de vue entre la naissance et l'âge de six ans à l'aide de tests univariés appropriés (test du chi deux, Fisher exact, Kruskal-Wallis ou Wilcoxon ranksum). Des modèles de régression linéaire multiple avec des intervalles de confiance bootstrapped à 95 % ont testé les principales associations entre l'exposition à l'infection parasitaire pendant la grossesse et les scores neurocognitifs et comportementaux continus des enfants à un et six ans. Diverses analyses de sensibilité, dont l'imputation multiple et les analyses de médiation, ont été menées pour garantir la robustesse des résultats de la régression. Les analyses portant sur les facteurs de confusion potentiels de la plombémie de l'enfant et de la dépression maternelle ont fait appel à des modèles de régression binomiale négative et logistique simple, dont les méthodes sont précisées dans chaque publication spécifique incluse dans cette thèse. Les analyses statistiques ont été réalisées à l'aide de STATA 13.1 (StataCorp. 2013. Logiciel statistique Stata : Release 13. College Station, TX : StataCorp LP) pour Windows et STATA 16 (StataCorp. 2019. Logiciel statistique Stata : Release 16. College Station, TX : StataCorp LLC) pour Mac. Tous les tests statistiques étaient

bilatéraux avec un risque alpha de 5%, sauf indication contraire dans le manuscrit ou dans les publications.

Résultats

La prévalence du SHIP dans notre population était de 12,6% à la 1ère CPN, 8,5% à la 2ème CPN et 1,2% à l'accouchement. Toutes les femmes ont reçu un total de 600 mg de mébendazole (100 mg deux fois par jour pendant 3 jours) à prendre après la 1ère CPN ; cependant, la prise n'a pas été directement observée. La prévalence de la MIP périphérique par goutte épaisse était de 15% à la 1ère CPN, de 3% à la 2ème CPN et de 10% à l'accouchement. A l'accouchement, 31% des femmes testées avaient un paludisme placentaire selon les analyses qPCR. Le SHIP diagnostiqué à chaque CPN et l'infection helminthique globale ainsi que la densité d'ankylostomes pendant la grossesse ne se sont pas avérés être associés au développement neurocognitif, à la fonction motrice ou au trouble de l'attention/hyperactivité chez les enfants à l'âge de six ans. Cependant, le SHIP a été associé à une altération du développement émotionnel, mesurée par le score d'intériorisation du SDQ. L'infection à helminthes lors de la 2e CPN/l'accouchement et à n'importe quel moment de la grossesse était associée à des scores d'intériorisation plus élevés, indiquant davantage de problèmes émotionnels, coefficient bêta [1,07 intervalle de confiance (IC) à 95 % (0,15, 2,00)] et [0,79 (0,12, 1,46)], respectivement, après ajustement pour les facteurs de risque identifiés dans les graphiques acycliques directs. L'imputation multiple des variables manquantes et des paires mère-enfant perdues de vue a confirmé ces résultats. Une analyse de médiation par des techniques de modélisation par équations structurelles a permis d'évaluer le rôle de facteurs médiateurs potentiels, tels que l'inflammation, dans cette association. Cependant, l'inflammation mesurée par la protéine C-réactive pendant la grossesse ne s'est pas avérée être un facteur médiateur significatif.

Alors que le MIP diagnostiqué au moins une fois pendant la grossesse n'a pas altéré de manière significative les scores ELC chez les enfants, il a altéré de manière significative les scores de développement de la motricité globale à un an après ajustement sur les facteurs de risque [-2,55 (-5,15, 0,05)]. En particulier, le paludisme diagnostiqué par qPCR et une densité parasitaire plus élevée à l'accouchement étaient associés à une altération significative des scores de développement de la motricité globale chez les enfants après ajustement, [-4,95 (-7,65, -2,24)] et [-1,92 (-3,86, 0,02)], respectivement. Le MIP diagnostiqué lors d'une CPN ou d'un accouchement par goutte épaisse n'était pas associé à une altération des scores de développement cognitif ou moteur chez les enfants à un an. Une densité parasitaire élevée à l'accouchement était associée à une altération des scores de l'IPM dans les modèles ajustés [-

1,25 (-2,46, -0,05)]. Le paludisme diagnostiqué par microscopie et la densité parasitaire élevée lors de la 2e CPN étaient tous deux associés de manière significative à une altération des scores INV du KABC-II dans les modèles ajustés [-2,57 IC (-4,86, -0,28)] et [-1,91 IC (-3,51, -0,32)], respectivement. Les infections palustres tardives lors de la deuxième CPN et de l'accouchement ont montré des tendances à la détérioration des scores de l'IPM et de l'INV à six ans, bien que statistiquement non significatives.

Concernant les objectifs secondaires de cette thèse, la plombémie médiane parmi les 204 enfants de nos analyses était de 56,50 (\pm 56,83) μ g/L, au-dessus du seuil d'intervention des Centers for Disease Control (CDC). Nos analyses ont révélé que la plombémie dans cette population d'enfants n'était pas associée à un plus grand nombre d'épisodes de paludisme entre 12 et 24 mois, même après ajustement sur d'autres facteurs de risque pertinents. Nos analyses de régression n'ont pas trouvé que la dépression maternelle un an après l'accouchement avait un impact sur le risque d'infection parasitaire chez les enfants. Cependant, nos analyses ont confirmé que la dépression maternelle était associée à une qualité et à une quantité moindre d'interactions parents-enfants, telles que mesurées par l'échelle HOME, comme l'ont montré des recherches antérieures pour avoir un impact sur le développement de l'enfant.

Discussion

Les études menées dans le cadre de cette thèse sont les premières du genre à explorer les conséquences neurocognitives à long terme d'infections parasitaires pendant la grossesse, ajoutant ainsi une contribution significative à la recherche épidémiologique existante. Nos analyses ont montré que le SHIP à n'importe quel moment de la grossesse était associé à une altération du développement comportemental de l'enfant six ans après la naissance. Nos analyses montrent une tendance à une association entre une infection palustre tardive et des scores neurocognitifs plus faibles chez les enfants à un et six ans. Plus important encore, nos analyses suggèrent que les infections palustres submicroscopiques et asymptomatiques sont plus présentes chez les femmes enceintes dans notre population. Cette thèse a également exploré les relations complexes entre l'infection parasitaire et les facteurs de risque potentiels tels que la plombémie et la dépression maternelle, qui pourraient finalement avoir un impact sur le développement neurocognitif de l'enfant.

La nature prospective de cette cohorte mère-enfant et les évaluations biologiques et neurocognitives complètes réalisées au sein de cette population sont des caractéristiques déterminantes des méthodes utilisées. Ces analyses sont les premières du genre à étudier les conséquences neurocognitives à long terme de l'infection parasitaire pendant la grossesse. Parmi les autres points forts de cette thèse, citons les différents outils utilisés pour évaluer le

développement des enfants à un et six ans, comme le KABC-II dont la validité de construction a été validé dans une étude pilote et le SDQ et BOT-2 qui ont été utilisés dans des études antérieures dans des populations similaires (21,22). Des analyses de sensibilité ont permis d'étudier d'autres facteurs intermédiaires et de confusion potentiels, tels que le groupe de traitement TPIp dans l'essai clinique, la carence en fer pendant la grossesse, la plombémie de l'enfant, l'inflammation pendant la grossesse, la naissance prématurée et le faible poids de naissance ; aucun de ces facteurs n'a modifié les résultats de manière significative.

L'imputation multiple a confirmé nos résultats, excluant ainsi tout biais potentiel lié à l'absence de données. Enfin, il n'y avait pas de différences en termes d'exposition aux infections parasitaires chez les femmes incluses et exclues des analyses, le biais de sélection est donc peu probable. Nos analyses présentent plusieurs limites, notamment la taille limitée de l'échantillon dans certaines analyses et la multitude de tests effectués dans cette population, ce qui augmente la probabilité que les résultats soient le fruit du hasard. Les femmes enceintes et les enfants ont été suivis de manière extensive et traités rapidement en cas d'infection parasitaire, ce qui pourrait sous-estimer les associations entre l'infection et le développement neurocognitif de l'enfant.

Au moment de l'essai clinique, seules deux doses d'TPIp-SP étaient recommandées pour prévenir le paludisme dans les zones de forte transmission de l'ASS, comme le Bénin. Nous avons vu dans notre suivi qu'il y avait une prévalence plus élevée d'infection par helminthes et de paludisme chez les femmes enceintes à l'accouchement par rapport à la 2e CPN, ce qui suggère un traitement préventif inadéquat pour ces femmes qui sont régulièrement exposées aux parasites responsables de ces infections. Le paludisme placentaire et la densité parasitaire à l'accouchement, diagnostiqués par qPCR, se sont avérés particulièrement préjudiciables aux scores neurocognitifs et moteurs des enfants. Cela pourrait s'expliquer par le fait que les infections palustres placentaires submicroscopiques peuvent être particulièrement délétères pour la croissance et le développement du fœtus (4,23,24). Alors que la technologie qPCR est capable d'identifier les infections palustres submicroscopiques indétectables par microscopie, les ressources de laboratoire ne sont pas facilement disponibles dans les pays à faible revenu comme le Bénin. Par conséquent, les femmes présentant des infections submicroscopiques dans notre population n'ont pas été immédiatement diagnostiquées et traitées au moment de l'accouchement et ces infections pourraient refléter des infections plus chroniques qui ne sont pas facilement diagnostiquées par les méthodes de microscopie et traitées rapidement.

Il a été démontré que l'infection maternelle pendant la grossesse active la réponse immunitaire inflammatoire de la mère, qui peut à son tour entraîner un risque accru de problèmes et de troubles du comportement chez la progéniture (25,26). Une étude en laboratoire a apporté la preuve d'un lien de causalité entre une infection expérimentale par le paludisme pendant la grossesse et une altération du développement neurocognitif du fœtus (13,27). Une étude précédente a spécifiquement révélé que des marqueurs élevés d'inflammation chez les femmes enceintes étaient associés à davantage de symptômes d'internalisation et d'externalisation chez leur progéniture plus tard dans l'enfance (28). Les mécanismes entre le SHIP et le développement comportemental ont été explorés dans les analyses ; cependant, ces mécanismes n'ont pas été explorés dans les modèles de paludisme. Les recherches sur le moment des infections maternelles pendant la grossesse et leur impact sur la santé du fœtus restent équivoques (6,7). Nos analyses montrent une tendance entre une infection palustre tardive et des scores neurocognitifs plus faibles chez les enfants à un et six ans.

Cette thèse apporte la preuve que l'exposition au plomb, un métal lourd neurotoxique, est toujours un problème de santé publique dans les milieux à faibles ressources comme le Bénin, même si l'exposition au plomb via la peinture et l'essence a été éradiquée dans les pays à hauts revenus pour la plupart (29). Il est essentiel d'assurer une surveillance régulière de l'exposition au plomb dans cette population car les munitions utilisées pour chasser la viande de brousse ou encore la présence de peinture dans les maisons étaient associées à une augmentation de la plombémie des enfants (30,31). Il est également intéressant pour la santé publique d'étudier l'impact de ces expositions environnementales persistantes sur des populations déjà vulnérables à plusieurs autres facteurs de risque de maladie et d'altération du fonctionnement cognitif. Au sein de la sous-cohorte d'enfants suivis pour une infection parasitaire après la naissance, des données ont été collectées par les chercheurs concernant des données environnementales (précipitations, type de sol, sources d'eau à proximité, indice de végétation) et biologiques (nombre d'habitants par pièce dans le ménage, utilisation de moustiquaires et/ou d'insecticides) afin de calculer un risque environnemental d'infection dépendant du temps et de l'espace, quantifiant l'exposition de chaque enfant aux vecteurs du paludisme à l'aide d'un modèle prédictif (31). Cela nous a permis de contrôler les fluctuations de la densité des moustiques entre les ménages, et donc le risque d'infection palustre, dans les analyses.

L'analyse du lien entre la dépression maternelle, les interactions parents-enfants et l'infection parasitaire a permis de fournir des informations précieuses sur les mécanismes

hypothétiques de l'impact de l'infection parasitaire pendant la grossesse et du développement neurocognitif de l'enfant. Une étude précédente avait trouvé une association entre la dépression maternelle et un risque accru de maladie diarrhéique chez l'enfant (15), en émettant l'hypothèse que les femmes déprimées ont plus de difficultés à prendre soin d'elles-mêmes et de leurs enfants, y compris à remarquer quand une maladie survient et à fournir un traitement à temps, ce qui entraîne un risque accru d'infection. Nos analyses de régression n'ont pas révélé que la dépression maternelle un an après l'accouchement avait un impact sur le risque d'infection parasitaire chez les enfants. Cependant, nos analyses ont confirmé que la dépression maternelle était associée à une qualité et à une quantité moindre d'interactions parents-enfants, telles que mesurées par l'échelle HOME, comme l'ont montré des recherches antérieures (32). De nombreux auteurs ont émis l'hypothèse que cette association a des effets négatifs sur le développement neurologique chez les enfants.

L'infection parasitaire dans les zones endémiques du monde, principalement dans les pays à revenu faible et intermédiaire, représente une charge importante de morbidité et de mortalité dans les groupes vulnérables et a des conséquences à long terme sur les survivants. La compréhension de ces conséquences à long terme par une approche interdisciplinaire en santé publique permet l'émergence d'une multitude d'initiatives et d'interventions innovantes. Les problèmes de santé publique d'aujourd'hui nécessitent des approches globales et synergiques pour y faire face (33). La participation d'intervenants de diverses disciplines permet donc une analyse et une action adéquates en matière d'intervention sanitaire. Si la morbidité maternelle liée aux infections parasitaires s'est améliorée au fil des ans, il reste encore beaucoup d'interventions à mener pour améliorer les résultats sanitaires et la qualité de vie de la mère et du fœtus tout au long de la vie.

Mots-clés: infection aux helminthes transmis par le sol ; paludisme ; grossesse ; développement neurocognitif de l'enfant ; Afrique subsaharienne

Introduction

Justification of thesis

Morbidity and mortality from parasitic infections in Sub-Saharan African (SSA) countries remain unacceptably high despite improvements in public health surveillance and treatment (34). Two of the most prevalent parasitic infections are soil-transmitted helminth infections, which impact 1.5 billion individuals every year (1), and malaria infection, the parasitic infection most commonly found in SSA where more than 94% of the 229 million cases diagnosed in 2019 originated from, with the other 6% of infections coming from Asia and South America (2). Pregnant women and children under five years of age are particularly vulnerable to these parasitic infections and their dire health consequences such as anemia, low birth weight, pre-term birth, fetal growth restriction, and malnutrition (4–7). The World Health Organization (WHO) currently recommends prophylaxis treatment of soil-transmitted helminth and malaria infection during routine antenatal care visits in SSA countries with high morbidity and mortality rates from parasitic infection. In Benin, a small French-speaking West African nation, pregnant women receive single-dose mebendazole (500 mg) or albendazole (400 mg) prophylaxis to prevent soil-transmitted helminth infection in pregnancy (SHIP) and three doses of intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) for malaria in pregnancy (MIP) (2,8). Even in the presence of routine antenatal care and prevention of these infections, for which adherence can often be low (9), morbidity in pregnant women and their surviving offspring continues to be of great international public health concern (10,11).

While the consequences of parasitic infection during pregnancy on maternal and birth outcomes have been widely studied, the consequences of these infections on fetal neurocognitive development remain largely unknown. One previous study found SHIP, particularly from hookworm species, to impair gross motor development in offspring one-year post-partum in a cohort of Beninese children (12). In terms of MIP, one laboratory study found a correlation between experimental malaria-exposed offspring and neurological deficits (13). Previous literature has hypothesized that maternal infection during pregnancy may lead to placental inflammatory immune responses that can alter brain development of the fetus and have long-term neurodevelopment consequences (15,35); however, this pathway as it relates to maternal infection from SHIP and MIP have yet to be investigated.

Additionally, pregnant women and children in low- and middle-income countries are exposed to a wide array of factors increasing their risk of parasitic infections, such as tropical

climates, iron deficiency, inadequate hygiene and sanitation, and conditions of poverty. Recent studies have shown novel results linking environmental exposure to lead (Pb) to a decreased risk of malaria infection (14), and maternal depression to an increased risk of infant infection (15). However, more research is needed in order to better understand the risk factors of parasitic infection in high-transmission areas. Given these gaps in the literature and in the epidemiological understanding of parasitic infection in pregnancy in areas of the world with a high burden of disease, we sought to address these questions within this thesis.

Objectives and hypotheses

The main objectives of this thesis were to study the impact of two parasitic infections during pregnancy, soil-transmitted helminth infection and malaria, on child neurocognitive and behavioral development in a mother-child cohort in the district of Allada, Benin. Secondary objectives of this thesis sought to investigate factors potentially related to risk of parasitic infection that could have an impact on associations between parasitic infection in pregnancy and child neurocognitive development in our population, but that have been widely unstudied in the literature. Below are the main objectives (1 & 2), along with their corresponding secondary objectives (a & b), each of which resulted in a publication within the confines of this thesis and served to explain the overall objective of this thesis: **to study the impacts of parasitic infection in pregnancy on child neurocognitive development in a mother-child cohort in Benin**. The secondary objectives investigated the potential confounding role of blood lead, maternal depression, and parent-child interactions in the relationship between helminth infections and malaria and child development.

Main objectives

1. To study the impact of soil-transmitted helminth infections in pregnancy on child neurocognitive and behavioral development at six years of age
2. To study the impact of *Plasmodium falciparum* malaria infection in pregnancy on child neurocognitive development at one and six years of age

Secondary objectives

- a. To investigate the association between maternal depression and parent-child interactions and risk of helminth infection and malaria.
- b. To investigate the association between child blood lead levels and risk of malaria infection.

We hypothesized that parasitic infection, both soil-transmitted helminth infection and malaria infection, during pregnancy would negatively impact child neurocognitive

development. Based on results by Mireku et al. (2015) that demonstrated an inverse association between SHIP and child gross motor scores as measured by the Mullen Scales of Early Learning (MSEL) at one year of age in this same cohort (12), we hypothesized that SHIP would continue to impair long-term neurocognitive and behavioral development measured by the Kaufman Assessment Battery in Children 2nd Edition (KABC-II) and the Strengths and Difficulties Questionnaire (SDQ), respectively, in this cohort at six years of age. Based on previous hypotheses of the role of maternal inflammation markers as the mechanism for this relationship, we hypothesized that maternal inflammation was a mediating factor between SHIP and impaired child development. There were no previous studies conducted on the impact of MIP on child neurocognitive development; however, one laboratory study on rodent-models found that experimental malaria-exposed offspring had persistent neurological deficits compared to the control group in their analyses (13). Due to this evidence, and the previously mentioned hypothesis that maternal infection may induce an inflammatory response that hinders fetal brain development, we hypothesized that MIP would be associated with impaired neurocognitive development measured by the MSEL and the KABC-II in children in our cohort at both one and six years of age, respectively.

The role of social and environmental factors on risk of parasitic infection was studied in a sub-cohort of 400 mother-child pairs for secondary objectives in order to identify whether these factors should be considered in our main analyses and in order to add to the limited existing epidemiological research on the link between parasitic infection and child development. We hypothesized that maternal depression and poorer quality of parent-child interactions as measured by the Edinburgh Postnatal Depression Scale (EPDS) and the Home Observation Measurement of the Environment (HOME), respectively, would be associated with higher risk of parasitic infection (15). One previous study had identified an inverse association between child blood lead level and risk of malaria infection in this cohort of Beninese children at 12 months of age (14), therefore we hypothesized that we would continue to see this association between high blood lead level and lower risk of malaria infection in a prospective follow-up of the same children from 12-24 months of age in this cohort.

Thesis Overview

This thesis is divided into seven chapters:

Chapter 1 presents background information regarding risk factors and consequences of parasitic infection during pregnancy from soil-transmitted helminths and from malaria, as well as relevant information on child development assessments and risk factors of impaired

development. This chapter provides adequate background knowledge and vocabulary necessary to understand the remainder of the thesis and its attached publications.

Chapter 2 details the materials and methods used within this thesis, describing the study design, population, and data collection of each ancillary study that pregnant women and their children participated in from the second trimester of pregnancy until six years post-partum. The assessment of parasitic infection during pregnancy, child neurocognitive and behavioral development at one and six years, and relevant risk factors such as socio-demographic information, maternal depression, and blood lead level is detailed within this chapter.

Chapter 3 presents the first article investigating the first objective of this thesis, the impact of soil-transmitted helminth infection during pregnancy on child development at six years of age, published in *Plos Neglected Tropical Diseases*.

Chapter 4 includes the second article of this thesis, corresponding to the second objective, assessing the impact of *P.falciparum* malaria during pregnancy on child development at one and six years of age, submitted for publication.

Chapter 5 presents the third article, showing the results of the secondary objective investigating the potential role of maternal depression and parent-child interactions on risk of parasitic infection in a sub-cohort of mothers and their children from our population. The resulting article was submitted for publication.

Chapter 6 presents the fourth article of this thesis, published in *Plos One*, which studied the association between blood lead level and subsequent risk of malaria infection from *P.falciparum* in a prospective sub-cohort of children from our population.

Finally, **Chapter 7** discusses the main findings for each objective of the thesis, the overall strengths and limitations of findings, and their implications across public health domains. Following this chapter is a conclusion that offers recommendations for policy and future research.

Chapter 1. Background: Parasitic infection, pregnancy, and child development

This first chapter provides background information necessary to understand the justification of the objectives of this thesis. The primary objectives sought to study the impact of two parasitic infectious diseases during pregnancy, soil-transmitted helminth infection and malaria, on child neurocognitive development. Secondary objectives explored hypothesized risk factors for parasitic infection that remain largely unstudied in order to assess their importance for models used in the primary objectives. This chapter explains the epidemiology of these two parasitic infections in pregnancy, as well as their known risk factors and consequences on maternal and infant health. Finally, this chapter discusses the factors known to influence child neurocognitive development, and the particular difficulties faced by children in low- and middle-income countries. The purpose of this chapter is to explain the existing literature related to parasitic infection in pregnancy and child neurocognitive development in order to highlight the gaps in the existing literature that this thesis addresses.

Soil-transmitted helminth infection

Soil-transmitted helminth (STH) infections are infections caused by several species of parasitic worms that affect almost a quarter of the world's population. Each year, more than 1.5 billion people, primarily from low- and middle-income countries, are infected by STH species, contributing to over 5 million disability-adjusted life years (DALYs) (36). Symptoms of these infections include gastrointestinal illness such as diarrhea and abdominal pain, as well as overall weakness and malaise. Morbidity from STH infections is directly related to the species of worm and the number of worms, or the parasite density, found in infected individuals. Those with a lighter parasitic density may not show symptoms; however, those with higher density infections may show severe symptoms which can lead to long-term health and developmental consequences (37). As their name suggests, these helminth species transmit infected eggs to individuals through contaminated soil in areas of the world with poor hygiene, sanitation, and access to water. The majority of those infected by STH each year are from Asian and African countries of low- and middle-income. Populations living in these areas of the world can exhibit polyparasitism, meaning individuals may be infected with more than one species of helminth at a time (38). Soil-transmitted helminth infections are among 17 diseases on the list of Neglected Tropical Diseases, established by the WHO as

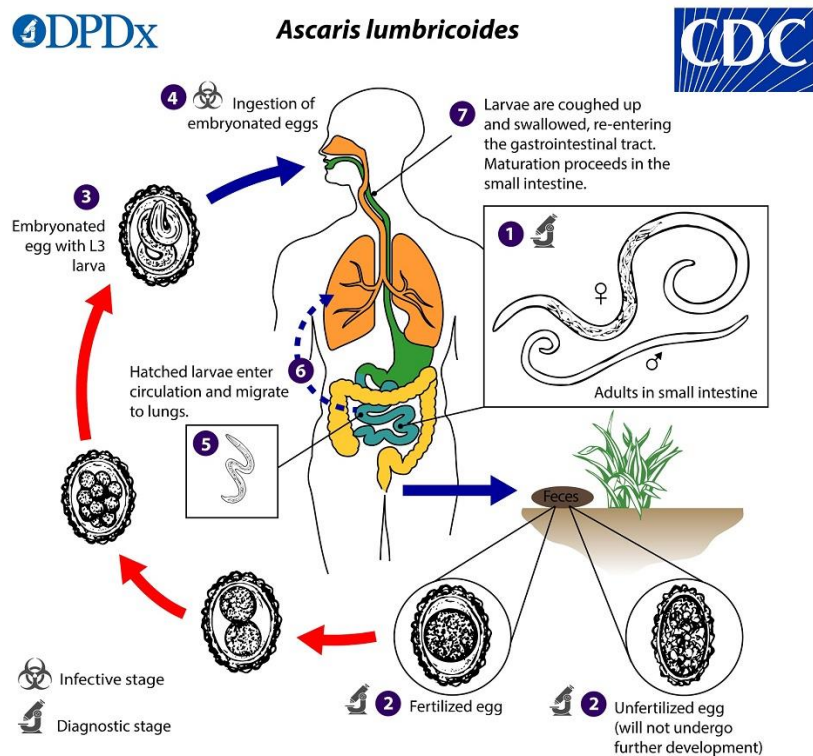
diseases that impact tropical and sub-tropical low-income countries and have not yet been eradicated due to low funding and low attention from public health stakeholders (39).

Life cycle and transmission

The term 'soil-transmitted helminths' refers to several species of parasitic worms, the most common of which are roundworms, *Ascaris lumbricoides*, whipworms, *Trichuris trichiura*, and hookworms, *Ancylostoma duodenale* and *Necator americanus* (3).

A.lumbricoides infects an estimated 800 million individuals per year, primarily children and adolescents (40). This species infects individuals through fecal-oral transmission of eggs, as seen in Figure 1 (41). Adult worms live in the small intestines of infected individuals, and female worms are able to produce hundreds of thousands of eggs per day which are then passed through feces during defecation. In areas of the world with poor sanitation and access to running water, defecation often occurs outside and in unprotected areas where feces can contaminate soil, which creates the perfect life-cycle environment for fertilized helminth eggs. Third-stage larvae develop to infectious levels in fertile eggs within a few weeks. Larvae-infected eggs in the soil are then swallowed, primarily by children playing in the soil, who ingest the soil or fruits and vegetables improperly washed beforehand that still contain traces of soil (42). Once these eggs are swallowed, the larvae hatch and travel from the intestinal mucosa into the lungs where larvae further mature and finally redescend back down into the small intestine to complete the life cycle and reach their adult form. Adult worms can live in the intestines of infected individuals for up to two years if left untreated.

Figure 1. Life cycle and fecal-oral transmission of *A.lumbricoides* species.

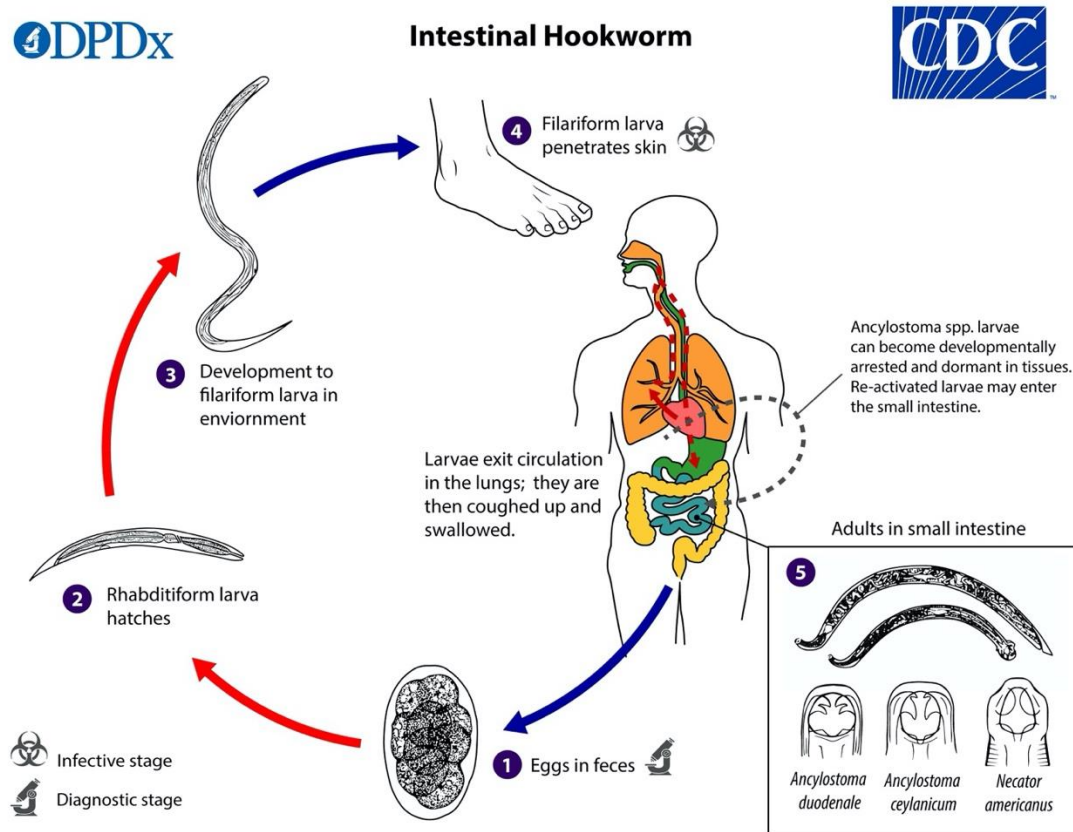


T.trichiura, named whipworm for its shape, infects close to 480 million people per year; again children are found to be the most heavily burdened (40). Similar to *Ascaris*, these whipworm species infect hosts through fecal-oral transmission. However, unlike *Ascaris*, *T.trichiura* larvae do not migrate to the lungs to reach their adult form, but rather attach to intestinal villi and mature into their adult forms within the ascending colon of the intestines (43).

Hookworm species *A.duodenale* and *N.americanus* infect an estimated 470 million people per year, and are most prevalent in adult populations rather than in children, although children may still become infected by these species (40). Hookworms have free-living larvae found in soil that penetrate human skin, usually bare feet, during transmission (Figure 2) (44). Adult worms living in the intestines of infected individuals pass their eggs through feces defecated in nearby soil. Larvae then hatch within a few days and are free-living within the contaminated soil and mature until they reach their infective larval stage. Once infective, these larvae can live in contaminated soil for several weeks until coming into contact with bare skin, normally bare feet of individuals walking in contaminated soil, where they penetrate the skin and travel through blood vessels to the heart and lungs. Once in the lungs, the larvae penetrate the pulmonary alveoli and ascend the bronchial tree to the pharynx where they are swallowed. Finally, the larvae reach the small intestines where they mature into their

adult worm stage and attach themselves onto the intestinal wall and can cause significant blood loss in hosts (45).

Figure 2. Life-cycle and transmission of hookworm species.



Diagnosis, prevention, and treatment

A variety of diagnostic methods are available to detect STH infections, ranging from microscopic methods to antigen/antibody assays to more advanced polymerase chain reaction (PCR) methods (46). The most widely-used method to date is the Kato-Katz technique, developed by Dr. Kan Kato and Naftale Katz in the mid 20th century, which is considered the gold standard for diagnosis of STH infection of multiple species by the WHO (47). This microscopy technique involves the examination of stool samples for the presence of helminth eggs under a light microscope; information on the number of eggs and the type of eggs are recorded in order to diagnose STH infection. The Kato-Katz technique is widely accepted for use in low-income countries with a high-burden of disease from STH due to its low-cost, ability to diagnose multiple species of STH, relatively simple application given the limited laboratory resources in low-income countries, easily learned techniques by laboratory technicians, and high specificity for detection of infection. However, there are some

limitations to this diagnostic tool, such as that it is less reliable in detecting certain species of helminths (*Strongyloidiasis*) and in detecting low egg densities, therefore potentially underestimating the true burden of disease from STH infection in endemic populations (48).

Several preventive measures are available for endemic populations to reduce the burden of disease from STH infection, such as Water, Sanitation, and Hygiene (WASH) campaigns carried out through UNICEF (49). WASH aims to provide clean water and sanitation to low-income areas to improve overall health and wellbeing of under-served populations. These campaigns, which include a focus on hand-washing practices, have been associated with a reduced odds of STH transmission by at least 33% according to a meta-analysis (50). Additionally, wearing adequate footwear while outside has been shown to prevent hookworm infection in particular, due to their mode of transmission via cutaneous penetration (50). While these preventive measures are long-term and require substantial political and societal involvement, systematic anti-helminthic prophylaxis is also used to prevent STH infection in the shorter term among high-risk groups of morbidity and mortality such as children under the age of five, women of reproductive age, and pregnant women.

For children ages 12 months to 12 years and for non-pregnant women of reproductive age, the WHO recommends a single-dose of albendazole (400 mg) or mebendazole (500 mg), two inexpensive and easy-to-administer treatments, given once a year when the baseline prevalence of STH infections in the community is over 20%, and twice a year when the prevalence in the community is over 50% (51,52). For pregnant women, the WHO recommends a single-dose of albendazole (400 mg) or mebendazole (500 mg) given to women at their first antenatal care visit after the first trimester of pregnancy in areas where the baseline prevalence of hookworm infection is 20% or more and where the prevalence of anemia in pregnant women is 40% or more (8), given that hookworm infection in particular can be a major cause of anemia. The prevention and treatment of STH infection in pregnancy is of particular public health importance due to the elevated risks of morbidity and mortality in pregnant women and their offspring.

Soil-transmitted helminth infection in pregnancy

Up to 30% of women in SSA are estimated to be diagnosed with SHIP at least once during routine antenatal care follow-up (53); however, this prevalence ranges between countries, with studies reporting 11% of women diagnosed in Benin and 32% in Ethiopia (54,55) for example. Preventive prophylaxis efforts target pregnant women in particular since

they and their fetus are most at risk of the various consequences of infection during the prenatal period.

Demographic and clinical characteristics have been shown to put certain pregnant women at higher risk of infection than others. A multitude of literature has shown that primigravida women, women who are pregnant for the first time, have an increased susceptibility to SHIP compared to multigravida women (56–59). Maternal age, education, and socio-economic status also influence infection, with lower maternal age being associated with increased infection risk and higher maternal education and socio-economic status associated with lower infection risk (12,59–61).

Consequences on maternal health

SHIP can have several negative consequences on maternal health, including increased risk of maternal anemia, co-infection with other parasites, inflammation, malnutrition, miscarriage, and mortality (7,62–64). Anemia is a deficiency in functional red blood cells and their hemoglobin concentration, which causes poor tissue oxygenation and can have serious health implications, particularly in pregnant women who are in greater need of blood supplies in order to adequately nourish mother and developing fetus. While multifactorial, one major cause of anemia is hookworm infection, due to chronic blood loss from the attachment of worms onto the intestines (62). In fact, while the estimated DALYs from STH infection are around 5 million (36), if taking into account anemia due to hookworm infection, those estimates increase 7-fold to more than 39 million DALYs (50) due to STH infection. Anemia in turn can contribute to low birth weight, pre-term birth, and other adverse pregnancy outcomes for mother and fetus (65).

Infections with helminth species and with malaria are two of the most prevalent parasitic infections in African nations and often overlap in their geographical distribution (66,67). Due to this, individuals are often co-infected with helminth and malaria species, particularly *P. falciparum*. However, previous research has found conflicting evidence on whether or not infection with one parasite increases, decreases, or has no change on the risk of infection with a subsequent parasite (57,68–70). A meta-analysis in 2013 pooled results from these existing studies and concluded that pregnant women infected with helminths had an increased risk of malaria infection compared to uninfected pregnant women (71). Researchers hypothesized that SHIP, which tends to be a more chronic infection than malaria, may compromise the maternal immune system and its ability to react to and fight off subsequent infections. Of course, due to the differing routes of transmission of different helminth species, their effect on risk of malaria

infection varies between species, which could be the reason for conflicting results on the impact of helminth infection on risk of malaria infection (68).

Exposure to helminths induces an inflammatory immune response in infected individuals, which can subsequently impact fetal development (60). Hookworm infection has been shown to increase systemic inflammation, more specifically increase erythrocyte sedimentation rate, a general marker of inflammation (62). Inflammation is thought to be the mechanism through which SHIP and other maternal infections can lead to adverse birth outcomes and impaired fetal brain development, which will be discussed later in this thesis.

Finally, SHIP can lead to malnutrition in chronically infected individuals in endemic regions of the world (72). Anemia and iron deficiency due to helminth infection in pregnancy greatly contribute to this burden of disease from malnutrition (73), which is why iron and folate acid supplements are vital during prenatal care in high-risk populations. Maternal malnutrition can subsequently impact fetal growth and development, as well as increase risk of miscarriage and mortality (72,74).

Consequences on birth outcomes

Several studies have shown a detrimental impact of SHIP on birth outcomes such as pre-term birth, low birth weight, and fetal growth restriction due to the increased risk of maternal anemia from infection (60,75,76). However, the mechanism behind this link remains unclear and several studies have in fact shown that the impact of helminth-malaria co-infection during pregnancy is primarily to blame for these negative birth outcomes (77). Many clinical trials have sought to investigate whether helminth prophylaxis in pregnancy can improve these birth outcomes, with some showing promising results (78–80). However, other clinical trials have found conflicting results that show no impact of helminth treatment during pregnancy on improving adverse birth outcomes (81–83).

Consequences on child health and development

Maternal infection with helminths can impact fetal and infant immunity to helminth and other parasitic infections. As previously stated, maternal infection with helminths elicits an immuno-regulatory response, which can impact the attached fetus in the placenta (84). Fetal exposure to maternal antibodies produced in response to infection could in fact increase susceptibility to helminth and other parasitic infection, compared to unexposed fetuses, according to some literature (84,85). This could be due to the fact that helminth infection has been shown to induce T-cell hyporesponsiveness, a down-regulation of the immune response, making for ideal physiological conditions for parasite survival within human hosts (86). However, other research has demonstrated that fetuses acquire immunity from infected mothers which prevents

them from experiencing severe forms of disease from helminth infection otherwise seen in infants without this acquired immunity (87,88). Subsequently, a large body of evidence is available on the detrimental impact of STH infection in childhood on child cognition and development (89,90). However, evidence on the ability of routine helminth treatment in children to negate or reverse these consequences remains insufficient (89,91).

Two previous studies, to our knowledge, have investigated the impacts of SHIP on child neurocognitive development. The first, within a Ugandan mother-child cohort in the early 2000s, found that maternal infection from *Mansonella perstans* and *Strongyloides stercoralis* worm species were associated with impaired early executive function and language in 15-month old infants (91). The second study took place in the same mother-child cohort analyzed within this thesis from Allada, Benin. This study found that SHIP diagnosed at the first antenatal care visit and at any point during pregnancy was associated with impaired gross motor scores and cognitive ability in children as measured by the MSEL at 12 months of age. Authors of these studies hypothesized that maternal inflammation and/or maternal anemia could be the mechanisms behind these associations, as both have been shown to impact fetal brain development (92,93). In addition to these studies, there is a growing body of literature showing negative associations between other types of maternal infection, such as human immunodeficiency virus (HIV) or influenza, and impaired cognitive and behavioral development in offspring (94,95). This research also suggests that the materno-fetal immuno-inflammatory response to infection disrupts placental vasculogenesis and angiogenesis necessary for fetal development (27).

In addition to the direct impact of SHIP on maternal and fetal outcomes, the use of preventive and treatment drugs for infection during pregnancy could also have consequences for the fetus; however, this subject remains highly debated amongst public health actors and research on the subject remains equivocal. Some studies have shown that helminth prophylaxis has no impact (either positive or negative) on adverse birth outcomes (91,96). While observational studies have shown benefits of anti-helminthic treatment on maternal and birth outcomes, no clinical trials have found such benefits (97).

Gaps in the research

Based on the existing literature of the consequences of SHIP, we found that evidence was needed to support the limited studies on its impacts on child neurocognitive development, especially in school-aged children. We also sought to confirm the negative associations between SHIP and child cognitive development seen in children at 12 months from this birth cohort in Benin prospectively at six years of age. Additionally, the hypothesized role of the maternal

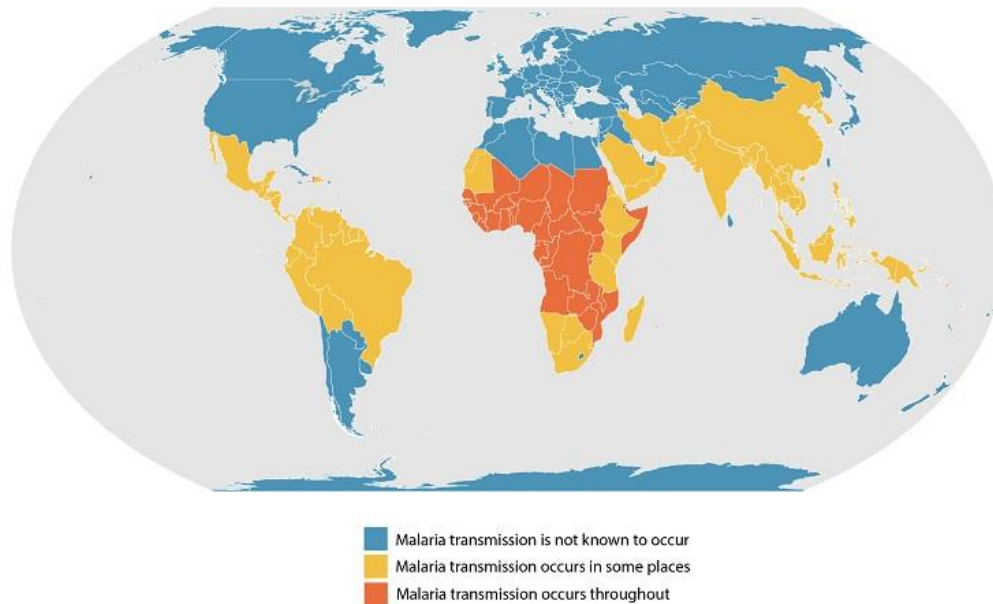
inflammatory response, particularly due to helminth infection, needed to be further investigated.

Malaria

Malaria is an infectious disease caused by parasites that are transmitted to humans through the bite of infected female *Anopheles* mosquito vectors (98). Malaria occurs in tropical and sub-tropical areas of the world, where wet and humid meteorological conditions provide a favorable environment for mosquito populations (2). In non-immune individuals, malaria symptoms include fever, chills, headache, and diarrhea, among other non-specific symptoms. These symptoms can occur anywhere from 9-30 days after being bitten by an infected mosquito. If not treated quickly with antimalarial drugs, infection can progress to severe illness, including cerebral malaria, and death. The progression of malaria in infected individuals depends highly on the immune status of the individual, the type of parasite they are infected with, and the use of antimalarial drugs after infection. In areas of the world where transmission of malaria is common, individuals acquire immunity after repeated infections of malaria and eventually exhibit asymptomatic infections (98).

According to the World Malaria Report in 2020, there were over 229 million cases of malaria reported in 2019, with 409,000 reported deaths due to malaria infection (99). An estimated 94% of these malaria infections and deaths occurred in SSA, with the remaining infections occurring primarily in South America and Asia, which is why these regions are known as the ‘malaria belt’ of the world (Figure 3) (100). The most at-risk populations in high-transmission areas are children under five years of age and pregnant women, as well as patients with HIV/AIDS (2). In 2019, over 67% of deaths due to malaria occurred in children under the age of five (99).

Figure 3. World map showing malaria transmission around the world.



Life cycle and transmission

As previously mentioned, malaria is transmitted through the bite of infected mosquito vectors from the *Anopheles* genus. There are over 400 different species of *Anopheles* mosquitoes, with 30 of these species capable of transmitting the malaria parasite to humans. One of five parasites from the *Plasmodium* genus can cause human malaria infection: *P. falciparum*, *P. vivax*, *P. ovale*, *P. knowlesi* and *P. malariae*. The geographical distribution of these mosquito and parasite species varies; however, *P. falciparum* parasites are found in almost all areas of the world where malaria transmission occurs and account for over 90% of malaria infections in SSA. This species of parasite is also most responsible for severe malaria infection and death (98).

The malaria parasite life cycle involves two hosts, the mosquito vector where the sexual phase of the life cycle occurs and the human host where the asexual phase occurs (Figure 4) (101). When a female *Anopheles* mosquito takes a blood meal from a human infected with malaria, they ingest the male and female gametocytes of the parasite along with their meal, which then make their way into the gut of the mosquito. From there, the male and female gametes of the parasite form zygotes, which continue through the sexual life cycle to eventually develop into oocysts which grow and divide to form sporozoites. This growth and division, known as the sporogonic phase, continues for 8-15 days until the oocysts burst and release these infectious sporozoites into the body of the mosquito, which then travel to the mosquito salivary

glands, ready to be injected into their hosts. This stage of the life cycle occurring within the mosquito vector does not lead to clinical infection in mosquitoes, in fact the malaria parasite and mosquito have a symbiotic, or mutually beneficial, relationship. When an infected mosquito draws a blood meal from a human host, the sporozoites that are present within the salivary glands are injected into the host (102).

Once within the human host, there are two parts of the malaria parasite life cycle that occur, one in the human liver and one in the red blood cells (RBCs). The injected sporozoites travel to and infect the liver cells of the human, usually within a few hours of biting, and mature into schizonts which contain tens of thousands of merozoites, the stage that invades the RBCs. This phase of the life cycle, known as the pre-erythrocytic phase, lasts 5-16 days and an infected individual does not experience clinical symptoms of infection at this point. (Side note: parasites from *P. vivax* and *P. ovale* species can remain dormant in the liver for months or years and cause repeated malaria attacks in infected individuals long after treatment of initial infection). When the schizonts in the liver are fully matured, they burst and release the merozoites that travel to and invade RBCs; a process that happens too quickly for the host immune system to react and defend itself from infection (103). Once within the RBCs, merozoites ingest hemoglobin to grow and multiply within the cell as they pass through the ring, trophozoite, and schizont stages of the life cycle. This part of the life cycle, known as the blood stage or the erythrocytic phase, is when infected hosts exhibit the clinical symptoms of malaria infection. At the end of this cycle, RBCs burst and release new merozoites to infect other RBCs within the human host, allowing parasite density to increase in the absence of treatment. A few of the parasites from the RBCs develop into the sexual male and female gametocytes which are then ingested by *Anopheles* mosquitoes during blood meals (Meibalan & Marti, 2017).

Another important factor of malaria transmission is mosquito vector behavior and environment. For example, the most prevalent malaria vector species in SSA, *A. gambiae*, breeds in small pools of stagnant water, meaning that during and just after rainy seasons are the periods of the year when transmission of malaria is highest, and therefore vector control strategies could be the most beneficial in order to prevent infection. Mosquitoes also tend to feed at night, when human hosts are sleeping, meaning that certain preventive measures can be taken to reduce the risk of being bitten by an infected mosquito vector at night. These methods of prevention and treatment will be discussed in detail later.

Diagnosis, prevention, and treatment

The prompt diagnosis of malaria is crucial in providing adequate care to infected individuals and reducing the risk of severe infection or death, since malaria is a curable disease if diagnosed and treated effectively. There are several methods for malaria diagnosis, including clinical, laboratory, and molecular methods, and the use of one method over another largely depends on the availability of laboratory equipment and trained technicians, the cost, and the context in which malaria is being diagnosed (i.e., an endemic area vs. non-endemic area) (104). The clinical manifestations of malaria can be used to diagnose disease in settings with sparse laboratory equipment and technicians; however, due to the non-specific symptoms of malaria illness that may overlap with other febrile illnesses common in high-transmission areas, this clinical diagnosis sometimes leads to over-diagnosis of malaria and over prescription of antimalarial medications (105). The gold standard for malaria diagnosis is the microscopic examination of thick and thin blood smears to identify the presence of malaria parasites in a laboratory (106). This method of diagnosis is relatively simple to carry out and provides results within a few hours of blood collection from an individual. The examination of thick and thin blood smears allows technicians to identify the presence of malaria parasites, including the species of parasite and the parasitemia (the quantity of parasites in the blood). In settings where laboratory equipment is not as readily available, and when diagnostic results are needed quickly in order to provide treatment, Rapid Diagnostic Tests (RDTs) can be used. These tests detect specific malaria antigens created in response to malaria infection using a blood sample; results can be obtained within 15 minutes of administering this test. While this method of diagnosis is beneficial in low-income, rural settings, it does not permit medical professionals to identify the parasite species or parasitemia in infected individuals, it simply identifies whether or not an individual is infected. Additionally, those with low parasitemia may have false negative RDT results and therefore go untreated for malaria infection. The Centers for Disease Control (CDC) recommends that all RDT diagnostic results, whether negative or positive, be followed up with microscopy techniques when available (107). Finally, the most sensitive and specific diagnostic method for malaria is the use of PCR-based techniques. These techniques require more sophisticated laboratory resources, which are not available in many low-income settings, and involve higher costs and more rigorous training for laboratory technicians to accurately carry out procedures. However, PCR methods are able to identify extremely low parasitemia, as few as 1-5 parasites/ μl of blood ($\leq 0.0001\%$ of infected red blood cells) compared with around 50-100 parasites/ μl of blood by microscopy or RDT (104).

The most effective ways to prevent malaria infection are vector control strategies, such as insecticide-treated bed nets (ITNs) and indoor residual spraying (IRS) with insecticides. Bed nets are the most commonly used vector control method because of this ease of use and cost effectiveness. The most-used class of insecticides on bed nets are pyrethroids, and ITNs must be retreated with this insecticide every 6-12 months in order to remain effective at preventing mosquito bites and killing mosquitoes that enter the home (108). Studies have shown that using ITNs reduces malaria incidence in children and pregnant women, the two most at risk groups of malaria and its consequences (109). The WHO now recommends the universal access and use of long-lasting insecticide-treated bed nets (LLITNs) which can last up to 3 years and have been shown to be more effective than simple ITNs (110). IRS control strategies involve the spraying of indoor walls and ceilings of homes with insecticides to kill mosquitoes that try to enter the home; IRS must be carried out every 4-6 months in homes in order to remain effective. Non-pyrethroid insecticides can be used in IRS campaigns, often in addition to the use of LLITNs in order to provide added protection against mosquito vectors (111). Unfortunately, evidence is now emerging of insecticide resistance within certain mosquito species, meaning that new vector control efforts must be prioritized along with existing strategies (112). Additionally, due to the toxic nature of pesticides in general, concerns about the safety and potential long-term consequences of insecticide-use for malaria vector control have become more frequent (113,114).

In addition to vector control, preventive antimalarial drugs can be administered to particularly at-risk groups, such as pregnant women and children under the age of five. There are several different types of antimalarial drugs available, and the type of drug used in a given population depends on the prevalent parasites in the area, as well as on the antimalarial drug resistance in that country. In Benin, the WHO recommendations call for intermittent preventive treatment in infants with sulfadoxine-pyrimethamine (IPTi-SP) to be given 3 times during the first year of life at approximately 10 weeks, 14 weeks, and 9 months of age, corresponding to the routine vaccination schedule of the Expanded Program on Immunization (EPI) (115). For pregnant women, the WHO recommends at least 3 doses of IPTp-SP during pregnancy, beginning in the 2nd trimester, with doses being given at least one month apart. As mentioned before, drug resistance of certain antimalarials is becoming more common, and researchers and doctors around the world are increasingly investigating new drugs or combinations that could help offset this drug resistance in high-transmission areas (16). WHO treatment guidelines for malaria infection from *P. falciparum* in SSA are displayed in Table 1 (116).

Table 1. WHO treatment guidelines for *P. falciparum* infection in high-transmission areas of SSA.

	Uncomplicated malaria	Severe malaria
Children over 25kg and adults (including pregnant women in 2nd and 3rd trimesters)	One of the following artemisinin-based combination therapies (ACTs) for 3 days: Artemether + lumefrantrine Artesunate + amodiaquine Artesunate + mefloquine Dihydroartemisinin + piperazine Artesunate + sulfadoxine-pyrimethamine (SP)	Intravenous or intramuscular artesunate for at least 24 hours and until they can tolerate oral medication, then complete treatment with 3 days of ACT
Infants and children under 25kg	Minimum of 2.5 mg/kg body weight per day of dihydroartemisinin and 20mg/kg body weight per day of piperazine daily for 3 days	A higher dose of artesunate (3mg/kg body weight per dose) than larger children and adults
Pregnant women in 1st trimester	7 days of oral quinine + clindamycin	Parental quinine

According to the WHO, there is currently only one vaccine against malaria that has been proven in clinical trials to be effective in reducing malaria illness, specifically severe, life-threatening malaria, in children in medium- and high-transmission areas (117). The RTS,S malaria vaccine acts against *P. falciparum* malaria infection and has been shown to prevent 4 in 10 malaria cases in children over a 4-year period (2). Pilot programmes for the administration of the vaccine has begun in some SSA countries. More research and clinical trials are needed to continue the search for effective vaccines to decrease the burden of malaria around the world and especially in endemic areas, particularly for high-risk populations like children under five and pregnant women.

Malaria in pregnancy

An estimated 125 million pregnancies occur in malaria-endemic regions of the world each year, and research demonstrates that one in four women in these regions are infected with malaria over the course of pregnancy (118,119). In SSA, pregnant women are at an increased risk of malaria infection from *P. falciparum* and *P. vivax* species due to immunological changes

during pregnancy and because a unique phenomenon occurs during pregnancy where *P. falciparum* erythrocytes sequester in the placenta in infected women (120). This type of placental malaria infection allows parasites to evade the host immune response and is typically characteristic of more chronic maternal malaria infection that can be particularly harmful for mothers and the developing fetus (118).

The risk of MIP differs between low- and high-transmission regions, with pregnant women in low-transmission areas more susceptible to severe malaria infection and death due to their insufficient immunity to malaria infection. Additionally, women of all gravidities in low-transmission areas are at equal risk of infection, while primigravidae women are the most at risk of malaria infection in high-transmission areas (118,120) since immunity is acquired with increasing numbers of pregnancies in these areas. While MIP is more common in high-transmission areas, women do not necessarily exhibit pathological symptoms of disease, particularly in the case of placental malaria infection (120). Younger maternal age has been shown to be independently associated with increased risk of malaria infection in pregnancy in high-transmission areas (121) and HIV infection also increases susceptibility to malaria infection in pregnant women, as it decreases variant-specific immunity and impairs cytokine response to malaria (120,122). Studies have shown that pregnancy trimester may also impact risk of infection, with women in the second trimester most at risk of infection (118). However, there is little available research on malaria during the first trimester, especially since women in SSA often do not attend antenatal care clinics before the second trimester (123).

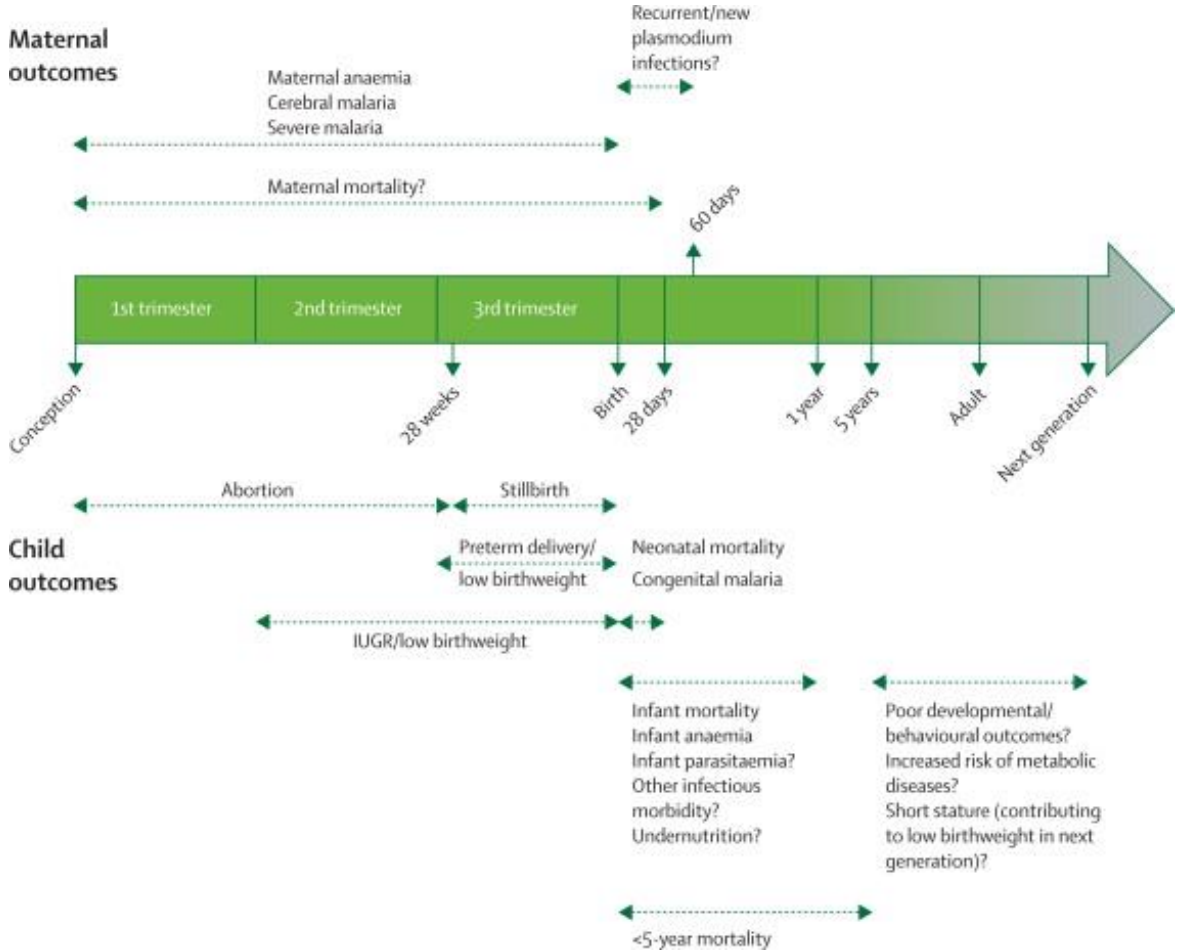
Diagnosis of MIP is commonly done using microscopy and PCR methods, where laboratory settings permit such sophisticated techniques. As previously mentioned, PCR tests are able to detect low parasitemia thresholds, lower than those detected by microscopy or RDTs, and in pregnant women from high-transmission areas often reflect chronic, asymptomatic, submicroscopic infections. Placental histology is also used to detect malaria in pregnant women, and involves the examination of placental tissue collected at delivery in order to diagnose past or current malaria infections through identification of malaria pigment leukocyte infiltrates (124).

Consequences on maternal health

MIP, both peripheral and placental, contributes to an estimated 200,000 infant deaths and 10,000 maternal deaths each year (120,125); however, mortality rates differ significantly between low- and high-transmission areas (118). In low-transmission areas, pregnant women are at increased risk of severe malaria infection, often leading to cerebral malaria and/or death (126). Overall, pregnant women are three times more likely to develop severe malaria than non-

pregnant women, and therefore more at risk of severe anemia, hypoglycemia, respiratory distress, renal failure, and cerebral malaria (127). The consequences of MIP, including maternal and infant outcomes, vary depending on the time of infection and the immune status of the pregnant woman, as illustrated in Figure 5 (118).

Figure 5. Effects of malaria in pregnancy through the life course.



Maternal malaria infection is also a major cause of maternal anemia during pregnancy, defined by the WHO as a hemoglobin level of less than 11 g/L (128), which subsequently greatly increases the risk of maternal mortality and negative birth outcomes like low birth weight and fetal growth restriction (129). Studies suggest that placental malaria, which reflect more chronic malaria infections, is particularly associated with increased risk of severe maternal anemia (130,131). The mechanisms behind this association are well-understood and primarily involve the destruction of parasitized RBCs and specific and non-specific immune responses which shorten RBC survival, increasing the risk of anemia (132). Iron deficiency, defined by the WHO as serum ferritin levels of less than 15 µg/L (133), remains the main cause of anemia and may also interact with malaria based on emerging evidence. Observational studies have shown iron deficient individuals to have a decreased risk of malaria infection in

endemic regions (134,135). Hypotheses for this observation suggest that iron uptake into RBCs influences the host immune response and inhibits the parasite life cycle (134).

Consequences on birth outcomes

MIP has been associated with increased risk of stillbirth, pre-term birth, fetal growth restriction, and low birth weight (136,137). In malaria-endemic regions, MIP contributes to up to 20% of stillbirths and risk of stillbirth is even higher still in areas of low endemicity (138,139). An estimated 20% of infants born with low birth weight, defined as weight at birth less than 2500g, can be attributable to placental malaria in SSA (140). MIP increases the risk of low birth weight due to a multitude of factors including maternal anemia, intrauterine growth restriction (IUGR), and pre-term birth (127). MIP is thought to impair oxygen and nutrient delivery to the fetus, resulting in IUGR (141). Malaria also substantially contributes to pre-term birth in endemic areas, up to 36% of pre-term births being attributable to infection, presumably due to the hosts immune response to infection which triggers early labor (139). Additionally, women in malaria endemic-regions are more likely to be malnourished, and the interaction of these two factors can increase risk of low birth weight (142).

MIP, particularly placental malaria, has been shown to impede placental vascular development, a process essential for a healthy pregnancy and proper development of the fetus (143). In cases of placental malaria, parasitized erythrocytes sequester in the placental intervillous space and induce a systemic inflammatory immune response in the mother (144). This increase in inflammation as a result of infection has been shown to dysregulate vasculogenesis and angiogenesis pathways between mother and fetus necessary for proper fetal development (145), therefore increasing risk of low birth weight, pre-term birth, and stillbirth. In addition, the impairment of these processes in the placenta could impact overall fetal growth and development and have long-term consequences on infant and child growth and development (13).

Consequences on child health and development

As previously mentioned, MIP is estimated to contribute to over 200,000 infant deaths, and can also continue to impact the lives of surviving offspring after birth in several ways (120). Pregnant women infected with malaria have infants at greater risk of congenital malaria, when *P. falciparum* parasites infect the cord or peripheral blood of newborns within the first seven days after birth (146). Newborns, who have underdeveloped immune systems, can develop severe symptoms from congenital malaria and have an increased risk of death (147). Placental malaria has also been shown to increase the risk of malaria disease in infants in the first few months of life through interference of the transfer of maternal antibodies to the developing

fetus. Infants exposed to malaria *in utero* have been shown to acquire a tolerance to malaria blood-stage antigens that continues into childhood, and therefore increases their susceptibility to malaria infection (148). However, recent literature suggests that fetal exposure to malaria may in fact prime the malaria-specific T-cell immune response in infants, therefore providing a type of acquired immunity to malaria in infants in the first few months of life (149).

This increased risk of malaria infection in early childhood is of significant public health importance due to the fact that children under five years of age are the most at risk of mortality from malaria, with the WHO estimating almost 300,000 infant deaths per year due to childhood malaria infection (115). Research has also shown that children are at a greater risk for severe malaria, which can present as cerebral malaria, and can have long-term implications for neurocognitive development in children (150). Novel research has also shown an association between child malaria infection and lead poisoning, lead being a neurotoxic heavy metal known to impair child growth and development (14). This research suggests that children living in areas burdened by both malaria and lead exposure may face significant challenges in terms of sufficient neurocognitive development. MIP has also been found to increase risk of anemia in children after birth, with placental and peripheral malaria being found as the main contributors to hemoglobin concentrations in infants in the first year of life (151).

As discussed earlier, maternal infection with malaria activates the inflammatory immune response which can subsequently impair placental vasculogenesis vital for proper fetal growth and development (145). Placental malaria also involves the complementary immune response, which is known to play a role in fetal neurodevelopment (152). Recent research has investigated this mechanism and found that experimental malaria-exposed offspring had persistent neurocognitive deficits in memory and affective-like behavior compared to the unexposed controls (13), providing evidence that placental malaria disrupts this complement immune response, particularly the C5a receptor, and has long-term implications for fetal neurocognitive development.

Gaps in research

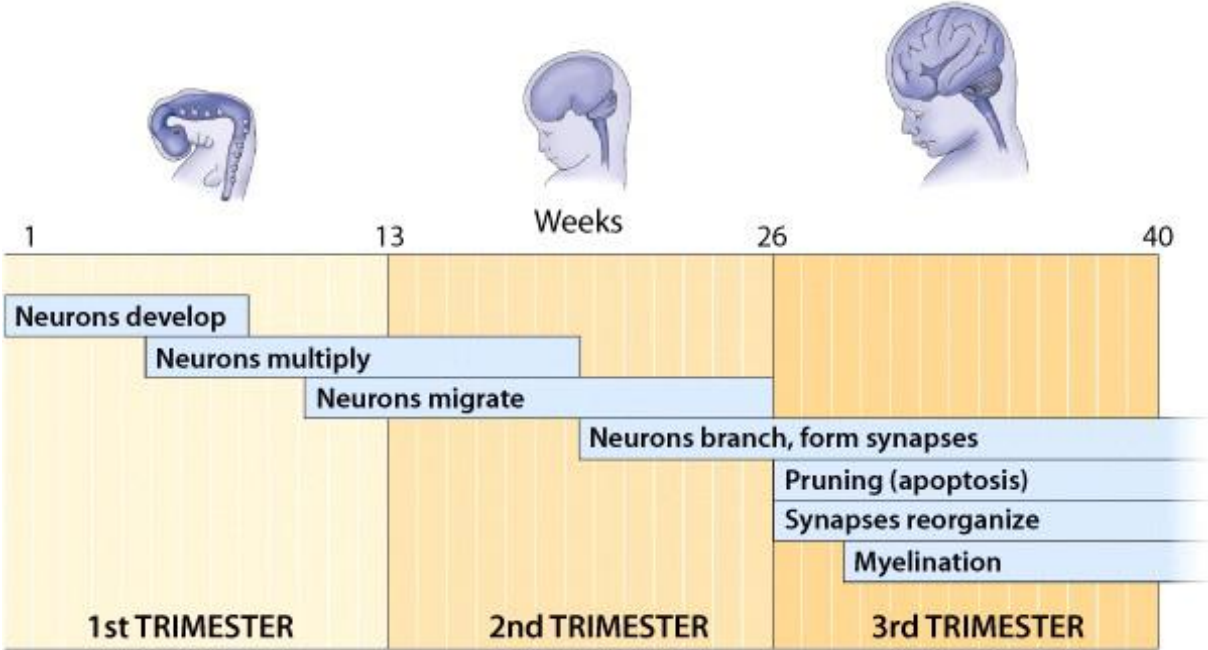
While MIP is associated with several negative consequences in terms of maternal and fetal outcomes, its impacts on fetal neurocognitive development remain widely unknown. Recent research provides laboratory evidence of a negative association between placental experimental malaria and fetal neurocognitive development; however, studies of this association in large mother-child cohorts have yet to be carried out. This thesis provides the first known study to investigate the relationship between MIP and child development in a large mother-child cohort from a malaria-endemic area in SSA.

Child neurocognitive development

Neurocognitive development refers to the processes in the brain involved in the development of neurological pathways necessary for cognitive, behavioral, and emotional functioning in individuals (153). These processes begin during the prenatal phase of life and continue for the first several years after birth, with the first 8 years of life considered to be the most critical period for this social, emotional, cognitive, physical, and behavioral development in children (154). Early models of child development by well-known developmental psychologists, such as Jean Piaget and his counterparts, consisted of age-specific stages governed primarily by maturation during childhood and biological processes (155). However, cognitive development begins at the fetal stage (156), as seen in Figure 6, and is influenced by a myriad of factors.

Within the first 10 weeks of conception, stem cells develop into neurons and glia, the primary cells that compose the brain. As these cells multiply, they then migrate to the part of the fetus where the brain will develop, the proper positioning of these cells being a crucial step in fetal brain development. As neurons continue to develop in the second and third trimesters, they develop branches, an axon, and undergo myelination, processes that allow for neurons to receive and transmit information throughout the body. Neurotransmitters, the chemicals responsible for communication between neurons, establish synapses between neurons to facilitate their communication. By the third trimester, neurons undergo “pruning”, otherwise known as apoptosis (cell death), when the brain signals that certain neurons make inappropriate or unneeded connections (154).

Figure 6. Fetal brain development over the course of pregnancy.

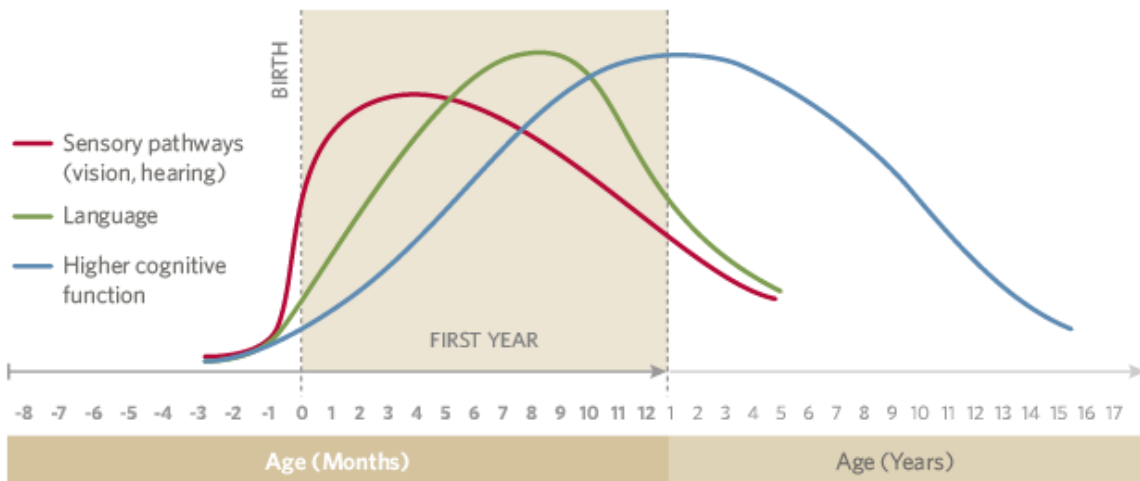


Duke University Wordpress (2020).

The foundations of child brain development continue throughout early and middle childhood, when up to 80% of the brain’s grey matter is created through an interplay of genes, environment, and life experiences (156) (Figure 7). Several factors including learning environment, social climate, family dynamics, educational opportunities, sensory stimulation, adverse life events, and socio-economic and social conditions can all play a role during this phase of development and have long-lasting implications for individuals. Further information on these influential factors for child development is detailed later in this chapter.

Figure 7. Cognitive development in early and middle childhood.

Human Brain Development



Source: Nelson, C. (2000). From *Neurons to Neighborhoods: The Science of Early Childhood Development*. Washington, DC: The National Academies Press.

Several tools are available to assess and measure sensory development, language ability, and cognitive and motor functioning in children of all ages. These assessments can be carried out by parents, educators, or health professionals in young children to assess various aspects of development within one tool, with infants receiving one or more developmental scores for each domain (157). For example, there is the Ages and Stages Questionnaire (ASQ), a parental screening tool for developmental delays in infants 1-66 months of age. Another well-known tool is the Bayley Scales of Infant and Toddler Development, a tool used to assess cognitive, motor, language, interaction, social-emotional, and adaptive behavior development in infants less than 1-42 months of age. Finally, the MSEL assesses child gross motor functioning and four cognitive scales to deliver a final, early learning composite (ELC) score. Once children are old enough, developmental assessment tools involve tasks and activities that children can complete under the supervision of test administrators to measure cognitive, motor, and behavioral processes. Among the most well-known are the Kaufman Assessment Battery for Children (KABC) and the Early Screening Inventory-Revised (ESI-R) tests that assess overall cognitive ability and processing in middle childhood (158).

Risk factors of impaired neurocognitive development

The Developmental Origins of Health and Disease (DOHaD) conceptual theory proposes that an array of factors, exposures, and experiences influence health throughout the life course, especially in adult life (159). There are several factors, both prenatal and postnatal, that begin to influence and negatively impact infant and child health and development, which can have long-term implications. Among these are socio-economic conditions, genetic predispositions, adverse birth outcomes, the home-learning environment, parental psychological well-being, environmental exposures, infectious disease, and nutritional deficiencies.

Socio-economic conditions

It is estimated that one third of children under five years of age in low- and middle-income countries are at risk of developmental impairments due to conditions of poverty and stunting from malnutrition (160). Populations in developing nations are more often exposed to political unrest, conflict, extreme poverty, migration, reduced access to education, and other stressful events that can all have an impact on growing infants and children, as well as on the population as a whole. Low socio-economic status is often linked with lower parent education levels and support, both of which have been shown to impact child development. Studies have shown that lower levels of parent education level, particularly that of the mother, are associated with impaired child development in developing countries (161,162).

The difficulties faced in developing nations are also a major contributor to decreased school attendance rates, according to available studies. In 2015, the Global Monitoring Report indicated that 58 million primary school-aged children were not enrolled in school in low-income countries (163). National statistics on school enrollment rates and child cognitive development in developing countries is extremely lacking, thus contributing to the issue of poor development for which there are no clear statistics or testing norms outside of available Western ones (160).

Genetics

As molecular biology continues to advance, scientists are identifying more and more genes associated with behavioral and cognitive disfunctions and disorders (164). The inheritance of these genes from parents to children can have a substantial effect on cognitive development in infants before they are even born. Some well-documented, but rare, genetic developmental disorders include Prader-Willi Syndrome (PWS) and Williams syndrome

(165,166). Novel research has also begun to show certain genetic markers increase risk of autism and attention deficient and hyperactivity disorder (ADHD) (167).

Adverse birth outcomes

Infants born pre-term (before 37 weeks' gestation) and/or with a low birth weight (under 2,500 grams) are at increased risk of developmental delays, particularly in terms of motor and cognitive functioning (168–170). While this delay greatly varies according to gestational age and weight at birth, evidence is unequivocal that premature and underweight infants perform worse in terms of cognitive ability, executive function, and gross and fine motor skills than term-born controls (168,171). These delays can even persist into adolescence and adulthood (172,173) through what is known as a “developmental cascade” originating from early developmental stages (174). Pre-term birth interrupts the development of the fetal brain, most notably cerebellar development, resulting in cognitive deficits in surviving infants (175,176). Similarly, infants born with a low or very low birth weight have growth and developmental deficits, sometimes as a result of intrauterine growth restriction during pregnancy (177,178).

Learning environment and parental well-being

In addition to genetic and obstetrical factors, the environment in which infants and children live and interact with others during the very early years of life are crucial for their development. Multiple studies have shown that stimulating home environments, in which there are a variety of toys and objects for infants to use to help with gross and fine motor skills and an active participation from family members to stimulate language and emotional skills, significantly increase the developmental capacities of growing infants, even in settings of poverty (179–181). One study even found that home environment contributed more to child intellectual performance than did socio-economic status or maternal education level (182). However, these factors more than likely act synergistically to impact child cognitive and behavioral development (15).

Maternal depression, also referred to as post-partum depression, that normally occurs within the first year after giving birth, has also been shown to negatively impact the cognitive and behavioral development of infants (183,184). Research has shown that mothers suffering from depression interact with their children differently than non-depressed mothers, and they provide less stimulation and attention to children that is needed for their neurocognitive and psychosocial development (185). The link between maternal depression and impaired child neurocognitive development is even thought to be mediated by the quality of the home environment and parent stimulation (186). Recent evidence has also emerged that maternal

depression can even impact the physical health of developing children, increasing their risk of diarrheal disease and febrile illness (15,187).

Environmental exposures

Environmental exposure from chemicals, pollutants, and heavy metals during pregnancy or childhood has been shown to negatively affect crucial developmental processes. The most studied contaminants in prenatal and postnatal exposure studies are persistent organic pollutants (organochlorinated pesticides, polychlorinated biphenyls), non-persistent pollutants (phthalates, bisphenol A), and toxic heavy metals (cadmium, lead and mercury) (188). Pre-natal exposure to these chemicals can occur through trans-placental passage, particularly in heavily polluted urban areas, or postnatally through air or dust inhalation or through hand-to-mouth ingestion (189,190). Studies have shown that even low levels of exposure to certain pollutants, such as organophosphate pesticides, during fetal developmental phases can impair cognitive, social, and behavioral development (191,192).

Lead (Pb) is a neurotoxic heavy metal well-known for its irreversible and highly detrimental effects on child health and development (193–195). Prenatal exposure to lead has been particularly linked to disruptions in child cognitive and neurobehavioral development (196). Pollutant co-exposures can also culminate in the body, primarily in individuals living in low-income and low-resource settings, thus further putting these populations at risk of severe neurodevelopmental disorders (197).

Infectious disease in childhood

While exposure to infectious disease during pregnancy and its association with child development have been understudied, there is evidence linking childhood exposure to infections with impaired neurocognitive and behavioral development. STH infection in school-age children has been found to impair academic performance in numerous studies, with these studies also demonstrating the cognitive and educational benefits of school-focused deworming programs in burdened populations (198,199). Cerebral malaria, as mentioned earlier in this thesis, can also be most common amongst children under the age of five and can have long-term neurocognitive consequences for survivors. Several cohort studies have shown persistent, significant cognitive impairments in children infected with severe forms of *P. falciparum* malaria, with one study referring to these impairments as the “hidden burden” of malaria in endemic countries (200–202). Finally, HIV-infected infants and children have been shown to have impaired growth and development compared to HIV-uninfected counterparts, even in the presence of antiretroviral therapies (203,204). Prenatal exposure to HIV and

materno-fetal transmission of HIV has also been linked to cognitive and developmental delays in surviving infants (205).

Nutritional deficiencies

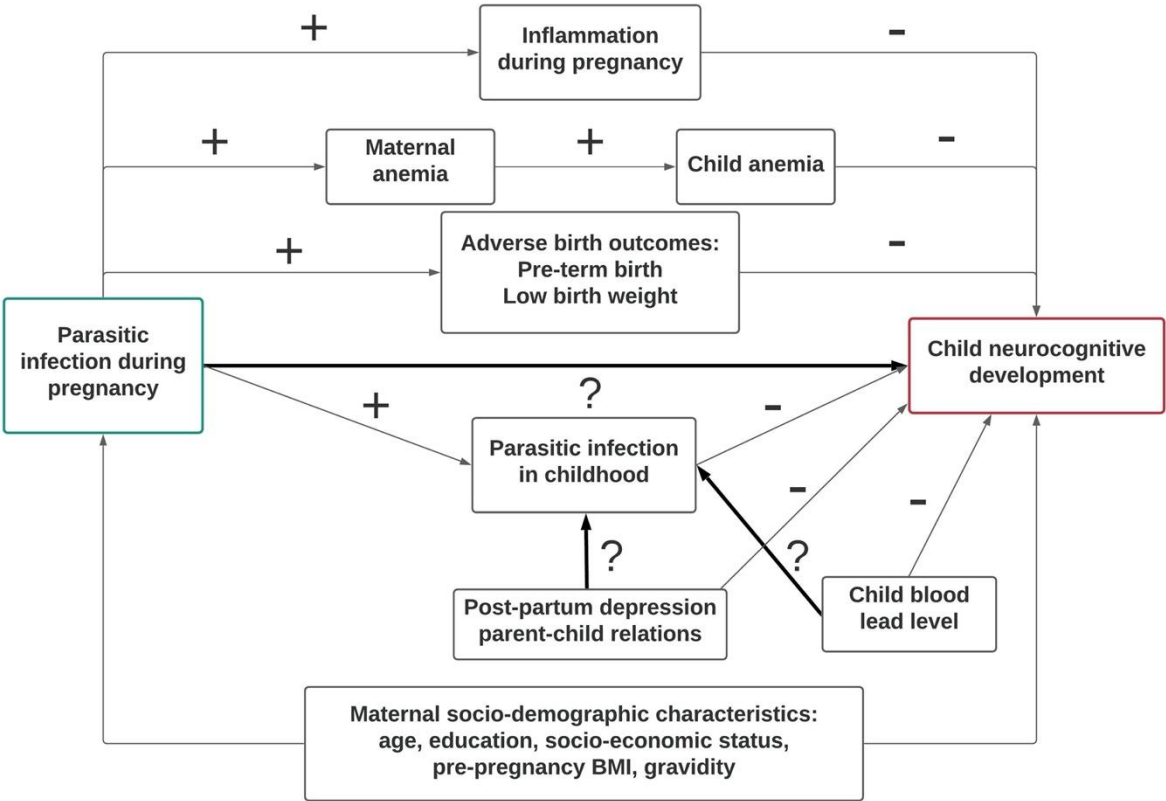
Pre- and post-natal nutrition is one of the major contributing factors for efficient child development. Maternal health and nutrition are vital for adequate fetal development and growth, with many studies demonstrating that proper maternal levels of folic acid, iron, vitamin D, zinc, and long-chain polyunsaturated fatty acids are particularly important in optimizing pregnancy and birth outcomes (206–208). The overall dietary nutrition of mothers has also been greatly studied, since inadequate or excessive gestational weight gain can be suboptimal for offspring development (209,210). Child nutrition has also been a widely studied public health issue and is known to be associated with cognition, with malnourished children more at risk of growth stunting and developmental impairments (208,211,212).

Nutritional deficiencies in iron are the most significant contributors to maternal and child anemia, which have been subsequently linked to impaired fetal and child development. Pregnant women have higher iron requirements than non-pregnant women, in order to provide adequate nutrition and blood flow to the fetus in the placenta. Therefore, iron deficiency and iron deficiency-anemia (IDA) are of great public health concern in this population, especially in low-income countries that may not have access to food sources with adequate iron stores (213). Studies have suggested that iron plays an integral role in fetal development, therefore establishing a connection between prenatal iron deficiency and IDA and impaired fetal growth and development (214). Prenatal anemia increases the risk of childhood anemia, which in turn can also have negative effects on child development if left untreated (215). Observational and experimental studies have shown that iron deficient children have long-term cognitive, social, and emotional deficits compared to iron-replete children (216,217).

Gaps in research

The influence of infectious disease during pregnancy has not been widely studied and is of interest to public health factors particularly in heavily burdened areas of the world. Figure 8 shows a Direct Acyclic Graph (DAG) demonstrating the complex documented pathways between parasitic infection in pregnancy and impaired child neurocognitive development, as well as the pathways that were previously unexplored in the literature and that were addressed within the objectives of this thesis to help better our epidemiological understanding of these relationships and the mechanisms behind them.

Figure 8. DAG showing pathways between parasitic infection during pregnancy and child neurocognitive development.



Chapter 2. Materials and Methods

The population of mothers and their children involved in this thesis are part of a larger, multi-study cohort from the district of Allada, Benin that was followed prospectively from the second trimester of pregnancy until offspring were six years of age. This chapter details the study sites, population, objectives, and procedures of the five studies from which data on participants were used for the purposes of this thesis. Biological, neurocognitive, socio-demographic, and anthropometric assessments of participating women and children are described for each study, as well as interventions given to participants. Statistical analyses used for each thesis objective are detailed individually in subsequent chapters and in attached publications.

Data sources

Information on biological, sociodemographic, and anthropometric data from pregnant women and their offspring was collected during two parallel studies: the *Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD)* randomized clinical trial (NCT00811421) and the *Anaemia in Pregnancy: Aetiologies and Consequences (APEC)* nested-cohort study. Additional biological data of a sub-cohort of infants was collected within the APEC study at birth, three, six, nine, and twelve months of age.

Parasitic infection and haematological factors were measured in a sub-population of infants from APEC between birth and twelve months of age in the *Tolérance Immunitaire et Paludisme (TOLIMMUNPAL)* prospective cohort study. The *TOVI* ('Child from the country' in Fon) cross-sectional study assessed postnatal characteristics and neurocognitive development in one-year-old children born to mothers from the MiPPAD and APEC cohorts. Six-year-old children were further assessed for long-term neurocognitive development in the *EXposition au PLomb et au manganèse et Risques pour l'Enfant (EXPLORE)* cross-sectional study.

The scientific context, objectives, population, and assessments of each of the previously mentioned studies are further detailed in the remainder of this chapter.

The MiPPAD clinical trial

Context & Implementation

In 2012, the WHO guidelines for malaria prevention in HIV-negative pregnant women living in SSA countries with stable transmission of *P. falciparum* recommended at least two

doses of IPTp-SP, in conjunction with the use of LLITNs, starting from the second trimester and at least one month apart . While IPTp-SP proved to be cost-effective and reduced maternal morbidity, parasite resistance to the drug had been documented and could rapidly gain in momentum if no alternative strategies were identified (218). Based on previous evidence, mefloquine (MQ) proved to be a valid substitute for SP in this population due to its long half-life, ability to be administered in a single dose, and acceptable reproductive toxicity profile (219). Single-dose MQ has been associated with mild secondary effects after administration and rare psychological and neurological effects (220); however, these effects were attenuated when MQ was given to individuals in a split dose in one previous study (221). Given the lack of adequate information on the efficacy of IPTp-MQ as a replacement for IPTp-SP, apart from two promising clinical trials in Benin (222,223), researchers conducted MiPPAD open-label randomized clinical trial comparing the safety and efficacy of IPTp-SP with IPTp-MQ full-dose and split-dose in HIV-negative pregnant women in the context of LLITNs across four countries in SSA. Researchers also recruited HIV-positive women from three other countries for the same comparison of IPTp; however, these women were not part of the cohorts described in this thesis.

The clinical trial recruited eligible pregnant women from four countries in SSA (Benin, Gabon, Mozambique, and Tanzania); only data from the women participating at the Benin study site is discussed within the remainder of this thesis. The trial was conducted from 2009 until 2013 in Allada, Benin, a semirural district about 50 kilometers north of the economic capital and major port city, Cotonou. The district is comprised of 84 villages with a total population of 127, 512 inhabitants according to a 2013 report by the Institut National de la Statistique et de l'Analyse Economique (224). Figure 9 shows a map of the study location in Benin.



Figure 9. Study site of Allada, Benin.

Source: <https://www.actualitix.com/carte-benin.html>

All eligible pregnant women from three sub-districts in Allada (Allada, Attogon, and Sékou) attending their first antenatal care visit (ANV) in the second trimester of pregnancy were invited to participate in the clinical trial. Inclusion criteria for recruitment into the trial were as follows: a permanent residence in the study area and agreement to deliver at one of the three participating maternity clinics in the sub-districts, gestational age ≤ 28 weeks, HIV-negative at recruitment, no known allergies to sulfa or mefloquine drugs, no treatment of malaria using these drugs within four weeks of recruitment, no intake of antihelminthic therapy or iron/folic acid or vitamin B12 supplements prior to the first ANV, and no history of severe renal, hepatic, psychiatric, or neurological disease (16). Women were then randomly stratified into one of three IPTp intervention arms of the clinical trial using a 1:1:1 ratio; (1) standard IPTp with SP, (2) IPTp with MQ (15 mg/kg) given once as a full dose, and (3) IPTp with MQ (15 mg/kg) split over two days.

As part of standard antenatal care guidelines in Benin, women received LLITNs (PermaNet, Vestergaard Fransen) at recruitment. Women were assessed for hemoglobin, HIV, and the syphilis rapid plasma regain test at recruitment per local standard procedures. Women also received ferrous sulphate-folic acid supplements at each ANV for anemia prevention.

Direct consumption of supplements was not monitored by study personnel. At the first ANV, women received 600mg of mebendazole (100mg to take twice daily for three days) for prevention of helminth infections and received treatment again at the 2nd ANV if diagnosed with helminth infection. At each ANV, women received their respective IPTp treatments according to their designated intervention arm. Women in the standard IPTp with SP group were treated with three tablets of the fixed combination therapy containing 500mg of sulfadoxine and 25mg of pyrimethamine (Malastop, Sterop).

Participants were encouraged to seek medical care at the ANV clinics whenever needed and a passive surveillance system was put into place to monitor symptoms of illness, malaria episodes, or other adverse events which were documented within the clinical trial. Clinical malaria episodes were treated with oral quinine in the first trimester or with artemether-lumefantrine in subsequent trimesters for uncomplicated malaria; severe malaria was treated with parental quinine (17). At delivery, if women were diagnosed as anemic, they were treated with oral ferrous sulphate 200 mg/8 hours for three months or by blood transfusion in severe cases (124).

Socio-demographic and anthropometric information during pregnancy

Participating pregnant women were seen at two ANVs in one of the three maternity clinic locations at least one month apart, as well as at delivery, for a total of three scheduled ANVs. At the first ANV, sociodemographic information was collected via a questionnaire administered to women. Information collected included maternal age, gravidity, marital status, occupation and education level of mother and father, area of residence, and information related to the use of LLITNs the previous night.

Maternal weight, height, and gestational age were measured at each ANV by trained health professionals. Weight was measured using an electronic scale to the nearest 0.1kg (SeaCorp, Hanover, Maryland) and height was measured using a body meter device to the nearest 0.1cm (Seca 206 Bodymeter, SeaCorp). Gestational age was determined by measuring fundal height using manual palpation at each ANV. Maternal pre-pregnancy Body Mass Index (BMI) was determined based on the weight, height, and gestational age assessed at recruitment by subtracting the expected weight gain during pregnancy (roughly 1kg per month from the end of the first trimester until delivery (225)) and calculating BMI in kg/m².

At delivery, in addition to the previously mentioned information collected for women, newborn length was measured to the nearest 1mm using a locally manufactured wooden measuring scale validated by the WHO (226) and newborn weight was assessed to the nearest

10g using an electronic baby scale (Seca type 354). Newborn sex was noted, as well as gestational age at birth according to the Ballard's score (227).

Malaria infection during pregnancy

At each ANV, 4mL of venous blood was collected from pregnant women and divided into two tubes for examination and storage; 2mL was introduced into an iron-free dry tube, and the other 2mL was stored in a tube with potassium Ethylenediaminetetraacetic acid (EDTA). Malaria infection by *P. falciparum* was determined at each ANV, including delivery, by thick and thin blood smear tests using the Lambaréné technique. From collected samples, 10 μ L of blood was smeared on a 1.8cm x 1cm rectangular slide and stained with Giemsa. Stained slides were read with an oil immersion lens at 1000X magnification. The number of parasites per μ L of blood was obtained by applying a multiplication factor to the average parasitemia/field.

In addition to microscopy techniques for malaria diagnosis, 50 μ L of maternal peripheral and placental blood was spotted onto filter papers for parasitological assessment at delivery. Filter papers were tested for *P. falciparum* presence and density by a real-time quantitative polymerase chain-reaction (qPCR) assay targeting 18S ribosomal deoxyribonucleic acid (rDNA) (19). Parasitemia was quantified by extrapolation of cycle thresholds, the value for which DNA is amplified, from a standard curve of *P. falciparum* ring-infected erythrocytes. Samples where no cycle thresholds were detected were considered negative, and a density of 2 parasites/ μ L was assigned if amplification was lower than the standard curve of 5 parasites/ μ L (124).

Hematological assessments

From blood samples taken at each ANV, 10 μ L was used to determine the concentration of Hemoglobin (Hb) using the Hemo-control photometer device (EKF Diagnostics, Barleben/Magdeburg, Germany). The device was calibrated daily for quality control and one in ten Hb concentrations was crosschecked by a Hematology analyzer (Erma Laboratory, Japan) at the Allada Central Hospital laboratory. Hb concentration was recorded in g/L and women with a concentration less than 110 g/L were considered anemic.

Iron stores in pregnant women were determined by serum ferritin concentration after samples were centrifuged for serum analysis. A rapid test (CRP Latex; Cypres Diagnostics Inc., Campbellville, Ontario, Canada) measured concentrations of C-reactive protein (CRP) in blood samples. Women were considered to have markers of inflammation if their CRP concentration was less than 5mg/L. Iron deficiency (ID) was defined as a serum ferritin concentration less

than 12µg/L in the absence of inflammation or a serum ferritin concentration less than 70µg/L in the presence of inflammation measured by CRP.

Funding & Ethics Statement

The MiPPAD clinical trial was funded by the European and Developing Countries Clinical Trials Partnership (EDCTP; IP.2007.31080.002), the Malaria in Pregnancy Consortium, as well as the following national agencies: Instituto de Salud Carlos III (PI08/0564), Spain; Federal Ministry of Education and Research (BMBF FKZ: da01KA0803), Germany; Institut de Recherche pour le Developpement (IRD), France.

The Ethics Committee of the Hospital Clinic of Barcelona in Spain, the IRD Consultative Ethics Committee in France, and the regulatory authorities and the National Ethics Review Committee of Benin approved study protocol and informed consent forms. Written informed consent was obtained by pregnant women in the presence of a witness, with thumbprints provided for consent if women could not read and/or write.

The APEC nested-cohort study

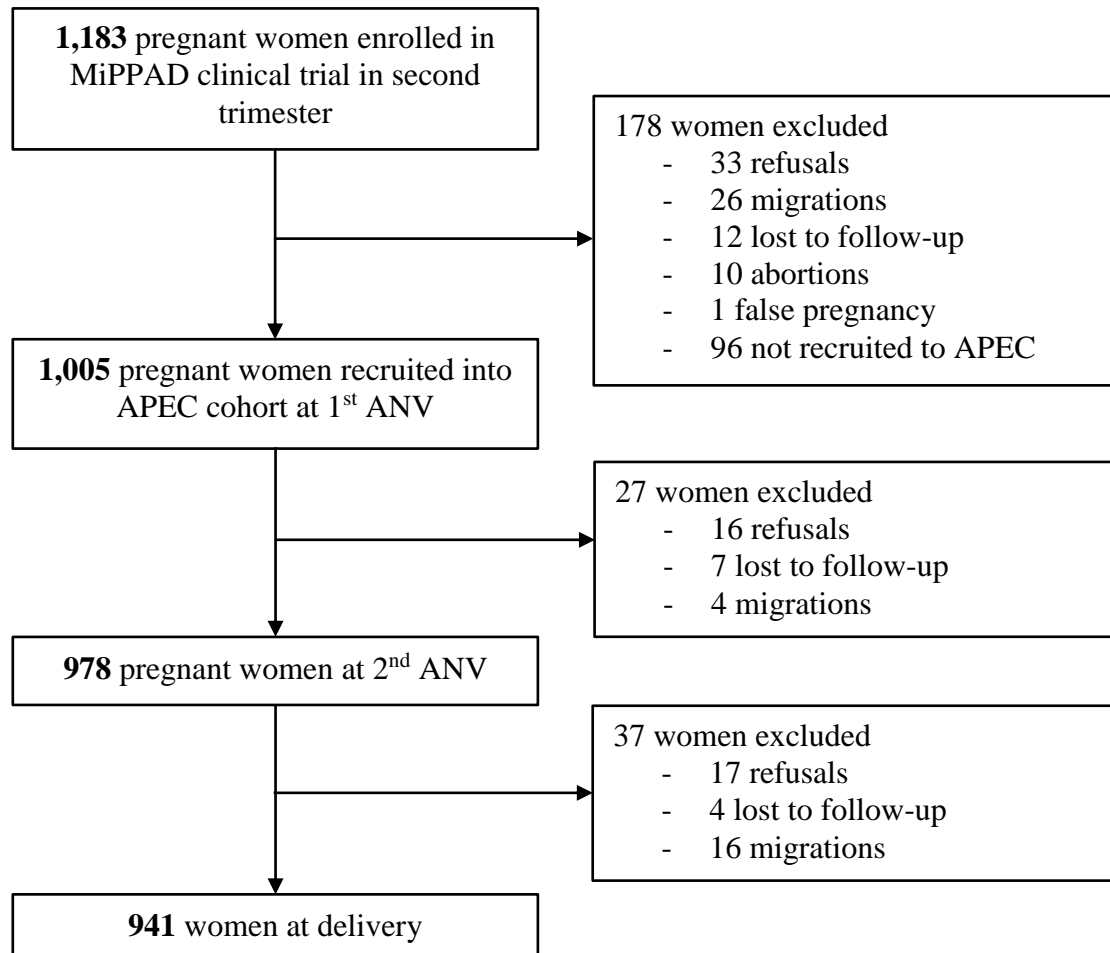
Context & Implementation

Pregnant women in developing countries are at increased risk of anemia during pregnancy due to a multitude of factors such as parasitic infections and nutritional deficiencies. A previous study found 60% of pregnant women in a Beninese cohort to be anemic (228). Even with prevention and treatment measures in place in high-risk populations in SSA endemic to malaria parasitic infection, the effectiveness of these interventions is still widely undocumented (229). Therefore, a nested prospective cohort study, APEC, was launched as an ancillary study to the MiPPAD clinical trial with the primary objective to investigate the etiological factors of anemia in pregnancy and its consequences on maternal and child health in a malaria endemic population.

The APEC study ran parallel to MiPPAD and enrolled the first 1,005 pregnant women who agreed to participate in the study at the first ANV within the clinical trial from January 2010 until May 2011. Follow-up of pregnant women from recruitment until delivery in the MiPPAD and APEC cohorts is depicted in Figure 10. In addition to the previously mentioned biological, socio-demographic, and anthropometric data collected from pregnant women in the MiPPAD trial, participants in APEC were also assessed for additional parasitic infection caused by soil-transmitted helminths and for vitamin B12 and folic acid concentrations to identify relevant nutritional deficiencies. A sub-cohort of 400 children were followed from birth to 12 months of age in the APEC study the impact of prenatal anemia in mothers on child malarial

and hematological statuses. Participants were chosen based on maternal anemia status; 200 infants were born to anemic mothers and 200 were born to non-anemic mothers.

Figure 10. Follow-up of pregnant women from second trimester until delivery.



Soil-transmitted helminth infection during pregnancy

At each ANV, pregnant women received a container to collect stool samples from the morning following the visit. Stool samples were also collected either 15 days prior to the expected delivery date of women or within a week after delivery. Containers were retrieved from pregnant women within six hours of defecation in order to test for the presence of soil-transmitted helminth eggs.

Thick smears of stool samples were prepared and assessed using the Kato-Katz technique to obtain a quantitative estimate of the intensity of helminth infection according to WHO standards (18). Soil-transmitted helminth eggs of *A.lumbricoides*, *N.americanus*, *A.duodenale*, and *T.trichiura* species were identified. Quality control was performed by two laboratory technicians who systematically and independently examined each of the two

prepared slides of thick smears under a microscope; the mean egg count of the two results was calculated. The number of eggs in each individual sample was calculated, recorded, and then the number of eggs per species was multiplied by a factor of 24 in order to obtain the standardized fecal egg count (FEC), expressed in eggs per gram (epg). An individual was considered to be infected with soil-transmitted helminths if the FEC was at least 24 epg for any species.

Nutritional assessments

From the venous blood collected from pregnant women at ANVs and centrifuged, 500µL of serum ferritin and vitamin B12 concentrations were measured using a microparticle enzyme immunoassay (MEIA) method. Folic acid concentrations were determined using fluorescence polarization immunoassay (FPIA) technique with an AxSym Immuno-Assay Analyzer (Abbott Diagnostics, Frankfurt, Germany) (229). Women were considered to have a folic acid deficiency if serum ferritin concentrations were less than 6ng/mL and were considered to be vitamin B12 deficient with vitamin concentrations less than 150 pg/mL.

Infant malaria assessments

The sub-cohort of 400 infants were followed from birth to 12 months and were systematically assessed for malaria status at birth, 3, 6, 9, and 12 months. Infants also had access to free health care services for passive detection of malaria during the follow-up period. At each systematic visit, 8 mL of venous blood was taken from infants and the Lambaréné technique was used to diagnose malaria on thick blood smears, described in detail within the MiPPAD clinical trial protocol. If diagnosed with malaria, infants were treated with artemether-lumefantrine according to existing Beninese guidelines at the time.

Funding & Ethics Statement

The APEC study was supported by the Malaria in Pregnancy Consortium, which is funded through a grant from the Bill and Melinda Gates Foundation to the Liverpool School of Tropical Medicine. Ethical consideration for the APEC nested cohort was approved by the French Comité Consultatif de Déontologie et d'Ethique and the Ethics Committee of the University of Abomey-Calavi in Benin. Informed consent was obtained from all human adult participants and from the parents or legal guardians of minors.

The TOVI study

Context & Implementation

Prenatal anemia is known to have severe consequences on maternal and birth outcomes, such as low birth weight, pre-term birth, and perinatal mortality (230); U-shaped fluctuations in maternal hemoglobin during pregnancy have also been associated with adverse birth outcomes (231). However, little is known about the impact of prenatal Hb concentrations on infant neurocognitive development (232). Given this research gap and the high prevalence of prenatal anemia in the population of pregnant women enrolled in the MiPPAD clinical trial, researchers sought to follow-up with singletons born to these women at 1 year of age and investigate the impacts of prenatal anemia on infant neurocognitive development scores through the TOVI study. This was a cross-sectional study conducted between June 2010 and June 2013 in the district of Allada, Benin.

All 863 surviving one-year-old singletons born to pregnant from the MiPPAD trial were eligible to participate in the TOVI study. Infant neurocognitive and motor development was measured by a trained research nurse at a scheduled 1-year post-partum visit at a local maternity clinic using the American Guidance Service edition of the MSEL (20). Within three days after cognitive assessments, a research nurse different from the one who administered the MSEL visited family homes to collect information related to socio-demographic factors, maternal depression, home environment, and environmental exposures that could impact infant development.

Mullen Scales of Early Learning (MSEL)

The MSEL is an individually administered, norm-referenced measurement tool used to assess cognitive and motor function in children from birth to 68 months of age. The tool consists of five scales to determine functioning: the gross motor scale, fine motor scale, receptive language scale, expressive language scale, and the visual perception scale. The gross motor scale is designed specifically for use in children age 1-36 months in order to assess basic mobility and balance important for young infants. Therefore, children in our population were assessed on all five scales of the MSEL. Raw scores of each scale were computed and then converted into normative *t* scores; *t* scores for the fine motor, receptive language, expressive language, and visual perception scales were converted into the Early Learning Composite (ELC) score, indicative of overall cognitive functioning in children. Only trained professionals are permitted to administer the tasks of the MSEL.

The MSEL, previously only available in English and never before used in Benin, was translated and adapted for use in this population prior to the onset of the study in four steps: linguistic translation, training, pilot testing, and review (22).

1. The MSEL was translated and back-translated between English and French by two separate psychologists and both English versions (translated and back-translated) were compared by study personnel and modified if needed. The back-translation was validated by Pearson Assessments, the copy-right holder of the official MSEL. An oral translation of the MSEL in the local Beninese language, Fon, was used for assessments.
2. Research nurses and the coordinating psychologist of the study were trained to administer the MSEL and other assessments used in the TOVI study by psychologist Michael Boivin, specialized in child development in SSA, for a two-week period.
3. During the two-week training period, a pilot study was conducted and trained staff administered the MSEL and other assessments to fifteen participating children and their mothers. Later, thirty-two additional children were assessed prior to the onset of the study. Administration and scoring of the MSEL by research nurses were audited and reviewed prior to child enrollment.
4. During the training and pilot study, research coordinators and staff reviewed and adapted assessments accordingly. Three items were adapted for use in this population: a potato chip was used instead of a cheerio to utilize local materials, children received one point if they handed the ball to their mother/guardian instead of throwing it underhand, and one question was changed from ‘Why do we have refrigerators?’ to ‘Why do we have lanterns?’ since most participants did not have refrigerators.

Quality control of assessment administration was completed during the study by the field coordinator and research nurses. Inter-rater reliability was assessed among the three research nurses who administered the MSEL; the gold-standard assessor, the nurse who administered the most assessments, was identified and ELC scores were compared among all three nurses. The correlation score for inter-rater reliability between the gold standard interviewer and other assessors ranged from 94 to 100% with a mean correlation score of 98% (22).

Socio-demographic information

During the home visit after the MSEL administration, a questionnaire was given to mothers or guardians of children to assess family possession scores. These scores were computed using a checklist of material possessions that families could own and indicated family

wealth: radio, television, bicycle, motorbike, car, at least two cows, and electricity. Maternal education level was also noted during this home visit.

Edinburgh Postnatal Depression Scale (EPDS)

Maternal post-partum depression symptoms were measured using the EPDS, a 10-item Likert-style questionnaire used to measure symptoms of depression by inquiring about feelings of sadness or hopelessness, changes in appetite or activity, and ideations of self-harm within the previous seven days (233). Each item of the EPDS is scored between 0-3, therefore total scores range between 0-30, with higher scores indicating the presence of depressive symptoms. The validated French version of the EPDS was translated into a written version in Fon and administered by trained research nurses. The EPDS was evaluated for adaptability and validity in this population during the previously mentioned pilot study, given that there was no previously identified gold standard clinical diagnostic test for maternal depression in Benin.

Home Observation for Measurement of the Environment (HOME)

The HOME inventory, developed by Bettye Caldwell and Robert Bradley, is a systematic assessment of the quantity and quality of stimulation, support, and structure available to a child in their home environment (234). The inventory for Infant-Toddler assessment is a combination of interview questions and observations of mother-child interactions in the home environment and consists of six subscales, detailed in Table 2 (235).

Table 2. Infant-Toddler HOME inventory sub-scales (ages 0 to 3).

Subscale	Description	Example
Responsivity	Verbal and affective interactions between caregiver and child	Caregiver spontaneously vocalizes to child at least twice during visit
Acceptance	How the caregiver disciplines the child	Caregiver does not express overt annoyance with or hostility towards the child
Organization	How the child’s time is organized outside of the home environment and what the child’s personal space is like	The child’s play environment appears safe and free of hazards
Learning Materials	Availability of age-appropriate toys and activities for the child	Child is provided pieces of equipment appropriate for their age (i.e. playpen, infant seat)
Involvement	How the caregiver interacts physically with the child	Caregiver tends to keep child within visual range and looks at them often
Variety	How the child’s daily routine incorporates variety and social	Family visits or receives visits from relatives approximately once per month

interactions with those other than the
mother

Adapted from (235).

The HOME was adapted for use in this population through formal training of research staff, a pilot study involving ten home visits, adaptation of certain items according to the findings of the pilot study, and a final review with research staff (22). Six items of the HOME inventory were adapted for use in this population: the ‘Learning Materials’ scale was reduced to four questions due to the fact that only one family in the pilot study owned a toy, a question related to the number of books owned by the child was changed from ‘At least two books are present or can be seen’ to ‘The child owns one or more books’, an item related to the amount of time the child spends outside was removed since children in this population spend most of their days outside. Assessors were trained to administer the adapted HOME inventory in French by trained research staff.

Infant blood lead levels

Nurses collected 8mL of venous blood from each child, with 4mL stored in a tube containing dipotassium EDTA and 4mL put into an iron-free dry tube. An aliquot of EDTA blood was diluted 20-fold in ammonia 0.5% v/v and 0.1% v/v surfactant Triton-X and analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan DRC II ICP-MS instrument) at the Centre de Toxicologie, Institut National de Santé Publique du Québec (Québec, Canada). The limit of detection for blood lead analysis was 0.2 µg/L (236).

Funding & Ethics Statement

The TOVI study was funded by the Eunice Kennedy Shriver National Institute of Child Health & Human Development (no. R21-HD060524). This study was approved by the institutional review boards of the University of Abomey-Calavi in Benin and New York University in the United States (IRB#09-1253). At recruitment, researchers obtained informed consent from all women and guardians who participated.

The TOLIMMUNPAL study

Context & Implementation

In malaria-endemic areas of the world, children and adults are regularly infected with malaria parasites throughout their lives, leading to an eventual acquired immunity over time that protects many adults from overt illness caused by malaria (237). Antibodies produced in response to *P. falciparum* parasites play an essential role in this acquired immunity (238); however, the specific antigens that illicit these antibody responses are not well known.

Understanding these antigens is vital for effective vaccine development against *P. falciparum* infection during the blood stages, particularly in areas like Benin. Therefore, the TOLIMMUNPAL study sought to study antibody responses to candidate vaccine antigens and malaria infection in children from birth to 24 months of age in Benin.

The study recruited the same 400 children, 200 anemic and 200 non-anemic, followed after birth in the APEC study between 2010 and 2013. TOLIMMUNPAL systematically collected information related to *P. falciparum* infection in childhood, as well as hematological and environmental factors relevant to risk of malaria infection in the study area. For the purposes of this thesis, only the data collected on malaria infection and related risk factors in children between 12-24 months was used.

Malaria infections in early childhood

Malaria infection was diagnosed during the 12-month follow-up (from 12 to 24 months of age) using both positive thick blood smear tests and RDTs. Participating children were visited by a nurse during a monthly systematic visit, where children were given both a thick blood smear and an RDT, whether they presented with symptoms or not. Children also had a systematic visit at a healthcare center in Benin at 18 and 24 months of age, where venous blood samples were drawn in addition to the administration of a thick blood smear and RDT. A nurse visited the homes of participating children every 15 days, during which a thick blood smear test was administered, regardless of whether children presented with symptoms or not, and an RDT if children had fever or history of fever in the previous 24 hours (temperature $\geq 37.5^{\circ}\text{C}$). Children had access to free emergency care and were able to present at the hospital anytime symptoms occurred, and a thick blood smear test and an RDT were administered to diagnose malaria. Malaria episodes were identified as symptomatic if children presented with fever at the time of diagnosis or within three days of diagnosis, and episodes were identified as asymptomatic in the absence of fever at time of diagnosis or in the three days following diagnosis. *P.falciparum* density was determined if children tested positive for malaria through thick blood smear or RDT.

Hematological Assessments

At 12 months, blood samples were collected from children to assess iron deficiency. Iron deficiency was defined as low ferritin concentration ($<12 \mu\text{g/L}$) corrected for CRP (239), and/or iron deficient tissue in the body indicated by soluble transferrin receptors (sTfR) in the blood ($>8.3 \text{ mg/L}$) (240).

Environmental Assessments

To account for the potential difference in risk of malaria outcome among children, researchers collected information on mosquito populations in 180 out of the 400 participating homes of children based on geographical equi-distribution criteria (31). CDC mosquito-light traps were installed in selected homes at intervals of two consecutive nights per month between April 2011 and February 2013, where mosquitoes were trapped and then analyzed by researchers for mosquito species and density. The average number of mosquitoes found per home per night was calculated for all children using multiple imputation.

At each visit, mothers were asked if their children slept under an LLITN the previous night. Based on the number of times children were said to have slept under a bed net during the follow-up, they were classified as having used LLITNS rarely, occasionally, frequently, or always.

Funding & Ethics Statement

The TOLIMMUNPAL study was funded by the Agence Nationale de la Recherche (ANR). Ethical approval was obtained from the Consultative Committee on Ethics and Deontology (CCDE) of the Institute of Research for Development (France) and the Ethics Committee of the Faculty of Health Sciences, University of Abomey Calavi, Benin (43/11/2010/CE/FSS/UAC).

The EXPLORE study

Context & Implementation

Lead (Pb), a neurotoxic heavy metal, can be found in gasoline, paint, out-of-date water pipes, ammunition, and contaminated soil, particularly in developing countries where these sources have yet to be eradicated (241). Child exposure to lead through inhalation or ingestion is known to have permanent and serious consequences on neurodevelopment, child growth, and risk of infection (14,242). The CDC recommends intervention when blood lead levels are 50 µg/L or more; however, even blood lead levels under this threshold can be detrimental to child health and development (193). However, the sources of lead and its burden in SSA countries has not been as widely studied as in Western countries. During the TOVI study, 58% of children had elevated blood lead levels ($\geq 50\mu\text{g/L}$) at one year, indicating high levels of exposure in this population.

Therefore, researchers conducted the EXPLORE study to follow-up with these children prospectively at six years of age to assess the impact of heavy metal exposure (from Pb and manganese (Mn)) on child neurocognitive and behavioral development, as well as to study the

sources of these heavy metals in this population. All children who participated in the TOVI study at one year of age were invited to participate in EXPLORE. The cross-sectional study took place in Allada, Benin from May 2016 until June 2018. Table 3 provides a summary of all studies that pregnant women and children participated in from the second trimester of pregnancy until six years of age, as well as the relevant collected and used within this thesis.

Table 3. Summary of all studies and variables collected during follow-up.

Timing	Pregnancy			Singleton Birth	3 months	6 months	9 months	12 months	12 months	24 months	6 years
	1 st ANV	2 nd ANV	Delivery						Systematic monthly follow-up		
Year	2009-2011				2009-2011			2011-2013	2011-2014		2016-2018
Study	MiPPAD & APEC				APEC			TOVI	TOLIMMUNPAL		EXPLORE
N	1,005	978	941	863	Sub-cohort of 400			747	Sub-cohort of 400		578
Soil-transmitted helminth infection											
<i>P. falciparum</i> infection											
Neurocognitive development											
Socio-demographic information											

Adapted from S. Ahmadi (2020).

Before recruitment in EXPLORE, study personnel were trained by the Principal Investigator and study psychologist to administer neurocognitive assessments to children, measure anthropometric factors, collect biological samples, and administer a questionnaire to mothers to evaluate maternal depression and socio-demographic factors. Validation of assessments and quality control was assured prior to the onset of the study. A common translation of oral instructions from French to Fon was validated by trained assessors and study nurses for all assessments given to mothers and their children. Participating children were treated for anemia, soil-transmitted helminth infection, and malaria infection if present according to existing national guidelines in Benin.

The day before assessments, participating families were visited by study personnel where protocol was explained to them and they were given containers to collect child stool samples. The following day, mothers and their children visited one of the three participating health centers in Allada (Allada, Attogon, Sékou) where all assessments were carried out. Upon arrival, if the child was visibly ill or had symptoms, their temperature was taken. If children had a fever, a blood sample was taken to test for parasitic infection and anemia, and appropriate treatment according to national guidelines was provided. Mothers and children were then asked

to return to the health center in a few days when the child was feeling better in order to complete the neurocognitive assessments.

Biological Assessments

The day of neurocognitive assessments at the health clinic, families arrived and returned the containers given to them the previous day with a child stool sample to be analyzed for helminth infection. The Kato-Katz technique was used to detect the presence of soil-transmitted helminth eggs, as described in detail within the APEC protocol. Eggs belonging to *A.lumbricoides*, *N.americanus*, *A.duodenale*, and *Taenia* species were identified.

Samples of 8mL of venous blood were collected from children; 4mL was stored in a tube containing dipotassium EDTA and 4mL put into an iron-free dry tube. Iron deficiency status was determined, with children considered as iron deficient with serum ferritin concentrations <15 µg/L corrected for CRP. *P. falciparum* malaria infection was diagnosed using a thick blood smear test and parasite density was calculated according to the average parasitemia/field.

Blood lead level of children was also assessed using inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan DRC II ICP-MS instrument) on an aliquot of EDTA blood diluted 20-fold in ammonia 0.5% v/v and 0.1% v/v surfactant Triton-X at the laboratory of the Ecole des Hautes Etudes en Santé Publique (EHESP) (Rennes, France). The limit of detection for blood lead analysis was 0.2 µg/L (236).

Anthropometric Assessments

Height and weight of six-year-old children was collected; height was measured by a locally manufactured length board and weight was determined using the TANITA analogue scale. Weight-for-age z-scores for children were calculated according to the WHO z-score classification system (243).

Developmental Assessments

Kaufman Assessment Battery for Children 2nd edition (KABC-II)

The KABC-II, first published in 2004, is a psychological diagnostic tool used to assess cognitive ability in children 3-12 years of age. The second edition of the tool measures simultaneous and sequential processing, as in the first edition, as well as planning and learning abilities in children. There are four subscales of the KABC-II, the Simultaneous Processing scale, the Sequential Processing scale, Learning ability, and Planning ability. For the purposes of assessments in this population, the Planning ability scale was not used and certain items from each scale that were not culturally appropriate were not included. The KABC-II is a Western measure of cognition that has been widely used in SSA countries, particularly in the context of

malaria-endemic populations (200,244). The construct validity of the French version of the KABC-II (by Les Editions du Centre de Psychologie Appliquée (ECPA); French translation published and distributed exclusively by ECPA with the permission of AGS Publishing, Pearson Products Inc.) was verified in this population in Benin during a pilot study (245), and instructions were orally translated from French to Fon by trained study personnel. The standardized Mental Processing Index (MPI), a measure of global cognitive ability, and the Non-Verbal Index (NVI), a non-verbal assessment of child processing and cognitive abilities, both generated by KABC-II scores, were used for the purposes of this thesis and its analyses. A quality control of the KABC-II was completed at the Global Health Assessment (GHA) center in Uganda. Videos of investigators administering the KABC-II were sent to the GHA center each month for six months and each investigator received individualized feedback regarding their assessment of children.

Bruininks-Oseretsky Test of Motor Proficiency Edition 2 (BOT-2)

The BOT-2 is a comprehensive, standardized, norm-referenced diagnostic tool used to assess gross and fine motor skills in school-aged children. The short form of the BOT-2 was used for motor skill assessment in this population, and included asking children to perform activities such as folding a piece of paper, jumping in place, and balancing on one leg. Final composite scores for fine and gross motor skills were standardized by age and sex and used for the purposes of this thesis. The BOT-2 was not validated for use in this population prior to the study onset; however, the BOT-2 short form has been used in other similar SSA populations to assess motor development in children (246).

Test of Variables of Attention (TOVA)

The TOVA, a non-language-based performance test, was administered to identify ADHD in children. Children were seated in front of a computer and completed the test by reacting to various stimuli that appeared on the screen. Children were instructed to press a certain button when a specified target would appear on the screen. Omission errors are the number of times the child does not click the button when the target appears on the screen and commission errors are the number of times the child clicks the button too early (before the target appears on the screen). Response time is the amount of time it takes the child to press the button once the target appears on the screen. Variability is the consistency in the child's response times. The final score was calculated based on response times, variability of response times, and rate of deterioration of responses to the computerized stimuli.

Strengths and Difficulties Questionnaire (SDQ)

Mothers and guardians received a questionnaire to fill out which included the SDQ, a brief 25-question behavioral screening tool to assess behavioral development in children 3-16 years of age, developed by child psychiatrist Robert N. Goodman (247). The questionnaire screens for issues related to five subscales: emotional problems, conduct problems, hyperactivity, peer problems, and pro-social behaviors; with the four former subscales used to generate an overall difficulties score. The SDQ further differentiates between emotional and behavioral conduct problems in children through internalizing and externalizing scores generated by its subscales. Scores from the conduct problems and hyperactivity subscales are combined to create the externalizing (behavioral issues) score, while emotional and peer problems are combined to form the internalizing (emotional issues) score. Higher scores indicate more behavioral problems in children.

EPDS

Within the maternal questionnaire, mothers completed the EPDS to measure symptoms of depression. Each item of the EPDS is scored between 0-3, therefore total scores range between 0-30, with higher scores indicating the presence of depressive symptoms. The validated French version of the EPDS was translated and the written version in Fon was administered by trained research nurses. If a woman indicated within the EPDS that they had had thoughts of self-harm, she was seen by a psychologist on the research team for further follow-up and aide.

Socio-demographic information

Similar to protocol used at the one-year follow-up, maternal questionnaires assessed family possession scores using a checklist of material possessions owned by the family to indicate wealth: a radio, television, bicycle, motorbike, car, at least two cows, and electricity. Women were also asked about their education level.

Funding & Ethics Statement

Funding for EXPLORE was provided by the Fondation de France (2015 00060746). Ethical approval was obtained by the Committee of Ethical Research of the Applied Biomedical Sciences Institute (CER-ISBA) in Benin. Written informed consent was obtained by mothers and guardians of children in the presence of a witness, with thumbprints provided for consent if women could not read and/or write.

Statistical Software

Statistical analyses were completed using STATA 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP) for Windows and STATA 16 (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC) for Mac. All statistical tests were two-tailed with an alpha risk of 5%, unless otherwise specified in the manuscript or in publications.

Chapter 3. The effect of soil-transmitted helminth infection in pregnancy on child neurocognitive development

This chapter presents the published article studying the impact of soil-transmitted helminth infections in pregnancy on child neurocognitive and behavioral development at six years of age. The analyses within this paper are a continuation of a previous article published by Mireku et al. (2015) that showed impaired gross motor function in one-year-old children born to mothers who were infected by hookworms during pregnancy in our population. Our results show that SHIP no longer impairs neurocognitive development in children long-term as measured by the KABC-II, BOT-2, and TOVA assessments at six years of age. However, further analyses show that SHIP was associated with impaired behavioral development in six-year-old children as measured by the SDQ, particularly from the internalizing score indicating emotional development in children. Mediation analyses explored the hypothesized mechanism of this association via activation of the maternal inflammatory immune response; however, the limited inflammation markers that are non-specific to helminth infection available for our analyses did not confirm this hypothesis. This is the first publication of its kind to address the long-term impacts of SHIP on child development, and to explore the mechanism of maternal inflammation. Additionally, this paper serves as evidence that SHIP and helminth infection in childhood carry a large burden of disease in this population, even in the context of a clinical trial and prospective follow-up of children, thus reinforcing the need for sufficient preventive and treatment options in heavily burdened areas of the world. This article was published in *Plos Neglected Tropical Diseases* as an original research article.

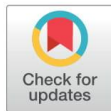
RESEARCH ARTICLE

Soil-transmitted helminth infection in pregnancy and long-term child neurocognitive and behavioral development: A prospective mother-child cohort in Benin

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OPEN ACCESS

Citation: Garrison A, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, et al. (2021) Soil-transmitted helminth infection in pregnancy and long-term child neurocognitive and behavioral development: A prospective mother-child cohort in Benin. *PLoS Negl Trop Dis* 15(3): e0009260. <https://doi.org/10.1371/journal.pntd.0009260>

Editor: Ayse Ercumen, North Carolina State University, UNITED STATES

Received: June 30, 2020

Accepted: February 21, 2021

Published: March 19, 2021

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Data Availability Statement: All relevant data related to this study are within the manuscript, its [Supporting Information files](#), and in an article published in *Plos Medicine* [González R, Mombona G, Ouédraogo S, Kakolwa MA, Abdulla S, et al. (2014) Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre Randomized Controlled Trial. *PLoS Med* 11(9): e1001733. doi:10.1371/journal.pmed.1001733]. Data from the MiPPAD clinical trial cannot be shared publicly

Abstract

Background

An estimated 30% of women in Sub-Saharan Africa suffer from soil-transmitted helminth infection during pregnancy (SHIP), which has been shown to increase risk of pre-term birth, low birth weight, and maternal anemia. A previous study in Benin found that SHIP was associated with impaired cognitive and gross motor development scores in 635 one-year-old children. The objective of the present study was to follow children prospectively to investigate whether the association between SHIP and child neurocognitive and behavioral development persisted at age six.

Principal findings

Our prospective child cohort included 487 live-born singletons of pregnant women enrolled in the *Malaria in Pregnancy Preventive Alternative Drugs* clinical trial in Allada, Benin. SHIP was assessed at three antenatal visits (ANVs) through collection and testing of stool samples. Neurocognitive and behavioral development was assessed in six-year-old children by trained investigators using the Kaufman Assessment Battery for Children 2nd edition and the parent-reported Strengths and Difficulties Questionnaire (SDQ). Multiple linear regression models generated coefficients and 95% confidence intervals and potential mediating factors were tested. Prevalence of SHIP was 13% at the 1st ANV, 9% at the 2nd ANV, and 1% at delivery. SHIP was not associated with low neurocognitive scores in children at six years.

because of ethical restrictions. Access to data from the MiPPAD study may be available upon request through The MiPPAD Executive Committee (Dr. Raquel Gonzalez at +34-932-275-400 or raquel.gonzalez@cresib.cat).

Funding: Research cohorts included in this study were funded by the following bodies: the Fondation de France (2015 00060746) (FBL), the Eunice Kennedy Shriver National Institute of Child Health & Human Development (NIH/NICHD- R21-HD060524) (FBL), the European and Developing Countries Clinical Trials Partnership (EDCTP-IP.07.31080.002) (MC), and the Bill and Melinda Gates Foundation (MC). Funding sources did not play any roles in the study design, data collection and analysis, decision to publish, or preparation of this manuscript.

Competing interests: The authors have declared that no competing interests exist.

Higher SDQ internalizing scores, indicating increased emotional impairments in children, were associated with helminth infection at the 2nd ANV/delivery 1.07 (95% CI 0.15, 2.00) and at least once during pregnancy 0.79 (95% CI 0.12, 1.46) in adjusted models. Mediation analysis did not reveal significant indirect effects of several mediators on this association.

Conclusions

Our study shows that while SHIP is not associated with impaired long-term neurocognitive development, infections may have significant negative impacts on emotional development in six-year-old children. SHIP remains a critical public health issue, and adequate prevention and treatment protocols should be enforced in low- and middle-income countries.

Author summary

Soil-transmitted helminth infections impact 1.5 billion individuals, primarily in low- and middle-income countries, each year and contribute to malnutrition, anemia, and impaired neurocognitive development in children. However, these infections in pregnancy and their impact on offspring have been less studied. One previous study found associations between soil-transmitted helminth infection during pregnancy and impaired cognitive functioning in offspring one year after birth. The current study aimed to follow these children prospectively until six years in order to confirm whether these associations persisted or not. Infections during pregnancy were no longer associated with cognitive or motor functioning in children; however, infections were associated with impaired behavioral development. Animal-based models have hypothesized maternal inflammation and poor birth outcomes to be the mechanisms behind this relationship; however, our findings did not support these mechanisms. This is one of very few prospective cohort studies in Sub-Saharan Africa to investigate these associations, and more research is needed to corroborate results. Limitations include limited power and the possibility that results are due to chance from multiple statistical tests. Adequate and accessible prevention and treatment efforts in pregnancy and childhood should be provided to populations in low- and middle-income countries at high risk of infection.

Introduction

Soil-transmitted helminths infect approximately 1.5 billion people worldwide annually [1], contributing to more than five million disability-adjusted life years (DALYs)[2]. Symptoms of these infections include diarrhea and abdominal pain, and infection can have long-term consequences on development and nutritional status [3]. The term 'soil-transmitted helminths' refers to several species of parasitic worms, the most common of which are roundworms, *Ascaris lumbricoides*, whipworms, *Trichuris trichiura*, and hookworms, *Ancylostoma duodenale* and *Necator americanus* [4]. Pregnant women and children are especially vulnerable to hookworm infection, a known risk factor for iron deficiency anemia, since these populations tend to have the lowest iron stores [5,6]. During their adult worm-stage, helminths live in the intestines of infected individuals and transmit their eggs within human feces. In areas with inadequate sanitation and plumbing, human feces may contaminate the soil which is ingested from improperly cleaned or prepared vegetables, contaminated water sources, or direct ingestion by

children [7]. Hookworm species infect individuals through penetration of the skin when it is exposed to larvae from hatched eggs in the soil [8]. Developing countries, particularly in Sub-Saharan Africa (SSA), are at higher risk of soil-transmitted helminth infection due to conditions of poverty, poor sanitation, limited resources, and an abundance of the population living in rural areas [9,10].

Prevalence of soil-transmitted helminth infection during pregnancy (SHIP) in SSA is estimated to range from 11–31%, according to previous studies [10–12]. Often, infected individuals in SSA countries display polyparasitism, meaning they are simultaneously infected with more than one species of helminth [13,14]. SHIP is known to have negative consequences on birth and maternal outcomes, such as pre-term birth, low birth weight, and maternal anemia [15,16]. The World Health Organization (WHO) recommends preventive chemotherapy in the form of a single dose of either 400mg of albendazole or 500mg of mebendazole for pregnant women during the first trimester in areas of the world with a high baseline prevalence of helminth infection (20%) and anemia (40%) in pregnant women [17].

Several studies have shown the detrimental impacts of soil-transmitted helminth infection during childhood on child growth and neurocognitive development [18,19]; however, little research has focused on how exposure to these infections during pregnancy may impact child neurocognitive development. Two studies from a birth-cohort in Uganda have shown that prenatal exposure to helminth infection did negatively impact early cognitive functioning in 15-month old infants [20]; however, anti-helminthic treatment did not reverse or impact cognitive development in these offspring in early or late childhood [21]. Evidence from some studies have shown that infections such as maternal Human Immunodeficiency Virus (HIV) and malaria during pregnancy remain associated with poor neurocognition in children even at 16 years of age [22,23], suggesting that infections during pregnancy can have long-term consequences on child development. A recent study in Benin found associations between hookworm infection during pregnancy and poor cognitive and gross motor scores, as measured by the Mullen Scales of Early Learning (MSEL), in children at one year of age [24]. However, we found no literature that explored these associations in school-aged children. Additionally, there is a growing body of literature on the consequences of prenatal infection on behavioral development in offspring [25–27]. We found no evidence of studies investigating how maternal parasitic infection, specifically SHIP, may impact long-term behavioral development in children.

Hookworm infection during pregnancy has been shown to increase systemic inflammation and more specifically increase erythrocyte sedimentation rate (ESR), a general marker of inflammation [28,29]. Rodent models have demonstrated that maternal infection leads to inflammatory immune responses that can alter brain development of the fetus and have long-term neurodevelopment consequences [30,31]. Existing evidence suggests that systemic maternal infection and subsequent inflammation response disrupts placental vasculogenesis and angiogenesis, which construct the materno-fetal interface during pregnancy, and in turn negatively impacts fetal growth and development [32]. We were unable to identify previous studies investigating the role of the maternal immunoinflammatory response from SHIP on long-term child neurocognitive development.

Therefore, the main objectives of our study were to investigate the associations between SHIP and neurocognitive and behavioral development outcomes in a population of children in Benin prospectively at six years of age. Infections from hookworms were analyzed separately from other helminth infections due to the significant susceptibility in this population to these infections and their subsequent consequences on iron stores. A secondary objective was to test the indirect effects of SHIP exposure on development through potential mediating factors identified in the literature to explore previously hypothesized mechanisms. Our hypothesis

was that SHIP would remain associated to impaired neurocognitive and behavioral development in this prospective cohort at six years of age, and that this relationship would be mediated by hypothesized factors such as inflammation.

Methods

Ethics statement

Ethical approval for all studies mentioned in this article was obtained by the institutional review boards of the Beninese Ethical Committee of the Faculté des Sciences de la Santé (FSS) and the Committee of Ethical Research of the Applied Biomedical Sciences Institute (CER--ISBA) in Benin, New York University in the United States of America, and the Research Institute for Development's (IRD) Consultative Ethics Committee in France. Written informed consent was obtained by mothers and guardians of children in the presence of a witness, with thumbprints provided for consent if women could not read and/or write.

Study population

Our prospective cohort of children included 487 live-born singletons of pregnant women enrolled in the *Malaria in Pregnancy Preventive Alternative Drugs* (MiPPAD) clinical trial (NCT00811421) comparing the efficacy of sulfadoxine-pyrimethamine and mefloquine, two intermittent preventive treatments of malaria in pregnancy (IPTp). The clinical trial took place in the district of Allada, Benin and recruited 1,005 women in the second trimester of pregnancy (before 29 weeks' gestation) who attended their first antenatal visit (ANV) located in one of three local health clinics in Sékou, Allada, or Attogon. Information on inclusion and exclusion criteria, and detailed recruitment procedures, of the clinical trial have been explained in a preceding publication [33]. Briefly, inclusion criteria for women were: a permanent residence in the study area, gestational age ≤ 28 weeks, HIV-negative at recruitment, no known allergies to sulfa or mefloquine drugs, no treatment of malaria using these drugs within four weeks of recruitment, and no history of severe renal, hepatic, psychiatric, or neurological disease. All 863 surviving, eligible singletons born to clinical trial participants were invited to participate in a follow-up study (TOVI, meaning 'child' in the local Benin language of Fon) at one year of age. At one year, 635 of these children were assessed for neurocognitive development, blood samples were taken, and demographic information was collected through questionnaires given to mothers [24]. Finally, 487 children from the birth cohort were followed-up prospectively at six years of age and assessed for neurocognitive and behavioral development within the *Lead and Manganese Exposure and Child Risk* (French acronym: EXPLORE) study.

Soil-transmitted helminth infection during pregnancy

The main exposure of interest in our study was SHIP, which was assessed during a nested-cohort study within the MiPPAD clinical trial, entitled *Anemia in Pregnancy: Etiologies and Consequences* (APEC) [34]. During follow-up, women were seen at a local health clinic on three occasions: the 1st ANV at recruitment, the 2nd ANV at least one month later, and at delivery. As part of the Benin ANV package, women were given malaria IPTp and iron and folic acid supplements at each visit, as well as 600 mg of mebendazole (100 mg twice daily for three days) at the 1st ANV, and again at the 2nd ANV if women tested positive for SHIP. Intake of mebendazole was not directly monitored by health professionals. Women also received insecticide-treated bed nets at recruitment. Containers were given to women at ANVs in order to collect stool samples the following morning to test for infection. Stool samples were also collected 15 days prior to the expected date of delivery, where possible, or within one week after delivery.

Thick smears of stool samples were prepared and assessed using the Kato-Katz technique as outlined by the WHO [35]. Each of the two slides prepared from each sample was systematically and independently examined by two laboratory technicians under a microscope and the mean of the two results was calculated. The standardized fecal egg count (FEC), expressed in eggs per gram (epg), for each stool sample was obtained by counting the number of eggs for each species and multiplying this number by 24. Helminth infection in this article is defined as the presence of at least one egg of any soil-transmitted helminth species found in the stool. Hookworm infection refers to infections caused by *A. duodenale* and *N. americanus* species. Analyses considered helminth and hookworm infection diagnosed at the 1st ANV, the 2nd ANV/delivery, and at least once during pregnancy. Helminth and hookworm infections diagnosed at the 2nd ANV and at delivery were considered together, with women being considered as infected if found to be positive at either visit; we hypothesized that infections during the third trimester would have similar effects on neurocognitive and behavioral development in children.

Child development at six years

The main outcomes considered in this study, child neurocognitive and behavioral development, were assessed at six years of age within the EXPLORE study, whose principal objective was to study the impact of lead and manganese exposure on neurocognitive development and growth in children. Participating mothers and their children visited one of two health centers, in Attogon or Sékou, where trained investigators obtained written consent from caregivers and subsequently administered questionnaires to mothers, collected blood and stool samples from children, and assessed them for neurocognitive and behavioral development. Child stool samples were collected in order to test for helminth infection. If a child was ill or had fever when they arrived at the clinic, blood samples were taken to determine presence of infection and children were asked to return at a later date for neurocognitive assessments. Children who were positively diagnosed with malaria, anemia, or helminth infection were treated according to existing national treatment guidelines at the time.

Neurocognitive development in children was assessed using several tests, including the Kaufman Assessment Battery for Children 2nd edition (KABC-II), a psychological diagnostic tool used to assess processing and cognitive ability, as well as planning and learning capabilities, in children. The KABC-II was available in French and for the purposes of this study was translated into the local language of Benin (Fon) by trained investigators. The construct validity of the KABC-II for use in Benin was verified during a pilot study within this population [36]. For the purposes of this study the Mental Processing Index (MPI), a measure of global cognitive ability generated by the KABC-II, was studied. The Bruininks-Oseretsky Test of Motor Proficiency Edition 2 (BOT-2) was administered to evaluate fine and gross motor skills in children. Final composite scores for fine and gross motor skills were standardized by age and sex and used in analyses. Finally, the Test of Variables of Attention (TOVA), a non-language-based performance test, was administered to identify attention deficits and hyperactivity disorders in children. The final score identifying Attention Deficit and Hyperactivity Disorder (ADHD) is calculated based on response times, variability of response times, and rate of deterioration of responses to computerized stimuli appearing on a screen in front of children during the test. Based on United States norms, scores below -1.80 are considered suggestive of ADHD, with higher scores indicating normal attention rates [37]. The TOVA and BOT-2 were not validated for use in this population; however, they have been validated within other populations of children in SSA countries [37]. For the purposes of our analyses, results within our population were not compared to Western norms and instead analyses focused solely on internal comparisons of scores among children.

Behavioral development of children was assessed through the parent-reported Strengths and Difficulties Questionnaire (SDQ) given to mothers [38]. The questionnaire screens for issues related to five subscales: emotional problems, conduct problems, hyperactivity, peer problems, and pro-social behaviors; with the four former subscales used to generate an overall difficulties score. The SDQ was translated into Fon by trained investigators for the purposes of this study. The SDQ further differentiates between emotional and behavioral conduct problems in children through externalizing and internalizing scores generated by its subscales. Scores from the conduct problems and hyperactivity subscales are combined to create the externalizing (behavioral issues) score, while emotional and peer problems are combined to form the internalizing (emotional issues) score. Higher SDQ scores indicate more behavioral and/or emotional issues in children. Due to the fact that illiteracy rate among mothers in this population was 75%, all assessment instructions and questionnaires were spoken aloud to children and caregivers by trained investigators. A common translation of oral instructions from French to Fon was validated by trained assessors and study nurses prior to the onset of assessments.

Demographic factors

Family socio-demographic information, such as maternal age, socioeconomic status, education level, and pre-pregnancy Body Mass Index (BMI) was collected from women at recruitment during the 1st ANV of the clinical trial. Malaria incidence during pregnancy, diagnosed using the gold standard thick blood smear test, was recorded in women during the trial. Infant sex, gestational age according to fundal height, and birth weight in grams were recorded at delivery. At the time of neurocognitive and behavioral assessments in children at six years, questionnaires were given to mothers to collect additional demographic information. A continuous score was generated for family possessions to indicate socioeconomic status; with scores given to items owned by the family of pregnant women such as electricity, a car, a motorcycle, a television, a radio, a bicycle, or cows. Parent-child interactions in the home were assessed using the Home Observation for Measurement of the Environment (HOME) test, which was adapted for use in Benin during a pilot study [39].

Factors included in mediation analyses were inflammation during pregnancy, weight-for-age z-scores in children at six years, gestational age at birth, and birth weight. Inflammation during pregnancy was defined as a C-reactive Protein (CRP) concentration higher than 5 mg/mL at one or more ANVs. Weight-for-age z-scores were calculated according to the WHO z-score classification system to represent the latent variable of child nutrition.

Statistical analysis

Baseline characteristics and exposure to SHIP within our population followed up six years post-partum were described and compared to those lost to follow-up since birth using the non-parametric Fisher exact test for categorical variables. Risk factors of helminth infection in pregnancy and impaired child neurocognitive and behavioral development were identified within the literature and direct acyclic graphs (DAGs). Adjusted models controlled for maternal age, education, pre-pregnancy BMI, family possession score, HOME score, child sex, child age at assessment, and incidence of malaria during pregnancy. Maternal age, family possession score, HOME score, and child age at assessment were continuous variables in regression models. Maternal education (some/none), child sex (male/female), and incidence of malaria during pregnancy (yes/no) were binary variables. Pre-pregnancy BMI was categorized as underweight (<18.5), normal (18.5–24.9), or overweight/obese (≥ 25). Multiple linear regression models were used to test associations between SHIP and hookworm density in pregnancy and child

neurocognitive and behavioral development at six years. The variance inflation factor (VIF) was used to detect multicollinearity between co-variables, if present.

For the secondary objective, mediation analysis using Structural Equation Modeling (SEM) further explored mechanisms of significant associations from multiple linear regressions. The total effects of SHIP on child emotional development were explained through the direct effect of the exposure on the outcome and the indirect effects (i.e. effects mediated by potential intermediate factors identified in the literature) of this association [40]. Mediation analyses adjusted for confounding factors included in regression models and bootstrapped, or bias-corrected, confidence intervals (CIs) are displayed.

Several sensitivity analyses were carried out to assess the robustness of analyses. Characteristics of the population analyzed were compared to those of the mothers and children lost to follow-up since birth. Linear regression models between SHIP and MSEL scores of the 487 children from EXPLORE seen at one year were tested to confirm results from the previous study conducted in this population. Multiple linear regression models tested for associations between helminth infection in children at six years and neurocognitive and behavioral development scores. Multiple imputation of missing variables, including outcome variables and confounding factors, and living mother-child pairs lost to follow-up between birth and six years ($n = 320$) was completed to minimize plausible selection bias. Blood lead level in children and history of hospitalization between one- and six-year follow-ups were tested in additional models for significance.

All statistical analyses were completed using STATA 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP). Statistical tests were two-tailed with an alpha risk of 5%. Supporting files contain relevant information regarding the epidemiological conduct of this paper (S1 STROBE Checklist) and data used in analyses (S1 Data).

Results

Of the 863 singletons born alive to mothers in the MiPPAD clinical trial, 487 (61%) of eligible children were assessed at 6 years of age and included in our analyses (Fig 1). Four children with severe neurological and mental disorders were excluded from analyses, as well as two children who presented with fever (temperature $\geq 37.5^{\circ}\text{C}$) at the time of neurodevelopment assessments.

Table 1 shows the comparison of maternal and infant demographic characteristics at birth and maternal exposure to helminths during pregnancy between children included and excluded in analyses. Mother-child pairs included in our analyses were of higher maternal age, more likely to be multigravida, and of higher parental education levels.

Prevalence of helminth infection in pregnant women during follow-up was 12.6% at the 1st ANV, 8.5% at the 2nd ANV, and 1.2% at delivery (Table 2). The majority of infections in pregnancy were not chronic, with 15% of infected women having more than one infection over the course of her pregnancy. The proportion of infections at each visit caused by hookworm species was 77.9%, 85%, and 100%, respectively. The prevalence of helminth infection in children at six years was 44.6%; 63.7% of infections due solely to hookworms, 27.4% due solely to roundworm, 8.2% due to polyparasitism of hookworm and roundworm species, and <1% due to tapeworm.

Crude and adjusted multiple linear regression models tested the associations between helminth infection and hookworm density in women during pregnancy and neurocognitive development outcomes in their children at six years of age (Table 3). Adjusted models were controlled for maternal age, education, pre-pregnancy BMI, family possession score, HOME score, child sex, age at the time of assessment, and malaria incidence during pregnancy.

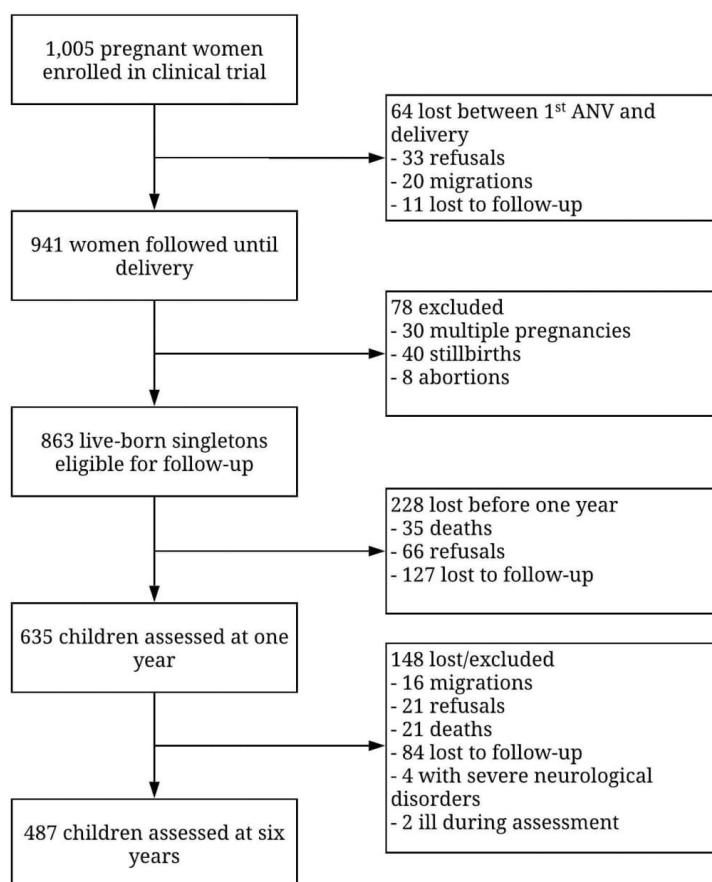


Fig 1. Population flowchart from second trimester of pregnancy until child follow-up at six years of age.

<https://doi.org/10.1371/journal.pntd.0009260.g001>

Helminth infection diagnosed in pregnant women at each ANV and overall helminth infection and hookworm density during pregnancy were not found to be associated with neurocognitive development, motor function, or attention/hyperactivity disorder in children at six years of age measured by the KABC-II, BOT-2, and TOVA tests, respectively. Helminth infection in children at the time of neurocognitive assessments was also not found to significantly impair test scores.

Table 4 illustrates that SHIP was not associated with a higher total SDQ score in crude or adjusted models. However, SHIP was associated with impaired emotional development as measured by the SDQ internalizing score. Helminth infection at the 1st ANV was associated with higher internalizing scores 0.82 (95% CI 0.02, 1.62) in the crude model. Helminth infection at the 2nd ANV/delivery was associated with higher internalizing scores in both crude 1.13

Table 1. Demographic and exposure characteristics of mothers and children included in analyses compared to those lost to follow-up.

	Included (N = 487) n (%)	Lost (N = 320) n (%)	p-value¹
<i>Demographic Characteristics</i>			
Maternal age			0.06
<25	196 (40)	147 (46)	
25–29	127 (26)	91 (28)	
30+	162 (34)	82 (26)	
Gravidity			0.001
Primigravida	78 (16)	83 (26)	
Multigravida	399 (84)	232 (74)	
Marital status			0.002
Not married	7 (1)	4 (1)	
Monogamous	298 (62)	233 (72)	
Polygamous	180 (37)	82 (27)	
Mother education (1 year)			0.02
No education	333 (69)	194 (61)	
Primary education/+	152 (31)	125 (39)	
Father education (1 year)			0.04
No education	251 (52)	141 (44)	
Primary education/+	234 (48)	178 (56)	
Child sex			0.54
Female	242 (50)	167 (52)	
Male	242 (50)	153 (48)	
Birth weight			0.85
Low (<2500g)	17 (3)	12 (4)	
Normal (≥ 2500g)	468 (97)	307 (96)	
Gestational age			0.43
Pre-term (<37 weeks)	16 (3)	14 (4)	
Term (≥ 37 weeks)	469 (97)	305 (96)	
<i>Exposure Characteristics</i>			
Helminth infection			0.32
Never infected	396 (82)	270 (84)	
Infected at least once	89 (18)	50 (16)	
Helminth infection			
1 st ANV	59 (12)	33 (10)	0.41
2 nd ANV/delivery	42 (13)	24 (12)	0.97
Hookworm infection			0.47
Never infected	411 (85)	277 (87)	
Infected at least once	74 (15)	43 (13)	
Hookworm infection			
1 st ANV	46 (10)	28 (9)	0.72
2 nd ANV/delivery	36 (11)	19 (10)	0.74

¹ P-values from Fisher exact tests

<https://doi.org/10.1371/journal.pntd.0009260.t001>

(95% CI 0.21, 2.05) and adjusted 1.07 (95% CI 0.15, 2.00) models. Helminth infection at any point in pregnancy was also associated with higher internalizing scores in crude 0.78 (95% CI 0.78, 1.45) and adjusted 0.79 (95% CI 0.12, 1.46) models. Infection did not seem to significantly

Table 2. Description of infection in women during pregnancy and in children at six years.

Parameter	N	n (%)
Helminth Infection		
1 st ANV	480	58 (12)
2 nd ANV	472	40 (9)
Delivery	334	4 (1)
Helminth species at 1st ANV	58	
Hookworms		45 (78)
<i>Ascaris lumbricoides</i>		7 (12)
<i>Trichuris trichiura</i>		5 (9)
Other		1 (2)
Helminth species at 2nd ANV	40	
Hookworms		34 (85)
<i>Ascaris lumbricoides</i>		4 (10)
<i>Trichuris trichiura</i>		3 (8)
Other		2 (5)
Helminth species at delivery	4	
Hookworms		4 (100)
<i>Ascaris lumbricoides</i>		–
<i>Trichuris trichiura</i>		–
Other		–
Hookworm density^{1,2}	73	
1 st ANV		72 (24–984)
2 nd ANV		72 (24–600)
Delivery		264 (72–624)
Number of helminth infections during pregnancy	487	
None		399 (82)
1 infection		74 (15)
2 infections		14 (3)
Co-infection with malaria during pregnancy	487	
1 st ANV		8 (2)
2 nd ANV		–
Delivery		1 (<1)
Helminth infection in children at six years	327	
No infection		181 (55)
Infection		146 (45)
Helminth species in children at six years	146	
Hookworms		93 (64)
<i>Ascaris lumbricoides</i>		40 (27)
<i>Trichuris trichiura</i>		–
<i>Strongyloides stercoralis</i>		–
<i>Enterobius vermicularis</i>		–
Hookworm/ <i>lumbricoides</i> co-infection		12 (8)
<i>Taenia</i>		1 (<1)

1 Hookworm density in eggs per gram (epg) after multiplication factor

2 Median (range)

<https://doi.org/10.1371/journal.pntd.0009260.t002>

Table 3. Multiple linear regression models between SHIP and child neurocognitive development scores at six years.

	<i>KABC-II Mental Processing Index</i>		<i>BOT-2 Motor Composite Score</i>		<i>TOVA ADHD Score</i>	
	Coefficient (95% CI)		Coefficient (95% CI)		Coefficient (95% CI)	
	Crude	Adjusted ¹	Crude	Adjusted ¹	Crude	Adjusted ¹
	N = 478	N = 456	N = 474	N = 452	N = 442	N = 422
<i>Helminth infection at 1st ANV</i>	-0.09 (-3.46, 3.28)	0.71 (-2.51, 3.92)	-1.82 (-4.34, 0.70)	-0.04 (-2.36, 2.27)	-0.48 (-1.29, 0.33)	-0.06 (-0.86, 0.73)
<i>Helminth infection at 2nd ANV/delivery</i>	-2.65 (-6.57, 1.26)	-2.04 (-5.82, 1.73)	0.22 (-2.72, 3.16)	-0.75 (-1.93, 3.43)	-0.02 (-0.95, 0.91)	0.02 (-0.89, 0.93)
<i>Helminth infection once in pregnancy</i>	-1.24 (-4.08, 1.60)	-0.71 (-3.41, 2.00)	-0.85 (-2.97, 1.28)	0.17 (-1.77, 2.11)	-0.27 (-0.95, 0.41)	-0.03 (-0.69, 0.64)
<i>Hookworm infection at 1st ANV</i>	0.82 (-2.94, 4.59)	1.74 (-1.86, 5.33)	-0.71 (-3.54, 2.12)	1.20 (-1.39, 3.80)	-0.45 (-1.36, 0.45)	-0.01 (-0.91, 0.89)
<i>Hookworm infection at 2nd ANV/delivery</i>	-3.75 (-7.96, 0.46)	-2.67 (-6.74, 1.41)	0.24 (-2.92, 3.40)	0.68 (-2.20, 3.57)	0.12 (-0.87, 1.12)	0.15 (-0.82, 1.11)
<i>Hookworm infection once in pregnancy</i>	-1.51 (-4.58, 1.55)	-0.78 (-3.70, 2.15)	-0.40 (-2.69, 1.89)	0.52 (-1.57, 2.62)	-0.24 (-0.97, 0.49)	-0.03 (-0.74, 0.68)
<i>Hookworm density during pregnancy</i>	0.01 (-0.01, 0.02)	0.01 (-0.01, 0.02)	0.01 (-0.01, 0.02)	0.01 (-0.01, 0.02)	-0.01 (-0.01, 0.01)	0.01 (-0.01, 0.01)
	N = 326	N = 307	N = 324	N = 305	N = 303	N = 288
<i>Child helminth infection at 6 years</i>	-0.71 (-3.28, 1.87)	-1.05 (-3.60, 1.50)	-1.34 (-3.24, 0.56)	-0.90 (-2.60, 0.79)	0.19 (-0.43, 0.80)	0.21 (-0.39, 0.82)

¹ Adjusted for maternal age, education, pre-pregnancy BMI, family possession score, HOME score, child sex, child age at assessment, and incidence of malaria during pregnancy

<https://doi.org/10.1371/journal.pntd.0009260.t003>

impair behavioral development measured by the SDQ externalizing scores. Hookworm density during pregnancy and helminth infection in children at the time of assessment were not found to be associated with SDQ scores.

Mediation analyses using SEM methods investigated potential mechanisms behind the significant association seen between SHIP at least once during pregnancy and impaired emotional development as measured by the SDQ internalizing score (Fig 2). Models were adjusted for potential confounding factors used in multiple linear regression and coefficients with 95% confidence intervals are shown. Residuals of mediator and outcome variables are represented by ϵ_{1-5} . Analyses did not reveal significant mediation through the indirect effects of each

Table 4. Multiple linear regression models between SHIP and child behavioral and emotional development scores at six years.

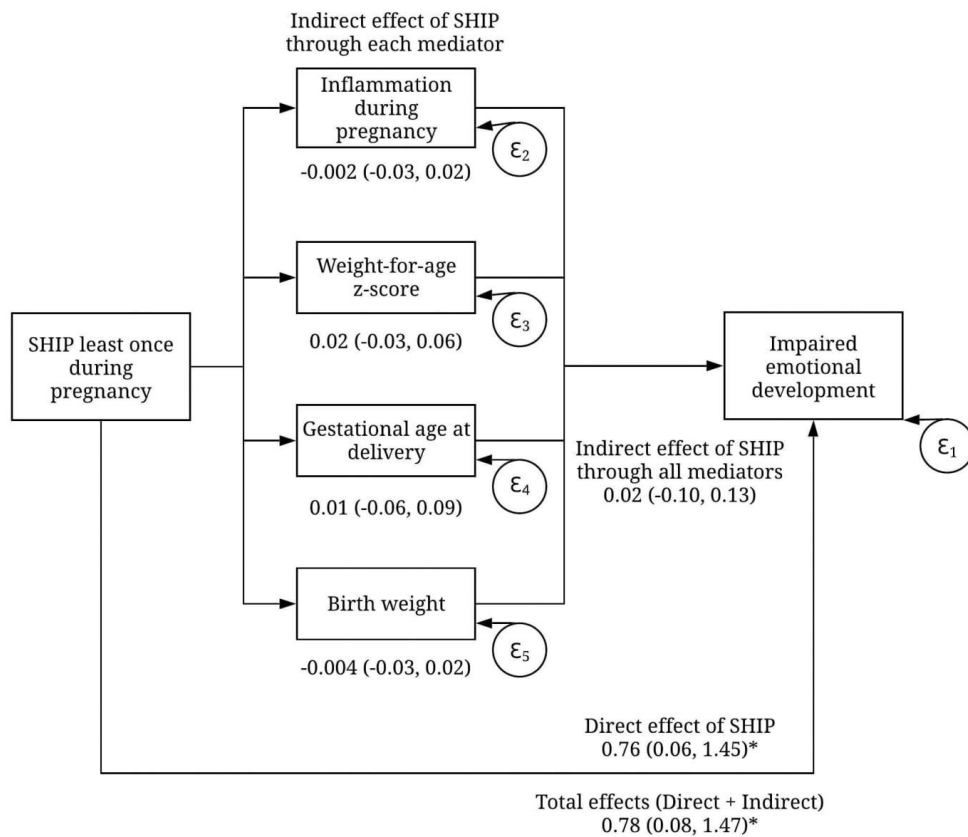
	<i>Strengths & Difficulties</i>		<i>Strengths & Difficulties</i>		<i>Strengths & Difficulties</i>	
	Total Score		Internalizing Score		Externalizing Score	
	Coefficient (95% CI)		Coefficient (95% CI)		Coefficient (95% CI)	
	Crude	Adjusted ¹	Crude	Adjusted ¹	Crude	Adjusted ¹
	N = 479	N = 458	N = 479	N = 458	N = 479	N = 458
<i>Helminth infection at 1st ANV</i>	0.83 (-0.67, 2.33)	1.02 (-0.52, 2.55)	0.82 (0.02, 1.62) *	0.77 (-0.04, 1.58)	0.01 (-1.00, 1.01)	-0.25 (-0.77, 1.26)
<i>Helminth infection at 2nd ANV/delivery</i>	1.68 (-0.07, 3.42)	1.45 (-0.32, 3.22)	1.13 (0.21, 2.05) *	1.07 (0.15, 2.00) *	0.55 (-0.62, 1.71)	0.38 (-0.80, 1.55)
<i>Helminth infection once in pregnancy</i>	0.81 (-0.45, 2.07)	0.88 (-0.40, 2.16)	0.78 (0.11, 1.45) *	0.79 (0.12, 1.46) *	0.03 (-0.81, 0.87)	0.09 (-0.76, 0.94)
<i>Hookworm infection at 1st ANV</i>	0.63 (-1.05, 2.31)	0.80 (-0.93, 2.52)	0.65 (-0.24, 1.55)	0.65 (-0.25, 1.56)	-0.02 (-1.14, 1.10)	0.14 (-0.99, 1.27)
<i>Hookworm infection at 2nd ANV/delivery</i>	1.30 (-0.58, 3.17)	0.87 (-1.05, 2.78)	0.95 (-0.03, 1.94)	0.85 (-0.15, 1.84)	0.34 (-0.91, 1.60)	0.02 (-1.24, 1.29)
<i>Hookworm infection once in pregnancy</i>	0.70 (-0.65, 2.06)	0.69 (-0.70, 2.07)	0.70 (-0.02, 1.42)	0.71 (-0.02, 1.43)	-0.01 (-0.91, 0.91)	-0.02 (-0.94, 0.89)
<i>Hookworm density during pregnancy</i>	0.01 (-0.01, 0.01)	0.01 (-0.01, 0.01)	0.001 (-0.01, 0.01)	0.01 (-0.01, 0.01)	0.01 (-0.01, 0.01)	-0.01 (-0.01, 0.01)
	N = 326	N = 308	N = 326	N = 308	N = 326	N = 308
<i>Child helminth infection at 6 years</i>	-0.37 (-1.53, 0.79)	-0.17 (-1.38, 1.05)	-0.17 (-0.81, 0.47)	-0.08 (-0.74, 0.57)	-0.20 (-0.98, 0.57)	-0.08 (-0.87, 0.71)

¹ Adjusted for maternal age, education, pre-pregnancy BMI, family possession score, HOME score, child sex, child age at assessment, and incidence of malaria during pregnancy

* $p \leq 0.05$

** $p \leq 0.01$

<https://doi.org/10.1371/journal.pntd.0009260.t004>



¹Coefficients (95% confidence intervals) shown are adjusted for maternal age, education, pre-pregnancy BMI, family possession score, HOME score, child sex, child age at assessment, and incidence of malaria during pregnancy
*P-value<0.05

Fig 2. Mediation analysis between SHIP at least once during pregnancy and impaired emotional development in children at six years¹.

<https://doi.org/10.1371/journal.pntd.0009260.g002>

mediator: inflammation during pregnancy -0.002 (95% CI -0.03, 0.02), child weight-for-age-z-score 0.02 (95% CI -0.03, 0.06), gestational age at delivery 0.01 (95% CI -0.06, 0.09), or birth weight -0.004 (95% CI -0.03, 0.02). The total effect of SHIP on impaired emotional development was statistically significant 0.78 (95% CI 0.08, 1.47), however less than 3% of the total effects could be explained by the indirect effects of potential mediators tested.

After multiple imputation of missing outcomes, co-variables, and children lost to follow-up between birth and six years of age, helminth infections at 1st ANV, 2nd ANV/delivery, and at least once during pregnancy were significantly associated to higher SDQ internalizing scores after adjusting for confounding factors (Table 5). Hookworm infections at the 2nd ANV/delivery and at least once during pregnancy were associated with higher internalizing scores in

Table 5. Crude and adjusted coefficients (95% CI) from multiple linear regression models after imputation of missing variables and children lost to follow-up.

	N	Neurocognitive development scores			Behavioral development scores		
		KABC-II Mental Processing Index	BOT-2 Motor Composite Score	TOVA ADHD Score	SDQ Total Score	SDQ Internalizing Score	SDQ Externalizing Score
Helminth infection at 1 st ANV	799	-0.19 (-3.08, 2.70)	-2.02 (-4.70, 0.67)	-0.63 (-1.39, 0.13)	1.19 (-0.28, 2.67)	1.03 (0.24, 1.82) **	0.18 (-0.81, 1.16)
		0.62 (-2.84, 4.07) ¹	-0.85 (-3.44, 1.73) ¹	-0.39 (-1.13, 0.35) ¹	1.16 (-0.34, 2.66) ¹	0.96 (0.16, 1.77) ¹ *	0.21 (-0.79, 1.21) ¹
Helminth infection at 2 nd ANV/delivery	794	-3.00 (-7.48, 1.48)	0.22 (-2.96, 3.39)	-0.17 (-1.18, 0.84)	1.97 (0.08, 3.86) *	1.28 (0.28, 2.28) **	0.68 (-0.59, 1.96)
		-1.78 (-5.91, 2.35) ¹	1.50 (-1.53, 4.53) ¹	-0.03 (-0.97, 0.92) ¹	1.76 (-0.08, 3.61) ¹	1.26 (0.28, 2.23) ¹ **	0.51 (-0.74, 1.76) ¹
Helminth infection once in pregnancy	810	-1.65 (-4.85, 1.55)	-0.91 (-3.30, 1.49)	-0.39 (-1.03, 0.26)	1.06 (-0.19, 2.32)	0.90 (0.24, 1.57) **	0.16 (-0.68, 1.00)
		-0.68 (-3.66, 2.30) ¹	0.09 (-2.18, 2.35) ¹	-0.22 (-0.84, 0.40) ¹	0.96 (-0.30, 2.22) ¹	0.89 (0.22, 1.55) ¹ **	0.08 (-0.76, 0.92) ¹
Hookworm infection at 1 st ANV	799	0.53 (-3.19, 4.24)	-0.89 (-3.79, 2.02)	-0.62 (-1.45, 0.22)	1.02 (-0.70, 2.73)	0.89 (-0.02, 1.81)	0.14 (-1.00, 1.29)
		1.55 (-1.98, 5.09) ¹	0.20 (-2.66, 3.06) ¹	-0.39 (-1.22, 0.44) ¹	0.99 (-0.74, 2.72) ¹	0.84 (-0.08, 1.76) ¹	0.17 (-0.98, 1.32) ¹
Hookworm infection at 2 nd ANV/delivery	794	-3.58 (-8.27, 1.10)	0.14 (-3.22, 3.49)	-0.01 (-1.00, 0.98)	1.62 (-0.42, 3.66)	1.13 (0.05, 2.21) *	0.50 (-0.87, 1.88)
		-2.34 (-6.75, 2.08) ¹	1.06 (-2.17, 4.29) ¹	0.08 (-0.87, 1.02) ¹	1.34 (-0.69, 3.37) ¹	1.13 (0.07, 2.20) ¹ *	0.23 (-1.14, 1.61) ¹
Hookworm infection once in pregnancy	810	-1.70 (-4.99, 1.60)	-0.50 (-3.06, 2.07)	-0.36 (-1.02, 0.29)	1.00 (-0.38, 2.37)	0.87 (0.14, 1.61) *	0.14 (-0.78, 1.06)
		-0.71 (-3.86, 2.45) ¹	0.34 (-2.13, 2.81) ¹	-0.23 (-0.87, 0.41) ¹	0.87 (-0.51, 2.26) ¹	0.87 (0.13, 1.60) ¹ *	0.03 (-0.90, 0.96) ¹
Hookworm density during pregnancy	119	-0.01 (-0.02, 0.02)	-0.01 (-0.01, 0.01)	-0.01 (-0.01, 0.01)	0.01 (-0.01, 0.01)	0.01 (-0.01, 0.01)	0.01 (-0.02, 0.02)
		-0.01 (-0.04, 0.04) ¹	0.01 (-0.02, 0.03) ¹	-0.01 (-0.01, 0.01) ¹	0.01 (-0.01, 0.02) ¹	0.01 (-0.01, 0.01) ¹	0.01 (-0.01, 0.01) ¹

¹ Adjusted for maternal age, education, pre-pregnancy BMI, family possession score, HOME score, child sex, child age at assessment, and incidence of malaria during pregnancy

* p<0.05

** p<0.01

<https://doi.org/10.1371/journal.pntd.0009260.t005>

crude and adjusted models. Blood lead level in children, known to impair long-term neurocognitive development [41,42], was considered in models in sensitivity analyses. Neither blood lead level measured in children at one year nor at six years had a significant impact on the association between SHIP and child development. Hospitalization of children between one and six years was not found to significantly impact associations between SHIP and child development. Analyses between SHIP and MSEL scores at one year were rerun and confirmed previous findings that hookworm infection in pregnancy was associated with impaired cognitive scores in this population.

Discussion

This study found that helminth infection during pregnancy was not associated with impaired cognitive ability, gross and fine motor skills, or hyperactivity disorder in children at six years of age. Our analyses did reveal that helminth infection was associated with impaired behavioral development, specifically emotional development in children, after controlling for risk factors. The observed association was not found to be mediated by inflammation during pregnancy, child weight-for-age z-score, or poor birth outcomes. Multiple imputation of missing variables and children lost to follow-up since birth confirmed that both helminth infection and hookworm infection during pregnancy were associated with impaired emotional development.

Our study is the first to our knowledge to investigate how helminth infection measured at different points during pregnancy may impact long-term child neurocognitive development, and is the first study to investigate how these infections during pregnancy impact behavioral development. There is an overall lack of existing literature investigating this subject because of the difficulty in extensive follow-up of SSA cohorts with advanced laboratory and neurocognitive assessment testing. A strength of this study is the prospective nature of the cohort of mothers and children followed from pregnancy until six years post-partum, which included several

biological measurements during pregnancy and child development assessments during the follow-up period. The construct validity of the KABC-II was verified in a pilot study within this population, and the SDQ has been used in several studies taking place in SSA countries [36,43,44]. A quality control of the SDQ test was completed at the Global Health Assessment center in Uganda before its use in this study. On-site investigators were trained by study Principal Investigators (PIs) MB and FBL before administering neurocognitive and behavioral assessments. Another strength of this study was the analysis of potential mediators such as gestational age at birth, birth weight, child growth, and inflammation during pregnancy.

Limitations of this study include the small sample size and insufficient power of analyses to show a meaningful difference in KABC-II scores between comparison groups. Given the mean and standard deviation of the KABC-II, a difference of 2.6 points as observed in our results would correspond to an effect size of 0.21; therefore, our study was underpowered to be able to show such a difference in our analyses. Although all investigators administering assessments and questionnaires received the same training and a universal oral translation of instructions was agreed upon, there is still the potential for measurement error. Also, the auto-evaluation of child behavior by parents using the SDQ could have introduced self-reporting bias into our study. However, this is often a bias from child behavioral assessments as many tools rely on observations from parents or teachers. The BOT-2 and TOVA assessments were not validated for use in this population; however, they have been validated for use in other SSA countries [37]. Another limitation of this study is the use of a multitude of statistical tests to explore associations between SHIP and development outcomes, thus the possibility that significant results seen among SDQ internalizing scores could be due to chance cannot be ruled out. Children lost to follow-up between birth and six years were also found to have differing sociodemographic characteristics, however there was no difference in exposure to helminths during pregnancy between these two groups. While this study did take into account several potential confounding and mediating factors, there is still the possibility that some factors were overlooked. For example, conditions of extreme poverty and low social support provided for parents could influence SHIP and neurocognitive development of children and were not included in analyses. Finally, the MiPPAD clinical trial and the APEC nested cohort study supplied helminth prophylaxis to infected women after diagnosis, thus potentially under-estimating exposure to helminths during pregnancy in our population.

A previous study involving 635 Beninese infants found that children with mothers infected by helminths at the 1st ANV and with mothers who had at least one helminth infection during pregnancy had significantly lower mean gross motor scores and cognitive ability than children with mothers who were not infected [24]. We sought to test whether these children still had lower neurocognitive test scores prospectively at six years of age, however our analyses revealed that helminth infection during pregnancy no longer had an impact on long-term child neurocognitive development. Within sensitivity analyses, we reran the same linear regression analyses conducted in this population of children assessed at one year [24] within the population of 487 children included in this paper. We found the same significant associations between SHIP and cognitive development in these children at one year as those found in the previous publication, thus ruling out the possibility that selection bias is responsible for the lack of significant association between SHIP and long-term neurocognitive development at six years (S1 Table). Helminth infection in childhood has been linked to impaired child development; however, results remain equivocal [19,21]. Additional analyses were conducted within this population to test for associations between helminth infection in children at the time of assessment at six years and impaired development scores; however, no significant associations were revealed. Due to its insignificance in univariate analyses, and the fact that helminth infection in pregnancy and in childhood may share common risk factors, authors did not further

evaluate childhood helminth infection in mediation analyses. Within our population, 45% of children were diagnosed with helminth infection at six years, despite the national recommendation in Benin to treat children with anti-helminthic prophylaxis every three months beginning at nine months of age. The high prevalence of hookworm infection in children, as opposed to *Ascaris*, could be attributed to water, sanitation, and hygiene (WASH) interventions that have been shown to significantly impact *Ascaris* infection, but have less impact on hookworm infection due to differing modes of transmission of these helminths [45]. This paper provides evidence that these national guidelines are often not respected due to poor governance of public health systems in low-income countries like Benin.

The importance of timing of maternal infection during pregnancy on risk of impaired birth and child outcomes has been widely questioned. Conclusions from previous studies seem inconsistent, with some studies citing no significant difference in child health outcomes based on infection timing during pregnancy [46,47] and others showing that various infections during the second trimester of pregnancy, as opposed to the third trimester, led to more severe neurocognitive and behavioral impairments in children [27,48,49]. Due to the limited existing research on the impact of SHIP on child neurocognitive development, we were unable to form concrete hypotheses for whether or not timing of infection during pregnancy would have a greater impact on neurocognitive scores in children at six years. While results from analyses at one year showed a trend that earlier helminth and hookworm infections (diagnosed in the 2nd trimester) were associated with lower cognitive and motor scores, we do not see the continuation of this trend in our analyses at six years. Further research within prospective cohorts should investigate the role of the timing of SHIP on child health and development outcomes to make clearer conclusions.

Additionally, our study investigated how SHIP could impact child behavioral development measured by the parent-reported SDQ. The hyperactivity and conduct problem scores are combined to form an externalizing score, indicating external behavioral issues, and the peer and emotional problem scores are used to generate an internalizing score to indicate overall emotional issues. We found that SHIP was associated with higher internalizing scores in children, indicating more emotional development problems in children. Several previous studies have found associations between maternal infection during pregnancy and increased risk of emotional and behavioral problems in children through disruption of blood vessel formation between placenta and fetus that have long-term implications on development [26,32,50]. Maternal infection in pregnancy activates the mothers' inflammatory immune response; several studies have identified this increase in inflammation as the mechanism that leads to increased risk of behavioral problems and disorders in offspring [51–53]. Rodent models have also shown that increased maternal inflammation during pregnancy can directly increase brain inflammation in the fetus and thus alter fetal brain development [25,52]. One previous study specifically found that elevated markers of inflammation in pregnant women were associated with more internalizing and externalizing symptoms in their offspring later in childhood [54]. Ours is the first study to investigate plausible mechanisms of the association between maternal intestinal worm infection and externalizing and internalizing symptoms in school-aged offspring.

Mediation analysis tested the indirect effects of potential mediators in the association between SHIP and impaired emotional development in children measured by the SDQ. SHIP is a known risk factor for birth outcomes such as pre-term birth [48] and low birth weight [55]. Helminths have also been attributed to stunted growth and wasting in children [2,56]. We examined how these mediators, along with inflammation in pregnancy measured by the presence of CRP, could indirectly affect the significant relationship seen between SHIP and impaired emotional development. However, our analyses did not reveal any significant

indirect effects from these mediators. Inflammation within mothers in our population could be the result of several different factors, such as malaria or other maternal infection [57] or increased maternal BMI [58]. Therefore, it is difficult to ascertain whether the cause of inflammation within this population is due primarily to helminth infection in pregnancy. Additionally, CRP was the only marker used to measure inflammation during pregnancy; previous studies have indicated eosinophils, IgE, and other cytokines could be more specific inflammatory markers of helminth infection [28]. Other serum cytokines known to be activated in the inflammatory immune response to helminth infection could be studied in future research to evaluate their role as mediators in this relationship. Sensitivity analyses were conducted to test mediation between hookworm infection and impaired emotional development through inflammation, since hookworm infection in particular has been previously linked to systemic inflammation [28], and similarly no significant results were found. Other potential mediating factors such as maternal anemia and child malnutrition were not considered in this paper. Malnutrition was not included due to the fact that it could be both a potential confounding factor and a mediating factor, and because there are several causes of malnutrition in low-income populations such as this. There are also several causes of anemia in at risk populations, and mechanisms between anemia and long-term child development are still unclear. A separate study exploring this complicated and multi-factorial relationship is currently underway within this population, and therefore was not expanded upon for the purposes of this paper.

This study does not confirm hypotheses that SHIP impacts long-term child neurocognitive development at six years. However, it provides evidence that these infections can influence behavioral development during childhood and beyond. The clinical manifestation of impaired neurocognitive development in SSA populations according to Western-based assessment scores is widely unknown, and studies with comparisons of symptoms within SSA populations are needed. Maternal infection by helminths remains a significant public health challenge in many areas of the world, and efforts to control these infections and understand the full scope of their burden on mother and child health are needed. Additionally, the high prevalence of helminth infection in six-year-old children in this study shows that national prevention guidelines for helminth infection may not be adequately followed and implementation of these guidelines must be strengthened to reduce the burden of these infections in school-aged children. Future research should be done to confirm our findings and further investigate how these parasitic infections can impair child development and the role that maternal inflammation may have on this association.

Supporting information

S1 STROBE Checklist. STROBE Checklist for cohort studies.
(DOC)

S1 Data. Soil-transmitted helminth infection during pregnancy and child development outcomes.
(XLS)

S1 Table. Associations between SHIP and MSEL scores among 487 children from EXPLORE at one year of age.
(DOCX)

Acknowledgments

We would like to thank the on-site researchers and public health nurses in Allada, Benin for their collaboration on all studies mentioned in this paper.

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References

1. Wright JE, Werkman M, Dunn JC, Anderson RM. Current epidemiological evidence for predisposition to high or low intensity human helminth infection: a systematic review. *Parasit Vectors*. 2018 31; 11(1):65. <https://doi.org/10.1186/s13071-018-2656-4> PMID: 29382360
2. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors*. 2014 Jan 21; 7:37. <https://doi.org/10.1186/1756-3305-7-37> PMID: 24447578
3. Organization WH. Soil-transmitted helminthiases: eliminating as public health problem soil-transmitted helminthiases in children: progress report 2001–2010 and strategic plan 2011–2020 [Internet]. World Health Organization; 2012 [cited 2019 May 10]. Available from: <https://apps.who.int/iris/handle/10665/44804>
4. Alelign T, Degarege A, Erko B. Soil-Transmitted Helminth Infections and Associated Risk Factors among Schoolchildren in Durbete Town, Northwestern Ethiopia. *J Parasitol Res* [Internet]. 2015;2015. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4487929/> <https://doi.org/10.1155/2015/641602> PMID: 26161265
5. Hotez PJ, Brindley PJ, Bethony JM, King CH, Pearce EJ, Jacobson J. Helminth infections: the great neglected tropical diseases. *J Clin Invest*. 2008 Apr 1; 118(4):1311–21. <https://doi.org/10.1172/JCI34261> PMID: 18382743
6. Hotez PJ, Brooker S, Bethony JM, Bottazzi ME, Loukas A, Xiao S. Hookworm Infection [Internet]. <http://dx.doi.org/10.1056/NEJMra032492>. 2009 [cited 2020 Jan 27]. Available from: https://www.nejm.org/doi/10.1056/NEJMra032492?url_ver=Z39.88-2003&rft_id=ori%3Arid%3Acrossref.org&rft_dat=cr_pub%3Dwww.ncbi.nlm.nih.gov
7. Soil-transmitted helminth infections [Internet]. [cited 2018 Oct 10]. Available from: <http://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>

8. Bethony J, Brooker S, Albonico M, Geiger SM, Loukas A, Diemert D, et al. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. *The Lancet*. 2006 May 6; 367(9521):1521–32.
9. Tefera E, Belay T, Mekonnen SK, Zeynudin A, Belachew T. Prevalence and intensity of soil transmitted helminths among school children of Mendera Elementary School, Jimma, Southwest Ethiopia. *Pan Afr Med J*. 2017; 27:88. <https://doi.org/10.11604/pamj.2017.27.88.8817> PMID: 28819509
10. Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J, Utzinger J. Effect of Sanitation on Soil-Transmitted Helminth Infection: Systematic Review and Meta-Analysis. *PLoS Med* [Internet]. 2012 Jan 24; 9(1). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3265535/> <https://doi.org/10.1371/journal.pmed.1001162> PMID: 22291577
11. Derso A, Nibret E, Munshea A. Prevalence of intestinal parasitic infections and associated risk factors among pregnant women attending antenatal care center at Felege Hiwot Referral Hospital, northwest Ethiopia. *BMC Infect Dis*. 2016 Sep 30; 16(1):530. <https://doi.org/10.1186/s12879-016-1859-6> PMID: 27716099
12. Ouédraogo S, Koura GK, Accrombessi MMK, Bodeau-Livinec F, Massougboji A, Cot M. Maternal anaemia at first antenatal visit: prevalence and risk factors in a malaria-endemic area in Benin. *Am J Trop Med Hyg*. 2012 Sep; 87(3):418–24. <https://doi.org/10.4269/ajtmh.2012.11-0706> PMID: 22826498
13. Mekonnen Z, Suleman S, Biruksew A, Tefera T, Chelkeba L. Intestinal polyparasitism with special emphasis to soil-transmitted helminths among residents around Gilgel Gibe Dam, Southwest Ethiopia: a community based survey. *BMC Public Health*. 2016 23; 16(1):1185. <https://doi.org/10.1186/s12889-016-3859-2> PMID: 27881115
14. Dawaki S, Al-Mekhlafi HM, Ithoi I. The burden and epidemiology of polyparasitism among rural communities in Kano State, Nigeria. *Trans R Soc Trop Med Hyg*. 2019 Apr 1; 113(4):169–82. <https://doi.org/10.1093/trstmh/try128> PMID: 30551211
15. Aderoba AK, Iribhogbe OI, Olagbuji BN, Olokori OE, Ojide CK, Ande AB. Prevalence of helminth infestation during pregnancy and its association with maternal anemia and low birth weight. *Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet*. 2015 Jun; 129(3):199–202. <https://doi.org/10.1016/j.ijgo.2014.12.002> PMID: 25733444
16. Larocque R, Casapia M, Gotuzzo E, Gyorkos TW. Relationship between intensity of soil-transmitted helminth infections and anemia during pregnancy. *Am J Trop Med Hyg*. 2005 Oct; 73(4):783–9. PMID: 16222026
17. WHO | Deworming in pregnant women [Internet]. WHO. [cited 2019 May 10]. Available from: http://www.who.int/elena/titles/deworming_pregnancy/en/
18. Kuong K, Fiorentino M, Perignon M, Chamnan C, Berger J, Sinuon M, et al. Cognitive Performance and Iron Status are Negatively Associated with Hookworm Infection in Cambodian Schoolchildren. *Am J Trop Med Hyg*. 2016 Oct 5; 95(4):856–63. <https://doi.org/10.4269/ajtmh.15-0813> PMID: 27573634
19. Pabalan N, Singian E, Tabangay L, Jarjanazi H, Boivin MJ, Ezeamama AE. Soil-transmitted helminth infection, loss of education and cognitive impairment in school-aged children: A systematic review and meta-analysis. *PLoS Negl Trop Dis*. 2018; 12(1):e0005523. <https://doi.org/10.1371/journal.pntd.0005523> PMID: 29329288
20. Nampijja M, Apule B, Lule S, Akurut H, Muhangi L, Webb EL, et al. Effects of Maternal Worm Infections and Anthelmintic Treatment during Pregnancy on Infant Motor and Neurocognitive Functioning. *J Int Neuropsychol Soc*. 2012 Nov; 18(6):1019–30. <https://doi.org/10.1017/S1355617712000768> PMID: 23158229
21. Ndibazza J, Mpairwe H, Webb EL, Mawa PA, Nampijja M, Muhangi L, et al. Impact of Anthelmintic Treatment in Pregnancy and Childhood on Immunisations, Infections and Eczema in Childhood: A Randomised Controlled Trial. *PLoS ONE*. 2012 Dec 7; 7(12):e50325. <https://doi.org/10.1371/journal.pone.0050325> PMID: 23236367
22. Le Doaré K, Bland R, Newell M-L. Neurodevelopment in children born to HIV-infected mothers by infection and treatment status. *Pediatrics*. 2012 Nov; 130(5):e1326–1344. <https://doi.org/10.1542/peds.2012-0405> PMID: 23118140
23. McDonald CR, Cahill LS, Ho KT, Yang J, Kim H, Silver KL, et al. Experimental Malaria in Pregnancy Induces Neurocognitive Injury in Uninfected Offspring via a C5a-C5a Receptor Dependent Pathway. *PLoS Pathog*. 2015 Sep; 11(9):e1005140. <https://doi.org/10.1371/journal.ppat.1005140> PMID: 26402732
24. Mireku MO, Boivin MJ, Davidson LL, Ouédraogo S, Koura GK, Alao MJ, et al. Impact of helminth infection during pregnancy on cognitive and motor functions of one-year-old children. *PLoS Negl Trop Dis*. 2015 Mar; 9(3):e0003463. <https://doi.org/10.1371/journal.pntd.0003463> PMID: 25756357
25. Simanek AM, Meier HCS. Association Between Prenatal Exposure to Maternal Infection and Offspring Mood Disorders: A Review of the Literature. *Curr Probl Pediatr Adolesc Health Care*. 2015 Nov 1; 45(11):325–64. <https://doi.org/10.1016/j.cppeds.2015.06.008> PMID: 26476880

26. den Haan PJ, de Kroon MLA, van Dokkum NH, Kerstjens JM, Reijneveld SA, Bos AF. Risk factors for emotional and behavioral problems in moderately-late preterms. *PLoS One*. 2019; 14(5):e0216468. <https://doi.org/10.1371/journal.pone.0216468> PMID: 31048855
27. Parker SE, Lijewski VA, Janulewicz PA, Collett BR, Speltz ML, Werler MM. Upper respiratory infection during pregnancy and neurodevelopmental outcomes among offspring. *Neurotoxicol Teratol*. 2016 Sep 1; 57:54–9. <https://doi.org/10.1016/j.ntt.2016.06.007> PMID: 27343815
28. Anderson AS, Trumble BC, Hové C, Kraft TS, Kaplan H, Gurven M, et al. Old friends and friendly fire: Pregnancy, hookworm infection, and anemia among tropical horticulturalists. *Am J Hum Biol*. 2020; 32(2):e23337. <https://doi.org/10.1002/ajhb.23337> PMID: 31642576
29. Kurtis JD, Higashi A, Wu H-W, Gundogan F, McDonald EA, Sharma S, et al. Maternal Schistosomiasis japonica is associated with maternal, placental, and fetal inflammation. *Infect Immun*. 2011 Mar; 79(3):1254–61. <https://doi.org/10.1128/IAI.01072-10> PMID: 21149589
30. Khan D, Fernando P, Cicvaric A, Berger A, Pollak A, Monje FJ, et al. Long-term effects of maternal immune activation on depression-like behavior in the mouse. *Transl Psychiatry*. 2014 Feb; 4(2):e363–e363. <https://doi.org/10.1038/tp.2013.132> PMID: 24548878
31. Smith SEP, Li J, Garbett K, Mirnics K, Patterson PH. Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6. *J Neurosci*. 2007 Oct 3; 27(40):10695–702. <https://doi.org/10.1523/JNEUROSCI.2178-07.2007> PMID: 17913903
32. Weckman AM, Ngai M, Wright J, McDonald CR, Kain KC. The Impact of Infection in Pregnancy on Placental Vascular Development and Adverse Birth Outcomes. *Front Microbiol* [Internet]. 2019 Aug 22 [cited 2020 Feb 17];10. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6713994/> <https://doi.org/10.3389/fmicb.2019.01924> PMID: 31507551
33. González R, Mombo-Ngoma G, Ouédraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multi-centre Randomized Controlled Trial. *PLOS Med*. 2014 Sep 23; 11(9):e1001733. <https://doi.org/10.1371/journal.pmed.1001733> PMID: 25247709
34. Ouédraogo S, Koura GK, Bodeau-Livinec F, Accrombessi MMK, Massougbojji A, Cot M. Maternal anaemia in pregnancy: assessing the effect of routine preventive measures in a malaria-endemic area. *Am J Trop Med Hyg*. 2013 Feb; 88(2):292–300. <https://doi.org/10.4269/ajtmh.12-0195> PMID: 23296448
35. Organization WH. Bench aids for the diagnosis of intestinal parasites [Internet]. World Health Organization; 1994 [cited 2019 Nov 14]. Available from: <https://apps.who.int/iris/handle/10665/37323>
36. Bodeau-Livinec F, Davidson LL, Zoumenou R, Massougbojji A, Cot M, Boivin MJ. Neurocognitive testing in West African children 3–6 years of age: Challenges and implications for data analyses. *Brain Res Bull*. 2018 Apr 6; <https://doi.org/10.1016/j.brainresbull.2018.04.001> PMID: 29630997
37. Chernoff MC, Laughton B, Ratswana M, Familiar I, Fairlie L, Vhembo T, et al. Validity of Neuropsychological Testing in Young African Children Affected by HIV. *J Pediatr Infect Dis*. 2018 Sep; 13(3):185–201. <https://doi.org/10.1055/s-0038-1637020> PMID: 30100780
38. Goodman R. The Strengths and Difficulties Questionnaire: A Research Note. *J Child Psychol Psychiatry*. 1997; 38(5):581–6. <https://doi.org/10.1111/j.1469-7610.1997.tb01545.x> PMID: 9255702
39. Koura KG, Boivin MJ, Davidson LL, Ouédraogo S, Zoumenou R, Alao MJ, et al. Usefulness of child development assessments for low-resource settings in francophone Africa. *J Dev Behav Pediatr JDBP*. 2013 Sep; 34(7):486–93. <https://doi.org/10.1097/DBP.0b013e31829d211c> PMID: 23899660
40. Miočević M, Gonzalez O, Valente MJ, MacKinnon DP. A Tutorial in Bayesian Potential Outcomes Mediation Analysis. *Struct Equ Model Multidiscip J*. 2018; 25(1):121–36. <https://doi.org/10.1080/10705511.2017.1342541> PMID: 29910595
41. Chiodo LM, Jacobson SW, Jacobson JL. Neurodevelopmental effects of postnatal lead exposure at very low levels. *Neurotoxicol Teratol*. 2004 Jun; 26(3):359–71. <https://doi.org/10.1016/j.ntt.2004.01.010> PMID: 15113598
42. Kashala-Abotnes E, Mumbere PP, Mishika JM, Ndjukendi AO, Mpaka DB, Bumoko M-MG, et al. Lead exposure and early child neurodevelopment among children 12–24 months in Kinshasa, the Democratic Republic of Congo. *Eur Child Adolesc Psychiatry*. 2016 Dec; 25(12):1361–7. <https://doi.org/10.1007/s00787-016-0860-3> PMID: 27165339
43. Kashala E, Lundervold A, Sommerfelt K, Tylleskär T, Elgen I. Co-existing symptoms and risk factors among African school children with hyperactivity-inattention symptoms in Kinshasa, Congo. *Eur Child Adolesc Psychiatry*. 2006 Aug; 15(5):292–9. <https://doi.org/10.1007/s00787-006-0536-5> PMID: 16583124
44. Springer PE, Slogrove AL, Kidd M, Kalk E, Bettinger JA, Esser MM, et al. Neurodevelopmental and behavioural outcomes of HIV-exposed uninfected and HIV-unexposed children at 2–3 years of age in Cape Town, South Africa. *AIDS Care*. 2019 Jul 7;1–9. <https://doi.org/10.1080/09540121.2019.1637506> PMID: 31280587

45. Coffeng LE, Nery SV, Gray DJ, Bakker R, Vlas SJ de, Clements ACA. Predicted short and long-term impact of deworming and water, hygiene, and sanitation on transmission of soil-transmitted helminths. *PLoS Negl Trop Dis*. 2018 Dec 6; 12(12):e0006758. <https://doi.org/10.1371/journal.pntd.0006758> PMID: 30522129
46. Lee BK, Magnusson C, Gardner RM, Blomström A, Newschaffer CJ, Burstyn I, et al. Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain Behav Immun*. 2015 Feb; 44:100–5. <https://doi.org/10.1016/j.bbi.2014.09.001> PMID: 25218900
47. Kalilani-Phiri L, Thesing PC, Nyirenda OM, Mawindo P, Madanitsa M, Membe G, et al. Timing of Malaria Infection during Pregnancy Has Characteristic Maternal, Infant and Placental Outcomes. *PLOS ONE*. 2013 Sep 18; 8(9):e74643. <https://doi.org/10.1371/journal.pone.0074643> PMID: 24058614
48. Mahande AM, Mahande MJ. Prevalence of parasitic infections and associations with pregnancy complications and outcomes in northern Tanzania: a registry-based cross-sectional study. *BMC Infect Dis*. 2016 Feb 13; 16:78. <https://doi.org/10.1186/s12879-016-1413-6> PMID: 26874788
49. Atladóttir HÓ, Thorsen P, Østergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal Infection Requiring Hospitalization During Pregnancy and Autism Spectrum Disorders. *J Autism Dev Disord*. 2010 Dec 1; 40(12):1423–30. <https://doi.org/10.1007/s10803-010-1006-y> PMID: 20414802
50. Murphy SK, Fineberg AM, Maxwell SD, Alloy LB, Zimmermann L, Krigbaum NY, et al. Maternal infection and stress during pregnancy and depressive symptoms in adolescent offspring. *Psychiatry Res*. 2017; 257:102–10. <https://doi.org/10.1016/j.psychres.2017.07.025> PMID: 28750213
51. Shi L, Tu N, Patterson PH. Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus. *Int J Dev Neurosci*. 2005 Apr 1; 23(2):299–305.
52. Meyer U, Feldon J, Fatemi SH. In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neurosci Biobehav Rev*. 2009 Jul 1; 33(7):1061–79. <https://doi.org/10.1016/j.neubiorev.2009.05.001> PMID: 19442688
53. Graham AM, Rasmussen JM, Rudolph MD, Heim CM, Gilmore JH, Styner M, et al. Maternal Systemic Interleukin-6 During Pregnancy Is Associated With Newborn Amygdala Phenotypes and Subsequent Behavior at 2 Years of Age. *Biol Psychiatry*. 2018 Jan 15; 83(2):109–19. <https://doi.org/10.1016/j.biopsych.2017.05.027> PMID: 28754515
54. Mac Giollabhui N, Breen EC, Murphy SK, Maxwell SD, Cohn BA, Krigbaum NY, et al. Maternal inflammation during pregnancy and offspring psychiatric symptoms in childhood: Timing and sex matter. *J Psychiatr Res*. 2019; 111:96–103. <https://doi.org/10.1016/j.jpsychires.2019.01.009> PMID: 30690329
55. Dreyfuss ML, Msamanga GI, Spiegelman D, Hunter DJ, Urassa EJ, Hertzmark E, et al. Determinants of low birth weight among HIV-infected pregnant women in Tanzania. *Am J Clin Nutr*. 2001 Dec; 74(6):814–26. <https://doi.org/10.1093/ajcn/74.6.814> PMID: 11722965
56. Ojurongbe O, Okorie PN, Opatokun RL, Ojurongbe TA, Mabayoje VO, Olowe OA, et al. Prevalence and associated factors of *Plasmodium falciparum* and soil transmitted helminth infections among pregnant women in Osun state, Nigeria. *Afr Health Sci*. 2018 Sep; 18(3):542–51. <https://doi.org/10.4314/ahs.v18i3.11> PMID: 30602986
57. Elphinstone RE, Weckman AM, McDonald CR, Tran V, Zhong K, Madanitsa M, et al. Early malaria infection, dysregulation of angiogenesis, metabolism and inflammation across pregnancy, and risk of preterm birth in Malawi: A cohort study. *PLoS Med* [Internet]. 2019 Oct 1; 16(10). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6772002/>
58. Aye ILMH, Lager S, Ramirez VI, Gaccioli F, Dudley DJ, Jansson T, et al. Increasing Maternal Body Mass Index Is Associated with Systemic Inflammation in the Mother and the Activation of Distinct Placental Inflammatory Pathways. *Biol Reprod* [Internet]. 2014 Jun; 90(6). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4094003/> <https://doi.org/10.1095/biolreprod.113.116186> PMID: 24759787

Chapter 4. The effect of malaria in pregnancy on child neurocognitive development

This chapter presents the second article of this thesis studying the impact of *Plasmodium falciparum* malaria infection in pregnancy on child neurocognitive development at one and six years of age. While the consequences of MIP on birth outcomes, such as pre-term birth, low birth weight, and fetal growth restriction, are well known, little is known about the long-term consequences on development of surviving offspring. Other types of maternal infection in pregnancy, including SHIP, influenza, and sexually-transmitted infections have been linked to increased risk of motor and behavioral impairments (12,248,249); however, only one previous study investigated experimental malaria infection in rodents and its risks for developmental impairment in offspring (13). This paper is the first to investigate this relationship in a large mother-child cohort in SSA and provides evidence that MIP, particularly later in pregnancy, can have detrimental impacts on child motor and neurocognitive development at both one and six years of age. Our article also shows that infections diagnosed by qPCR methods, often only available in areas with access to advanced laboratory techniques, are especially harmful for long-term child development. These infections reflect chronic, asymptomatic infections that often go untreated, this article therefore serves as evidence that diagnosis and treatment of these infections must be reinforced in low-income areas of the world like Benin in order to avoid their long-term consequences on child development. This article has been accepted for publication in *Clinical Infectious Diseases*.

Article 2

Manuscript

1 The effects of malaria in pregnancy on neurocognitive development in children at
2 one and six years of age in Benin: a prospective mother-child cohort

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16 **Keywords:** Malaria, pregnancy, child development, neurocognition, Sub-Saharan Africa

17 **Running Title:** Prenatal malaria and child development

18

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27

28 Summary

29 This paper provides the first evidence of an association between late-term peripheral and
30 placental malaria in pregnancy and impaired neurocognitive development in offspring at one and
31 six years of age in Benin, Sub-Saharan Africa.

32 **Abstract**

33 *Background*

34 Malaria in pregnancy (MiP) contributes significantly to infant mortality rates in Sub-
35 Saharan Africa and has consequences on survivors, such as pre-term birth and low birth weight.
36 However, its impact on long-term neurocognitive development in children remains unknown.

37 *Methods*

38 Our prospective cohort included pregnant women and their live-born singletons from the
39 *Malaria in Pregnancy Preventive Alternative Drugs* clinical trial. MiP was assessed using
40 microscopy and real-time quantitative polymerase chain-reactions (qPCR). Neurocognitive
41 development in children was assessed using the Mullen Scales of Early Learning and the
42 Kaufman Assessment Battery for Children 2nd edition (KABC-II) at one and six years of age,
43 respectively.

44 *Results*

45 Of 493 pregnant women, 196 (40%) were infected with malaria at least once; 121 (31%)
46 with placental malaria diagnosed by qPCR. Multiple linear regression beta coefficients showed
47 that impaired gross motor scores were associated with MiP at least once [-2.55 Confidence
48 Interval (CI) (-5.15, 0.05)], placental malaria by qPCR [-4.95 CI (-7.65, -2.24)], and high
49 parasite density at delivery [-1.92 CI (-3.86, 0.02)] after adjustment. Malaria and high parasite
50 density at the 2nd ANV were associated with lower KABC-II Non-Verbal Index scores at six
51 years [-2.57 CI (-4.86, -0.28)] and [-1.91 CI (-3.51, -0.32)], respectively.

52 *Conclusions*

53 This prospective cohort study provides evidence that MiP, particularly late-term, could
54 have important negative consequences on child development at one and six years of age.
55 Mechanisms behind this association must be further investigated and diagnostic methods in low-
56 income countries should be strengthened to provide adequate treatment.

57 **Introduction**

58 Malaria remains one of the greatest contributors to morbidity and mortality in areas of the
59 world with stable transmission of infection, particularly in Sub-Saharan Africa (SSA). Of the 25
60 million pregnant women in SSA countries at risk of malaria infection, an estimated 25% of them
61 will have evidence of *Plasmodium falciparum* placental malaria at the time of delivery [1] and
62 50% will have evidence of peripheral malaria in the blood during antenatal care follow-up [2].
63 This high prevalence is due to the fact that pregnant women are at increased risk of contracting
64 malaria compared to non-pregnant women of the same age [1].

65 While malaria in pregnancy (MiP) contributes to approximately 10,000 maternal deaths
66 and 200,000 fetal deaths [3], it can also have significant consequences on surviving mothers and
67 infants. MiP has been associated with increased risk of maternal anemia, fetal growth restriction,
68 low birth weight, pre-term birth, and later malaria infection in offspring [4,5]. Laboratory
69 simulations have demonstrated that experimental malaria-exposed offspring also had persistent
70 neurological deficits compared to the control group [6]. Researchers hypothesized that the
71 activation of the maternal inflammatory immune response may impact fetal brain development in
72 the placenta, therefore resulting in neurodevelopmental impairments [7]. To date, the direct link
73 between MiP and neurocognitive impairment in children has yet to be investigated prospectively
74 in SSA populations. Therefore, the main objective of this paper was to assess the impacts of
75 MiP, including peripheral and placental malaria, on neurocognitive and motor development
76 scores in surviving children at one and six years of age in a prospective mother-child cohort in
77 Benin.

78 **Methods**

79 *Ethics Statement*

80 Ethical approval for all studies mentioned in this article was obtained by the institutional
81 review boards of the Beninese Ethical Committee of the Faculté des Sciences de la Santé (FSS)
82 and the Committee of Ethical Research of the Applied Biomedical Sciences Institute (CER-
83 ISBA) in Benin, New York University in the United States of America (IRB#09-1253), and the
84 Research Institute for Development's (IRD) Consultative Ethics Committee in France. Written
85 informed consent was obtained or thumbprints were provided for consent if women could not
86 read or write.

87 *Study Population*

88 Pregnant women in this prospective mother-child cohort were enrolled in the *Malaria in*
89 *Pregnancy Preventive Alternative Drugs (MiPPAD)* clinical trial (NCT00811421) during their
90 second trimester. The objective of the trial was to compare the efficacy of two intermittent
91 preventive treatments of malaria in pregnancy (IPTp), sulfadoxine-pyrimethamine (SP) and
92 mefloquine (MQ), in three health centers in the Allada district in Benin (Sékou, Allada, and
93 Atogon). Information on inclusion and exclusion criteria are detailed in a preceding publication
94 [8]. Briefly, inclusion criteria for women were: a permanent residence in the study area,
95 gestational age ≤ 28 weeks, HIV-negative at recruitment, no known allergies to sulfa or
96 mefloquine drugs, no treatment of malaria using these drugs within four weeks of recruitment,
97 and no history of severe renal, hepatic, psychiatric, or neurological disease. Participating
98 pregnant women were recruited into a nested-cohort study, *Anemia in Pregnancy: Etiologies and*
99 *Consequences (APEC)* to be followed-up for parasitic infection during pregnancy. All eligible,
100 surviving singletons born to participants were invited to undergo neurocognitive assessments in
101 the TOVI (*child in Fon*) study one year after birth [9]. Surviving children were then followed-up
102 at six years of age for further neurocognitive assessments in the EXPLORE (*EXposition au*
103 *PLomb et au manganèse et Risques pour l'Enfant*) study.

104 *Malaria in Pregnancy*

105 As part of the Benin pregnancy package, women were given IPTp (1500/75 mg SP per
106 dose or 15 mg/kg MQ per dose) and iron and folic acid supplements during three antenatal care
107 visits (ANVs) within the clinical trial, as well as mebendazole to treat helminth infection if
108 present. Clinical malaria episodes were treated with oral quinine in the first trimester or with
109 artemether-lumefantrine in subsequent trimesters for uncomplicated malaria; severe malaria was
110 treated with parental quinine [10]. Malaria was detected using thick blood smears that were
111 stained and read according to standard quality-controlled procedures during pregnancy [11]. At
112 delivery, maternal peripheral and placental blood samples were collected. Blood samples were
113 stained on filter paper and tested for the presence and density of *P. falciparum* using real-time
114 quantitative polymerase chain-reaction (qPCR) tests targeting 18S rDNA. Parasitemia was
115 quantified by extrapolation of cycle thresholds, the value for which DNA is amplified, from a
116 standard curve of *P. falciparum* ring-infected erythrocytes. Samples where no cycle thresholds
117 were detected were considered negative, and a density of 2 parasites/ μL was assigned if
118 amplification was lower than the standard curve of 5 parasites/ μL [12].

119 *Child Neurocognitive Development*

120 At one year of age, trained research nurses assessed cognitive function in infants using
121 the Mullen Scales of Early Learning (MSEL) from April 2011 until November 2012. The MSEL
122 is comprised of five scales for gross motor, fine motor, visual reception, receptive language, and
123 expressive language. Quality assurance of assessments, including inter-rater reliability, was
124 established and the MSEL was adapted for use in this population in a pilot study [13].
125 Normalized age-specific t-scores of the visual reception, fine motor, receptive language, and
126 expressive language scales were combined to form the Early Learning Composite (ELC) score to
127 indicate overall early cognitive function. The ELC and gross motor scores were analyzed in this
128 paper. The Home Observation for Measurement of the Environment (HOME) inventory was
129 adapted for use in this setting and used to assess the quality of the home environment and parent-
130 child interactions at one year.

131 Neurocognitive development in children at six years was assessed using the Kaufman
132 Assessment Battery for Children 2nd edition (KABC-II), a psychological diagnostic tool that
133 assesses processing ability, cognitive ability, and planning and learning capabilities. The French
134 version of the KABC-II was orally translated into the local language by trained investigators and
135 the construct validity for use in this population was verified during a pilot study [14]. The Mental
136 Processing Index (MPI) and the Non-Verbal Index were used in analyses. The Bruininks-
137 Oseretsky Test of Motor Proficiency Edition 2 (BOT-2) was administered to evaluate fine and
138 gross motor skills in children. A final, composite score for fine and gross motor skills was
139 standardized by age and sex and used in analyses.

140 *Socio-Demographic Characteristics*

141 At recruitment, information on gravidity, iron deficiency, IPTp treatment, and socio-
142 demographic characteristics such as maternal age, education, and socio-economic status were
143 collected. Soil-transmitted helminth (STH) infection was diagnosed in stool samples of women
144 using the Kato-Katz technique. A score was generated to indicate socioeconomic status based on
145 material possessions owned by families. Pre-pregnancy Body Mass Index (BMI) was calculated
146 using the gestational age at inclusion and estimated expected weight gain of women during
147 pregnancy. At delivery, information on infant sex, gestational age according to the Ballard score,
148 and birth weight in grams was collected. Risk factors of MiP and impaired child neurocognitive
149 development were identified using Direct Acyclic Graphs.

150 *Statistical Analysis*

151 The statistical analysis of this paper involved the description of socio-demographic and
152 clinical characteristics of women during pregnancy and infant birth outcomes. Characteristics are
153 shown for mother-child pairs included in analyses, lost to follow-up, and deceased children.
154 Next, univariate analyses were used to test the significance of common risk factors for exposures
155 and outcomes identified in the literature. Kruskal-Wallis, Wilcoxon Rank-sum, and chi-squared
156 tests were used where appropriate for categorical and continuous data. Linear regression models
157 with bootstrapped confidence intervals tested associations between malaria diagnosed during
158 pregnancy by microscopy and qPCR and neurocognitive development outcomes in children at
159 one and six years. Sensitivity analyses were conducted to ensure the robustness of results,
160 including the multiple imputation of missing variables. Analyses were conducted using STATA
161 16 (StataCorp. 2019. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp
162 LLC). Statistical tests were two-tailed with an alpha risk of 5%, unless otherwise specified.

163 **Results**

164 Of the 1,183 pregnant women recruited into the MiPPAD clinical trial, the first 1,005
165 women were asked to participate in the APEC nested-cohort study to be followed for parasitic
166 infection during pregnancy (Figure 1). At delivery, 863 eligible singletons were born alive and
167 635 of them were assessed for neurocognitive development at one year of age. Finally, 493 of
168 these children were further assessed for neurocognitive development at six years.

169 *Description of Population*

170 Socio-demographic and clinical characteristics of mother-child pairs in our analyses
171 during pregnancy and at birth are described and compared with those lost to follow-up and
172 deceased before six years of age in Table 1. Prevalence of peripheral MiP was 15% at the 1st
173 ANV, 3% at the 2nd ANV, and 10% at delivery. At delivery, 31% of women tested had placental
174 malaria according to qPCR analyses. There were no significant differences in malaria infection
175 or parasite density between those included in analyses and lost to follow-up. Children who died
176 between birth and six years of age were more likely to have been born primigravida, been pre-
177 term (<37 weeks' gestation), and have low birth weight (<2,500 grams). There was no difference
178 in MiP exposure between children followed at six years and those deceased.

179 *Univariate Analyses*

180 Factors associated with MiP and cognitive development identified in the literature are
181 shown in Tables 2 and 3. Pre-pregnancy BMI, STH infection during pregnancy, maternal
182 education, family possession score, the HOME score, low birth weight, and malaria in childhood
183 were found to be significantly associated with both cognitive functioning and gross motor scores
184 in children at one year. Maternal age, pre-pregnancy BMI, maternal education, family possession
185 score, the HOME score, and child sex were associated with cognitive and motor development in
186 children at six years. Additionally, gravidity was associated with motor development in children
187 at one and six years.

188 *MiP and Child Neurocognitive Development*

189 Beta coefficients and bootstrapped 95% confidence intervals (CIs) for associations
190 between MiP and child neurocognitive and motor development measured by the MSEL at one
191 year are shown in Figure 2. While MiP diagnosed at least once during pregnancy did not
192 significantly impair ELC scores in children [0.15 (-2.72, 3.01)], it did significantly impair gross
193 motor development scores at one year before and after adjustment of risk factors, [-2.63 (-5.24, -
194 0.02)] and [-2.55 (-5.15, 0.05)] respectively. In particular, malaria diagnosed by qPCR and higher
195 parasite density at delivery were associated with significantly impaired gross motor development
196 scores in children after adjustment, [-4.95 (-7.65, -2.24)] and [-1.92 (-3.86, 0.02)] respectively. MiP
197 diagnosed at any ANV or delivery by thick blood smear was not associated with impaired
198 cognitive or motor development scores in children at one year. Figure 3 shows that MiP at least
199 once and high parasite density at the 2nd ANV were associated with impaired overall cognitive
200 development measured by the MPI at six years in crude models, [-2.17 (-4.27, -0.07)] and [-3.27 (-
201 6.23, -0.32)] respectively; however, these associations were no longer statistically significant after
202 adjustment. High parasite density at delivery was associated with impaired MPI scores in
203 adjusted models [-1.25 (-2.46, -0.05)]. Malaria diagnosed by microscopy and high parasite density
204 at the 2nd ANV were both significantly associated with impaired NVI scores from the KABC-II
205 in adjusted models [-2.57 CI (-4.86, -0.28)] and [-1.91 CI (-3.51, -0.32)], respectively. Late-term
206 malaria infections at the 2nd ANV and delivery showed trends of impairing both MPI and NVI
207 scores at six years, although statistically insignificant. Finally, no measure of MiP was associated
208 with impaired motor composite scores in children at six years (Supplemental Figure 1).

209 *Sensitivity Analyses*

210 Multiple imputation of missing variables (>20%) confirmed results seen in principal
211 analyses (Supplemental Table 1); however, associations between NVI scores and malaria
212 diagnosed at 2nd ANV were borderline significant. Regression models controlling for pre-term
213 birth and low birth weight confirmed principal analyses (Supplemental Table 2). Other factors,
214 such as IPTp treatment, iron deficiency, child blood lead levels, and STH infection were tested
215 and did not influence results.

216 **Discussion**

217 This paper reveals that prenatal exposure to *P.falciparum* malaria has long-term
218 consequences on neurocognitive development in surviving offspring in our cohort. MiP at least
219 once during pregnancy, placental malaria by qPCR, and high parasite density at delivery are
220 associated with impaired gross motor development scores in children at one year. MiP diagnosed
221 by microscopy and high parasite density at the 2nd ANV negatively impacted NVI scores of the
222 KABC-II, and analyses show a trend that infections occurring later in pregnancy more negatively
223 impact KABC-II scores than earlier infections. Placental malaria by qPCR was borderline
224 significantly associated with impaired KABC-II scores, and parasite density at delivery had a
225 negative impact on scores after adjustment of risk factors.

226 The prospective nature of this mother-child cohort and the comprehensive biological and
227 neurocognitive assessments carried out within this population are defining characteristics of this
228 study. This is the first study to our knowledge to investigate the relationship between MiP and its
229 long-term impacts on child neurocognitive development in such a large cohort from SSA. The
230 validity of child development assessments was confirmed and assessments were adapted for use
231 in this population prior [13,14]. The validity and reliability of MSEL scores at one year to predict
232 neurocognitive development at six years in this population was also verified in a preceding
233 publication [15]. Sensitivity analyses investigated other potential confounding and intermediate
234 factors, such as the IPTp treatment group, iron deficiency in pregnancy, child blood lead levels,
235 helminth infection in pregnancy [16], and pre-term birth and low birth weight; none of which
236 significantly altered results. This provides evidence of associations between MiP and impaired
237 cognition in children regardless of pre-term birth status and low birth weight, two previously
238 hypothesized mechanisms due to their significant impact on neurocognitive development and
239 their associations with submicroscopic malarial infections during pregnancy [17–19]. Multiple
240 imputation confirmed our results, thus ruling out potential bias from missingness. Finally, there

241 were no differences in terms of MiP exposure in women included and excluded from analyses,
242 therefore attrition bias is unlikely.

243 There are several limitations to our analyses, including the limited sample size in certain
244 analyses and the multitude of tests conducted in this population, increasing the likelihood that
245 results could be found by chance. Pregnant women and children were followed extensively and
246 treated promptly in the case of malaria infection, therefore potentially underestimating
247 associations between MiP and child neurocognitive development. Due to this close follow-up,
248 our study was also unable to investigate the impacts of severe malaria during pregnancy and
249 childhood, known to have detrimental neurological consequences on survivors [20]. However,
250 sensitivity analyses also tested the impact of repeated malaria episodes in children between one
251 and six years of age on main associations, and found no changes. Lastly, pregnant women were
252 not recruited until the second trimester of pregnancy and so this study was unable to expand
253 upon the limited existing literature on the impact of infections in the first trimester on poor birth
254 outcomes [21] and neurocognitive development in offspring.

255 Research on the timing of maternal infections during pregnancy and its impact on fetal health
256 remains equivocal [22,23]. Our analyses show a trend between late-term infection and lower
257 neurocognitive scores in children at one and six years. The World Health Organization recently
258 revised their standard recommendation for IPTp in high-transmission areas from two doses
259 during pregnancy to three doses to help improve birth outcomes [24]. Therefore, the higher
260 proportion of women diagnosed with malaria at delivery could reflect chronic infections due to
261 women not receiving tertiary IPTp doses between the 2nd ANV and delivery in the clinical trial.

262 Previous studies have shown the effect of maternal infection during pregnancy on fetal
263 neurodevelopmental abnormalities and risk of psychiatric disorders [25–27], with authors
264 hypothesizing that maternal immune activation in response to infection can have severe
265 consequences on neurological development of the fetus. However, these studies either
266 overlooked the effect of malaria entirely or malaria was assessed as a composite of overall
267 maternal infection during pregnancy. A laboratory-based study provided evidence of a causal
268 link between experimental malaria infection in pregnancy and impaired neurocognitive
269 development in the fetus [6,7]. Similar to previously mentioned hypotheses, their research
270 suggests that complement factors of the innate maternal immune response to infection, in
271 particular C5a, initiate pro-inflammatory and anti-angiogenic pathways that alter neurovascular

272 development of the fetus in the placenta. Our analyses did not investigate the mechanistic role of
273 maternal inflammation; however, future cohort studies should do so in order to add to the limited
274 existing research.

275 Placental malaria and parasite density at delivery, diagnosed by qPCR, were found to be
276 particularly detrimental to neurocognitive and motor scores in children. One explanation for this
277 could be that submicroscopic placental malaria infections may be particularly deleterious to fetus
278 growth and development [19,28,29]. While qPCR technology is able to identify submicroscopic
279 malarial infections undetectable by microscopy, laboratory resources are not readily available in
280 low-income countries like Benin. Therefore, women with submicroscopic infections in our
281 population were not immediately diagnosed and treated at the time of delivery and these
282 infections could reflect more chronic infections that are not easily diagnosed by microscopy
283 methods and treated rapidly.

284 This research provides evidence of neurocognitive consequences on fetuses exposed to
285 peripheral and placental malaria during pregnancy and expands on existing epidemiological
286 knowledge of malaria in pregnancy in endemic areas. Submicroscopic, asymptomatic malarial
287 infections pose a high risk to pregnant women in malaria-endemic countries like Benin, and
288 sufficient IPTp during antenatal care, as well as in early childhood [30], is vital to address its
289 risks on fetal development. This work reinforces the need for routine antenatal care visits to
290 adequately diagnose and treat *P. falciparum* infections in pregnant women. Further studies in
291 large cohorts should be conducted to confirm our analyses and expand upon hypothesized
292 mechanisms.

293 **Funding**

294 This work was supported by the Fondation de France [2015 00060746 to FBL], the
295 Eunice Kennedy Shriver National Institute of Child Health & Human Development
296 [NIH/NICHD- R21-HD060524 to FBL], the European and Developing Countries Clinical Trials
297 Partnership [EDCTP- IP.07.31080.002 to MC], and the Bill and Melinda Gates Foundation [to
298 MC].

299 **Acknowledgements**

300 We would like to sincerely thank the on-site researchers and public health nurses in
301 Allada, Benin for their collaboration on all studies mentioned in this paper. We also thank
302 Nicaise Tuikue Ndam for their contribution in qPCR analyses.

303 **References**

- 304 1. Desai M, ter Kuile FO, Nosten F, et al. Epidemiology and burden of malaria in pregnancy.
305 *Lancet Infect Dis.* 2007;7(2):93–104.
- 306 2. Bauserman M, Conroy AL, North K, Patterson J, Bose C, Meshnick S. An overview of
307 malaria in pregnancy. *Semin Perinatol.* 2019;43(5):282–90.
- 308 3. Schantz-Dunn J, Nour NM. Malaria and Pregnancy: A Global Health Perspective. *Rev*
309 *Obstet Gynecol.* 2009;2(3):186–92.
- 310 4. Guyatt HL, Snow RW. Impact of malaria during pregnancy on low birth weight in sub-
311 Saharan Africa. *Clin Microbiol Rev.* 2004;17(4):760–9.
- 312 5. Le Port A, Cottrell G, Martin-Prevel Y, Migot-Nabias F, Cot M, Garcia A. First malaria
313 infections in a cohort of infants in Benin: biological, environmental and genetic
314 determinants. Description of the study site, population methods and preliminary results.
315 *BMJ Open.* 2012;2(2):e000342.
- 316 6. McDonald CR, Cahill LS, Ho KT, et al. Experimental Malaria in Pregnancy Induces
317 Neurocognitive Injury in Uninfected Offspring via a C5a-C5a Receptor Dependent
318 Pathway. *PLoS Pathog.* 2015;11(9):e1005140.
- 319 7. Weckman AM, Ngai M, Wright J, McDonald CR, Kain KC. The Impact of Infection in
320 Pregnancy on Placental Vascular Development and Adverse Birth Outcomes. *Front*
321 *Microbiol.* 2019 Aug 22;10.
- 322 8. González R, Mombo-Ngoma G, Ouédraogo S, et al. Intermittent Preventive Treatment of
323 Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre
324 Randomized Controlled Trial. *PLOS Med.* 2014;11(9):e1001733.
- 325 9. Bodeau-Livinec F, Gloennec P, Cot M, et al. Elevated Blood Lead Levels in Infants and
326 Mothers in Benin and Potential Sources of Exposure. *Int J Environ Res Public Health.*
327 2016;13(3).
- 328 10. Ouédraogo S, Accrombessi M, Diallo I, et al. Placental impression smears is a good
329 indicator of placental malaria in sub-Saharan Africa. *Pan Afr Med J.* 2019;34(30).
- 330 11. Swysen C, Vekemans J, Bruls M, et al. Development of standardized laboratory methods
331 and quality processes for a phase III study of the RTS, S/AS01 candidate malaria vaccine.
332 *Malar J.* 2011;10:223.
- 333 12. Ndam NT, Mbuba E, González R, et al. Resisting and tolerating *P. falciparum* in pregnancy
334 under different malaria transmission intensities. *BMC Med.* 2017 17;15(1):130.
- 335 13. Koura KG, Boivin MJ, Davidson LL, et al. Usefulness of child development assessments
336 for low-resource settings in francophone Africa. *J Dev Behav Pediatr JDBP.*
337 2013;34(7):486–93.

- 338 14. Bodeau-Livinec F, Davidson LL, Zoumenou R, Massougbodji A, Cot M, Boivin MJ.
339 Neurocognitive testing in West African children 3-6 years of age: Challenges and
340 implications for data analyses. *Brain Res Bull.* 2018.
- 341 15. Boivin MJ, Zoumenou R, Sikorskii A, et al. Neurodevelopmental assessment at one year of
342 age predicts neuropsychological performance at six years in a cohort of West African
343 Children. *Child Neuropsychol.* 2021;0(0):1–24.
- 344 16. Mireku MO, Boivin MJ, Davidson LL, et al. Impact of helminth infection during pregnancy
345 on cognitive and motor functions of one-year-old children. *PLoS Negl Trop Dis.*
346 2015;9(3):e0003463.
- 347 17. Elphinstone RE, Weckman AM, McDonald CR, et al. Early malaria infection,
348 dysregulation of angiogenesis, metabolism and inflammation across pregnancy, and risk of
349 preterm birth in Malawi: A cohort study. *PLOS Med.* 2019;16(10):e1002914.
- 350 18. Sania A, Sudfeld CR, Danaei G, et al. Early life risk factors of motor, cognitive and
351 language development: a pooled analysis of studies from low/middle-income countries.
352 *BMJ Open.* 2019;9(10):e026449.
- 353 19. Cottrell G, Moussiliou A, Luty AJF, et al. Submicroscopic *Plasmodium falciparum*
354 Infections Are Associated With Maternal Anemia, Premature Births, and Low Birth
355 Weight. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2015;60(10):1481–8.
- 356 20. Boivin MJ, Bangirana P, Byarugaba J, et al. Cognitive impairment after cerebral malaria in
357 children: a prospective study. *Pediatrics.* 2007;119(2):e360-366.
- 358 21. Accrombessi M, Yovo E, Fievet N, et al. Effects of Malaria in the First Trimester of
359 Pregnancy on Poor Maternal and Birth Outcomes in Benin. *Clin Infect Dis Off Publ Infect*
360 *Dis Soc Am.* 2019;69(8):1385–93.
- 361 22. Kalilani-Phiri L, Thesing PC, Nyirenda OM, et al. Timing of Malaria Infection during
362 Pregnancy Has Characteristic Maternal, Infant and Placental Outcomes. *PLOS ONE.*
363 2013;8(9):e74643.
- 364 23. Mahande AM, Mahande MJ. Prevalence of parasitic infections and associations with
365 pregnancy complications and outcomes in northern Tanzania: a registry-based cross-
366 sectional study. *BMC Infect Dis.* 2016;16:78.
- 367 24. WHO | WHO policy brief for the implementation of intermittent preventive treatment of
368 malaria in pregnancy using sulfadoxine-pyrimethamine (IPTp-SP) [Internet]. WHO. World
369 Health Organization; Available at:
370 http://www.who.int/malaria/publications/atoz/policy_brief_iptp_sp_policy_recommendation/en/
371
- 372 25. al-Haddad BJS, Jacobsson B, Chabra S, et al. Long-term Risk of Neuropsychiatric Disease
373 After Exposure to Infection In Utero. *JAMA Psychiatry.* 2019;76(6):594–602.

- 374 26. Canetta SE, Bao Y, Co MDT, et al. Serological Documentation of Maternal Influenza
375 Exposure and Bipolar Disorder in Adult Offspring. *Am J Psychiatry*. 2014;171(5):557–63.
- 376 27. Knuesel I, Chicha L, Britschgi M, et al. Maternal immune activation and abnormal brain
377 development across CNS disorders. *Nat Rev Neurol*. 2014;10(11):643–60.
- 378 28. Huynh B-T, Fievet N, Gbaguidi G, et al. Influence of the timing of malaria infection during
379 pregnancy on birth weight and on maternal anemia in Benin. *Am J Trop Med Hyg*.
380 2011;85(2):214–20.
- 381 29. Uneke CJ. Impact of Placental *Plasmodium falciparum* Malaria on Pregnancy and Perinatal
382 Outcome in Sub-Saharan Africa. *Yale J Biol Med*. 2007;80(3):95–103.
- 383 30. Bigira V, Kapisi J, Clark TD, et al. Protective Efficacy and Safety of Three Antimalarial
384 Regimens for the Prevention of Malaria in Young Ugandan Children: A Randomized
385 Controlled Trial. *PLOS Med*. 2014;11(8):e1001689.
- 386
- 387

388 **Table 1.** Comparison of maternal and birth characteristics during pregnancy in mother-child pairs
 389 included in analyses, lost to follow-up, and children deceased between birth and six years of age.

Parameter	Missing	Included	Lost to follow-up	p-value	Deceased	p-value
	n/493 (%)	N=493 n (%)	N=310 n (%)		N=60 n (%)	
Age at delivery (years)	0			0.10		0.47
<25		255 (52)	179 (58)		34 (57)	
>25		238 (48)	131 (42)		26 (43)	
Gravidity	0			0.04		0.05
Primigravida		75 (15)	65 (21)		15 (25)	
Multigravida		418 (85)	245 (79)		45 (75)	
Pre-pregnancy BMI (kg/m²)	2 (<1)			0.12		0.50
Underweight		49 (10)	43 (14)		3 (5)	
Normal		353 (72)	219 (72)		44 (76)	
Overweight/obese		89 (18)	44 (14)		11 (19)	
IPTp treatment	0			0.31		0.38
SP		161 (33)	112 (36)		23 (38)	
MQ		332 (67)	198 (64)		37 (62)	
Iron deficiency^a	0			0.08		0.12
No		173 (35)	90 (29)		15 (25)	
Yes		320 (65)	220 (71)		45 (75)	
STH infection^b	2 (<1)			0.12		0.70
No		396 (81)	260 (85)		48 (83)	
Yes		95 (19)	46 (15)		10 (17)	
Maternal education	0			0.04		0.06
None		337 (68)	190 (61)		48 (80)	
Primary+		156 (32)	120 (39)		12 (20)	
Family possession score (quartile)	0			0.004		0.02
1		147 (30)	122 (39)		15 (25)	
2		105 (21)	74 (24)		15 (25)	
3		201 (41)	89 (29)		18 (30)	
4		40 (8)	25 (8)		12 (20)	
Pre-term (<37 weeks' gestation)	10 (2)			0.75		0.01
No		450 (93)	274 (93)		48 (83)	
Yes		33 (7)	22 (7)		10 (17)	
Low birth weight (<2,500 grams)	38 (8)			0.46		<0.001
No		417 (92)	253 (90)		39 (71)	
Yes		38 (8)	28 (10)		16 (29)	
Child sex	0			0.27		0.26
Male		252 (51)	146 (47)		26 (43)	
Female		241 (49)	164 (53)		34 (57)	
Thick blood smear at 1st ANV	0			0.61		0.97
Negative		420 (85)	260 (84)		51 (85)	
Positive		73 (15)	50 (16)		9 (15)	
Thick blood smear at 2nd ANV	6 (1)			0.41		0.48
Negative		470 (97)	285 (95)		53 (95)	
Positive		17 (3)	14 (5)		3 (5)	
Thick blood smear at delivery	26 (5)			0.70		0.41
Negative		418 (90)	245 (90)		53 (93)	

Positive qPCR of placenta	49 (10)	26 (10)	4 (7)		
Negative	100 (20)			0.69	0.93
Positive	271 (69)	136 (67)	32 (70)		
MiP at least once in pregnancy	121 (31)	66 (33)	14 (30)		
No	0			0.57	0.48
Yes	297 (60)	193 (62)	39 (65)		
Parasite density at 1st ANV^c	196 (40)	117 (38)	21 (35)		
	0	587 (9-35,940)	570 (12-100,391)	0.95	1,301 (12-8,291)
Parasite density at 2nd ANV^c	0	231 (3-63,090)	1,128 (3-19,700)	0.93	24 (3-1,1230)
Parasite density at delivery^c	0	84 (0.5-859,446)	165 (2-1,185,727)	0.98	798 (2-352,534)

390 ^aIron deficiency at recruitment, defined as ferritin serum concentration <12µg/L in the absence of
391 inflammation or a serum ferritin concentration <70µg/L in the presence of inflammation measured by C-
392 Reactive Protein.

393 ^b Women considered to be infected with soil-transmitted helminths if the fecal egg count (FEC) was at
394 least 24 epg for any species.

395 ^cParasite density in parasites/µL; parasite density at delivery determined through qPCR. Median (range)
396 of parasite densities shown.

397

398 **Table 2.** Associations between risk factors and neurocognitive development measured by the MSEL
 399 Early Learning Composite (ELC) and the gross motor composite score at one year.

Parameters	Neurocognitive Assessments at one year			
	MSEL ELC^a	p-value^b	Motor composite^a	p-value^b
<u>Maternal Characteristics</u>				
Age at delivery (years)		0.68		0.16
≤25	100		48.5	
>25	100		49	
Gravidity		0.34		0.04
Primigravida	102		45.5	
Multigravida	99		49	
Pre-pregnancy BMI (kg/m²)		0.10		0.06
Underweight	102		55	
Normal	99		49	
Overweight/obese	98		44	
IPTp treatment		0.20		0.29
SP	101		49	
MQ	99		49	
Iron deficiency^c		0.76		0.81
No	100		49	
Yes	100		49	
STH infection^d		0.002		0.03
No	101		49	
Yes	94.5		48	
Maternal education		<0.001		<0.001
None	97		48	
Primary+	104		49	
Family possession score (quartile)		0.001		0.01
1	97		44.5	
2	95		48	
3	102		49	
4	103		49	
EPDS score^e		0.31		0.75
≤10	99		49	
>10	101		49	
HOME score		<0.001		0.002
≤27 (mean)	98		47.5	
>27	102		49	
<u>Child Characteristics</u>				
Pre-term (<37 weeks' gestation)		0.02		0.31
No	101		49	
Yes	93.5		48	
Low birth weight (<2,500 grams)		0.06		0.008
No	100		49	
Yes	94.5		43.5	
Malaria infection^f		0.02		0.02
No	100		49	
Yes	97		44	
Sex		0.11		0.25
Male	99		49	

Female	101	48	
Blood lead levels^g		0.17	0.44
≤50ug	101	49	
>50ug	96.5	49	

400 ^a Median assessment scores shown for each category

401 ^b Wilcoxon rank-sum and Kruskal Wallis analyses used for binary and ordinal variables, respectively

402 ^c Iron deficiency at recruitment, defined as ferritin serum concentration <12μg/L in the absence of
403 inflammation or a serum ferritin concentration <70μg/L in the presence of inflammation measured by C-
404 Reactive Protein.

405 ^d Women considered to be infected with soil-transmitted helminths if the fecal egg count (FEC) was at
406 least 24 epg for any species.

407 ^e Women with a score of ≥10 considered to have symptoms of post-partum depression.

408 ^f Malaria infection at the time of neurocognitive assessment, diagnosed by thick blood smear.

409 ^g Blood lead levels measured at the time of neurocognitive assessment using inductively coupled plasma
410 mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan DRC II ICP-MS instrument).

411

412

413

414 **Table 3.** Associations between risk factors and neurocognitive development measured by the KABC-II,
 415 MPI and NVI, and BOT-2 standard score at six years.

Parameters	Neurocognitive Assessments at six years					
	KABC-II MPI^a	p-value^b	KABC-II NVI^a	p-value^b	BOT-2^a	p-value^b
Maternal Characteristics						
Age at delivery (years)		0.14		0.74		0.05
≤25	59		51		37	
>25	59		51		39	
Gravidity		0.84		0.38		0.07
Primigravida	59		49		36	
Multigravida	59		51		38	
Pre-pregnancy BMI (kg/m²)		0.04		0.78		0.04
Underweight	60		52		40	
Normal	59.5		51		38	
Overweight/obese	57		51		36.5	
IPTp treatment		0.15		0.02		0.12
SP	58		50.5		37	
MQ	59		52		38	
Iron deficiency^c		0.16		0.70		0.31
No	60		51		39	
Yes	58		51		37	
STH infection^d		0.32		0.55		0.42
No	60		51		38	
Yes	58		51		37	
Maternal education		<0.001		0.01		0.005
None	58		51		37	
Primary+	64		54		40	
Family possession score (quartile)		<0.001		0.004		0.01
1	56		49		36	
2	57		51		36.5	
3	62		52		39	
4	67.5		54		40	
EPDS score^e		0.03		0.07		0.62
≤10	58		51		38	
>10	62		52		38	
HOME score		0.005		0.08		0.08
≤27 (mean)	58		51		37	
>27	62		52		39	
Child Characteristics						
Pre-term (<37 weeks' gestation)		0.88		0.79		0.87
No	59		51		38	
Yes	58.5		52		38.5	
Low birth weight (<2,500 grams)		0.70		0.74		0.23
No	59		51		38	
Yes	58.5		52		36	
Malaria infection^f		0.25		0.37		0.46
No	59		51		38	
Yes	60		52		37	
Sex		0.07		0.94		<0.001
Male	60		51		42	

Female	58		52		35
Blood lead levels^g		0.26		0.22	0.95
≤50ug	61		51		37
>50ug	59		51		38

416 ^aMedian assessment scores shown for each category

417 ^bWilcoxon rank-sum and Kruskal Wallis analyses used for binary and ordinal variables, respectively

418 ^cIron deficiency at recruitment, defined as ferritin serum concentration <12μg/L in the absence of
419 inflammation or a serum ferritin concentration <70μg/L in the presence of inflammation measured by C-
420 Reactive Protein.

421 ^dWomen considered to be infected with soil-transmitted helminths if the fecal egg count (FEC) was at
422 least 24 epg for any species.

423 ^eWomen with a score of ≥10 considered to have symptoms of post-partum depression.

424 ^fMalaria infection at the time of neurocognitive assessment, diagnosed by thick blood smear.

425 ^gBlood lead levels measured at the time of neurocognitive assessment using inductively coupled plasma
426 mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan DRC II ICP-MS instrument).

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430

431 **Figure Legends**

432

433 **Figure 1.** Population follow-up of women and children from pregnancy until six years of age.

434

435 **Figure 2.** Linear regression beta coefficients and confidence intervals of association between MiP and
436 child neurocognitive development at one year of age.

437 --◇-- Unadjusted beta-coefficients and 95% bootstrapped confidence intervals.

438 --◆-- Adjusted beta-coefficients and 95% bootstrapped confidence intervals; controlling for maternal
439 age and education, gravidity, pre-pregnancy BMI, family possession score, HOME score, and child sex
440 and age at time of neurocognitive assessment.

441

442 **Figure 3.** Linear regression beta coefficients and confidence intervals of association between MiP and
443 child neurocognitive development at six years of age.

444 --◇-- Unadjusted beta-coefficients and 95% bootstrapped confidence intervals.

445 --◆-- Adjusted beta-coefficients and 95% bootstrapped confidence intervals; controlling for maternal
446 age and education, gravidity, pre-pregnancy BMI, family possession score, HOME score, and child sex
447 and age at time of neurocognitive assessment.

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Figure 1.

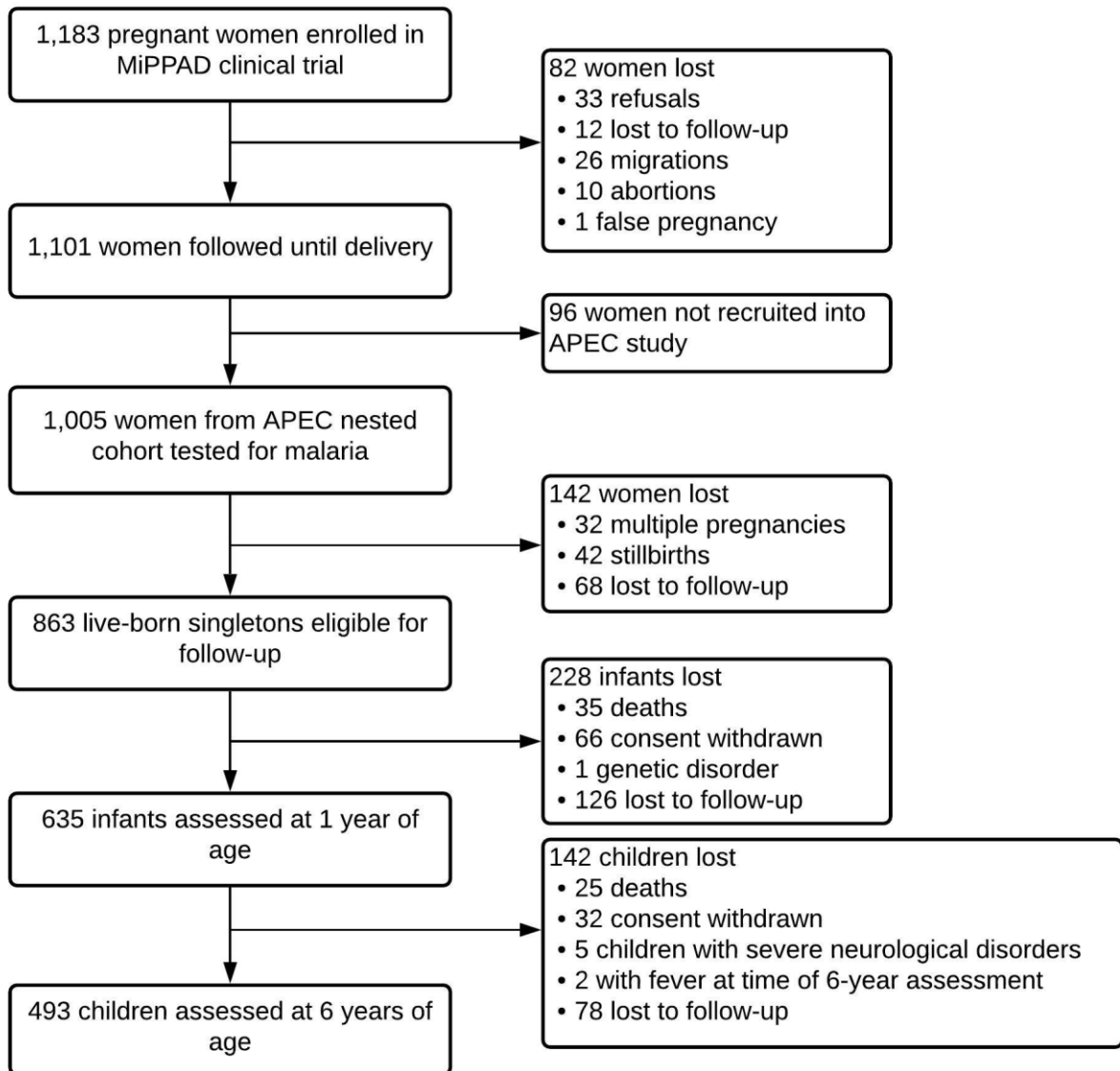


Figure 2.

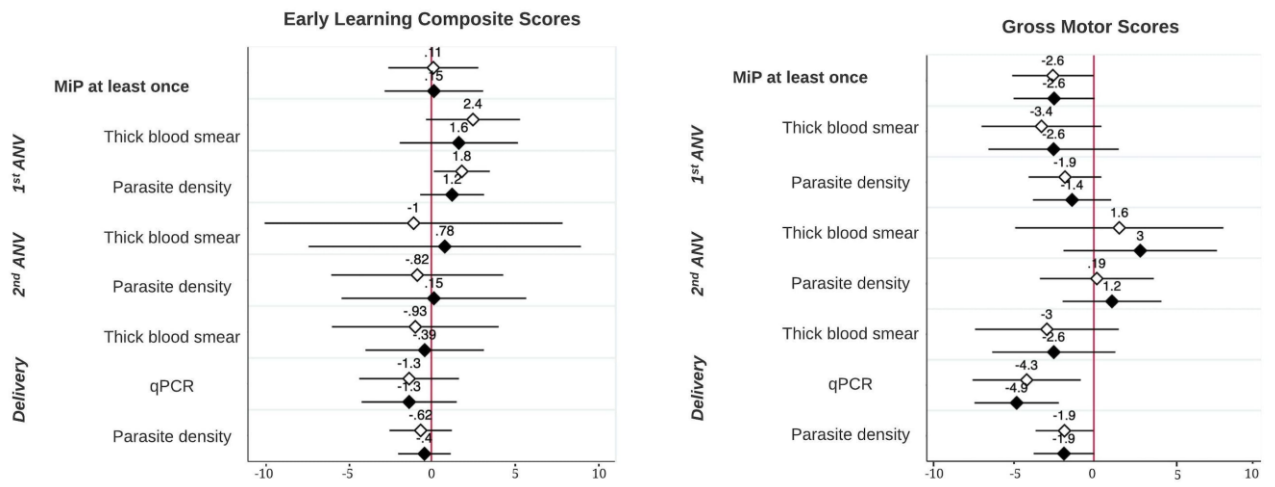
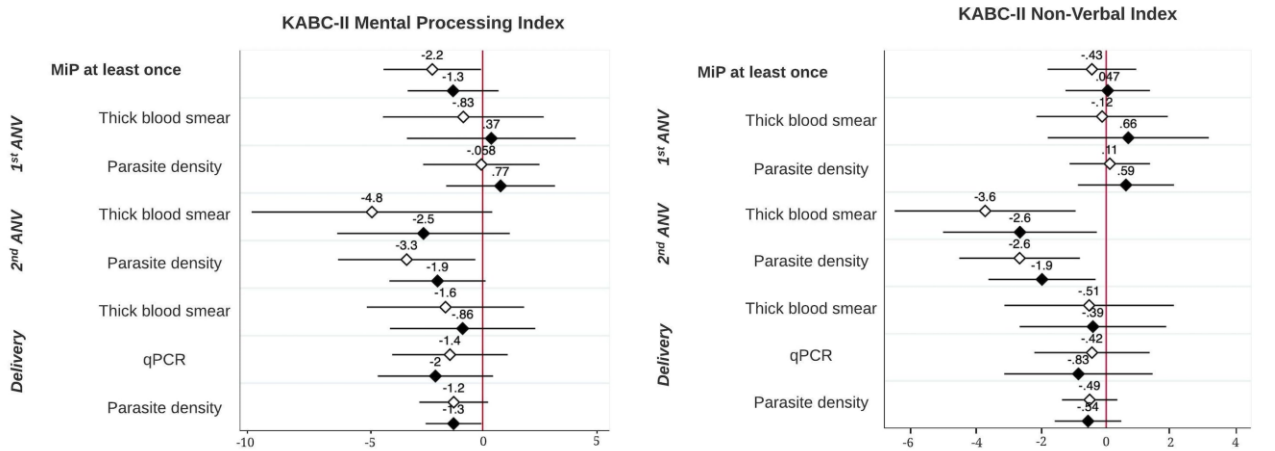


Figure 3.



Chapter 5. Maternal depression and parasitic infection

This chapter includes the third article of this thesis investigating the potential confounding role of maternal depression and parent-child interactions on risk of soil-transmitted helminth and malaria infection. Maternal depression has been shown to impact child development, with maternal psychological wellbeing and quality of care given to children two important determinants of healthy child development (186,250). However, the role of maternal depression and parent-child interactions on subsequent infection has been less studied. We conducted analyses within a sub-cohort of mothers and children from our birth cohort in order to test the hypothesis that women with more depressive symptoms and lower quality parent-child interactions would be associated with higher risk of parasitic infection in children. This hypothesis was based off of previous work by Rahman et al. (2007) which demonstrated an association between post-partum depression in mothers and increased risk of diarrheal disease in their infants (15). Our analyses revealed that while maternal depression, as measured by the EPDS, was associated with lower HOME scores, indicating lower quality of parent-child interactions and home learning environment of children, neither of these factors were found to influence subsequent parasitic infection in children. These analyses add to our understanding of the mechanisms behind the relationship between parasitic infection during pregnancy and child development, since parasitic infection during pregnancy has been found to increase the risk of infection in surviving offspring according to some studies (86,251). Understanding how maternal depression and parent-child interactions may influence risk of childhood infection, which subsequently is known to impair neurocognitive and motor development (199,252,253), is important for better interpreting the results of the primary objectives of this thesis. This quantitative work was done in tandem with a qualitative study exploring the experiences, barriers, and perceptions of risk of pregnant women in this cohort regarding prevention and treatment of parasitic infection during their pregnancies. This qualitative study, involving semi-direct interviews of participating women, also explored the role of maternal depression in the prevention and treatment of parasitic infection in children in this population. These interviews are set to take place in June 2021, with a qualitative analysis of interviews taking place shortly thereafter. The quantitative analyses for this subject are currently under review for publication.

Article 3

Title Page w/ ALL Author Contact Information

The impact of maternal depression and parent-child interactions on risk of parasitic infections in early childhood: a prospective cohort in Benin

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Abstract

Objectives

Maternal depression occurs in 13-20% of women from low-income countries, which is associated with negative child health outcomes, including diarrheal disease. However, few studies have investigated its impact on child risk of infectious disease. We studied associations between maternal depressive symptoms, and parent-child interactions, and the risk of *Plasmodium falciparum* malaria and soil-transmitted helminth infection in Beninese children.

Methods

Our population included mothers and children enrolled in a clinical trial during pregnancy (MiPPAD) in Benin. The Edinburgh Postnatal Depression Scale (EPDS) assessed maternal depressive symptoms and the Home Observation Measurement of the Environment (HOME) assessed parent-child interactions. Blood and stool sample analyses diagnosed child malaria and helminth infection at 12, 18, and 24 months. Negative binomial and Poisson regression models with robust variance tested associations.

Results

Of the 302 mother-child pairs, 39 (12.9%) mothers had depressive symptoms. Median number of malaria episodes per child was 3 (0-14) and 29.1% children had at least one helminth infection. Higher EPDS scores were associated with lower HOME scores; relative risk (RR) 0.97 (95% Confidence Interval (CI) 0.95, 0.99), particularly with lower acceptance, involvement, and variety subscales; RR 0.92 (95% CI 0.85, 0.99), RR 0.82 (95% CI 0.77, 0.88), RR 0.93 (95% CI 0.88, 0.99), respectively. However, neither exposure was associated with risk of parasitic infection in children.

Conclusions for Practice

Maternal depressive symptoms are associated with poor parent-child interactions, particularly acceptance of behavior, involvement with children, and variety of interactions, but these exposures do not impact risk of parasitic infection in children.

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Significance

What's already known?

Maternal depression increases risk of child morbidities like diarrheal disease and febrile illness, under the hypothesis that mothers have more difficulty caring for and preventing disease in their children. Previous studies have not considered how these exposures may increase parasitic infectious disease in children living in high-transmission settings.

What this study adds?

Maternal depressive symptoms impair the way mothers discipline children, interact with them, and provide stimulation and social interactions. Neither maternal depressive symptoms nor impaired parent-child interactions increase the risk of parasitic infection in children in the presence of regular screening and treatment of disease.

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Keywords

Postpartum Depression; Parent-Child Relations; Malaria; Helminths; Child Health

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Objectives

Post-natal mental health disorders are a significant public health concern as they have negative consequences not only on maternal health, but also on child well-being and development (Villegas et al., 2011). Women may experience symptoms of maternal depression post-partum such as feelings of sadness, tearfulness or hopelessness, loss of energy or appetite, and suicidal ideations from 2 weeks to 12 months after giving birth (Stewart, 2005). Maternal depression is estimated to affect 13-20% of women globally, according to the World Health Organization (WHO) (*World Health Organization*, n.d.). Women in developing countries, such as Sub-Saharan African (SSA) countries, are at higher risk of developing depression after pregnancy due to their lower socioeconomic status, lower social support, reduced access to mental health services, and higher rates of maternal morbidities compared to women in higher income countries (Sawyer et al., 2010).

Maternal depression is linked to several consequences on child health and development, such as stunting and underweight (Avan et al., 2010). A meta-analysis in 2017 concluded that maternal depressive symptoms are also associated with lower cognitive scores in infancy (Liu et al., 2017). Depressed mothers may interact differently with their infants compared to non-depressed mothers; they may be more withdrawn and provide less stimulation for children necessary for neurocognitive and psychological development (Azak & Raeder, 2013). Poor parent-child interactions in the home environment are thought to be the mediator between maternal depression and child cognitive development (Bass et al., 2016). Few studies have investigated how maternal depression and parent-child interactions independently impact child morbidity from infectious disease. One study found that post-natal depression increases risk of diarrheal illness in one-year-old children in a low-income setting (Rahman et al., 2007). Another linked post-natal depression to increased risk of febrile illness in Ghanaian and Ivorian infants one year after birth (Guo et al., 2013).

There are several hypothesized mechanisms for the relationship between maternal depression and child morbidity, including the role of various biological, hormonal, cultural, and social factors (Coughlin, 2012; Weinstock, 2005). Maternal depression could impact a mother's ability to complete daily activities, care for, and provide support for her child, therefore putting them at higher risk of morbidity (Patel et al., 2002). Poverty and low parental education could also contribute to the mechanism between maternal depression and risk of infection in developing

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countries (Rahman et al., 2007). Previous studies have found a link between poor parent-child interactions in early childhood and increased risk of child obesity (Anderson & Keim, 2016), however there are currently no existing studies, to our knowledge, on the impact of parent-child interactions on child parasitic infection.

In Benin, malaria caused by the *Plasmodium falciparum* parasite is the leading cause of mortality in children under five years (President's Malaria Initiative, n.d.). Transmitted by the bite of infected *Anopheles* mosquitoes, malaria infection can lead to repeated episodes of fever, vomiting, sweats, and in severe cases seizure and death (World Health Organization, n.d.). Infections from soil-transmitted helminths due to insufficient hygiene and sanitation systems also contribute to the burden of disease in this part of the world, with one study showing a prevalence of 23% in a population of children in Benin (Ibikounle et al., 2018). Soil-transmitted helminths comprise several species of parasitic worms, the most prominent species in Benin being the hookworms *Ankylostoma duodenale* and *Necator americanus*. The WHO currently recommends the use of intermittent preventive treatment in infants with sulfadoxine-pyrimethamine (IPTi-SP) during the first year of life and intermittent preventive treatment in pregnancy with sulfadoxine-pyrimethamine (IPTp-SP) beginning in the second trimester to prevent malaria, along with the use of insecticide-treated bed nets (ITNs) (World Health Organization, n.d.). Guidelines for the prevention and treatment of soil-transmitted helminth infection recommend the use of albendazole or mebendazole bi-annually in populations with disease prevalence over 20% (World Health Organization, 2012).

These parasitic infections have serious consequences for child health and are leading causes of anemia in children in SSA (Mulu et al., 2014). However, despite the increased interest in studies investigating the links between mental and physical health in mothers and children, no such studies exist on the impact of maternal depression and parent-child interactions on parasitic infection in early childhood. Similar to the hypothesis made by Rahman and colleagues (Rahman et al., 2007), we hypothesized that women suffering from symptoms of maternal depression, and women who interact with and stimulate their children less, may also have more difficulties caring for their children and providing them with necessary preventive measures against parasitic infection, therefore resulting in higher risk of malaria and helminth infection. The aims of this paper were to study the impacts of maternal depressive symptoms and parent-child interactions,

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independently, measured at one year postpartum on the risk of *P. falciparum* malaria and soil-transmitted helminth infections in children from a Beninese cohort.

Methods

Cohort selection

Our study population included women and their children who participated in the *Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD)* clinical trial comparing the efficacy of sulfadoxine-pyrimethamine and mefloquine used for IPTp in women (NCT0081142). Further information on the study protocol and inclusion criteria for this clinical trial may be found in a previous publication (González et al., 2014). The trial took place in the semi-rural area of Allada, about 40 kilometers outside of Cotonou, Benin, in Sub-Saharan Africa. All eligible, liveborn singletons of women were invited to participate in a follow-up study (TOVI) at 12 months of age, whose aim was to study associations between maternal anemia and neurodevelopment in children (Bodeau-Livinec et al., 2016). A sub-cohort of these children was then selected to participate in the prospective study *Immune Tolerance associated with Malaria: Consequences for the protection of pregnant women and children (TOLIMMUNPAL)*, which followed children from 12-24 months to measure *P. falciparum* malaria and soil-transmitted helminth infection.

Maternal depressive symptoms and parent-child interactions

Maternal depression was assessed in women during a home visit one year postpartum through the Edinburgh Postnatal Depression Scale (EPDS), a 10-item Likert-style questionnaire used to measure symptoms of depression by inquiring about feelings of sadness or hopelessness, changes in appetite or activity, and ideations of self-harm within the previous seven days. The French version of the EPDS was translated by local investigators into Fon, the language spoken in Allada. The EPDS score was recorded and analyzed as a continuous score, with higher scores indicating the presence of depressive symptoms. A pilot study evaluated the adaptability and validity of the EPDS in this setting, given that there are no gold standard clinical diagnostic tests for maternal depression in Benin (Koura et al., 2013). Parent-child interactions were assessed during this visit through the Home Observation Measurement of the Environment (HOME), which was adapted for use in this setting during the same pilot study. The HOME test is a combination of interview questions and observations of parent-child interactions in the home environment and consists of several subscales. The adaptations made to the version of the HOME

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used in our population included the removal of one question asking how much time the child spends outside, as children in this population spend most of their time outside of the home. Items related to the number of books or toys that children owned was modified to ask if children owned at least one book or if books could be seen in the home, and no questions were asked about toys since only one child seen by investigators owned a toy. The six sub-scales included in the adapted version of the HOME test were responsivity (verbal interactions between parent and child), acceptance (how the parent disciplines the child), organization (how the home and child's personal space is organized), learning materials (toys and activities available for child development), involvement (interactions between parent and child), and variety (opportunities for variety and social interactions in the child's daily routine).

Parasitic infection

The main outcomes of interest in our study, *P.falciparum* malaria and soil-transmitted helminth infection, were repeatedly measured in children from 12-24 months of age through the collection and analysis of blood and stool samples. Malaria was diagnosed by a Rapid Diagnostic Test (RDT) and/or a thick blood smear test administered systematically in children bi-weekly during the follow-up period as well as during emergency visits to a health clinic if children presented with fever ($\geq 37.5^{\circ}\text{C}$). Helminth infection was measured at 12, 18, and 24 months in children, using the Kato-Katz technique on a thick smear of stool sample (World Health Organization, 1994). Children found to be positive for either malaria or helminth infections were subsequently treated within the confines of the study; adherence to helminth prophylaxis was not directly monitored by researchers.

Covariates

Socio-demographic information was collected through a questionnaire given to women during the home visit one year post-partum. Maternal age, education, and marital status were noted. A family possession score was generated to determine socio-economic status; this score was comprised from a list of possessions owned by the family such as a motorcycle, a car, two cows, a bicycle, and electricity. Household size, the number of people living in the same house as the child, was noted. Women were asked about their overall hygiene practices, hand-washing habits, and accessibility to latrines in a transversal study (*Study of birthweight as a predictive factor for child health status; EPOPEE*); this information was used to create a synthesized hygiene rating using Multiple Correspondence Analysis (MCA) as outlined by Cortinovis et al

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(1993). Environmental risk of malaria was calculated as a time- and space-dependent assessment of environmental risk of infection that quantified each child’s exposure to malaria vectors using a predictive model (Lysaniuk et al., 2015). Environmental risk was log-transformed for statistical analyses. During each visit, parents were asked if their child had slept under a mosquito-net the previous night; child were then classified as sleeping under a mosquito net always, frequently, occasionally, or never. Participating children also had available information on malaria episodes prior to 12 months.

Statistical Analysis

First, Fisher exact and Wilcoxon rank-sum tests were used to compare characteristics of our population to the population of children excluded from the 12-month parasitic infection follow-up. Next, univariate analyses were conducted to investigate associations of potential confounding factors identified in the literature and in Direct Acyclic Graphs (DAGs) with child parasitic infection. Linear regression models tested the association of maternal depressive symptoms on the total HOME score, as well as the HOME subscales. Linear models controlled for the socio-economic factors known to influence maternal depression and parent-child interactions (Patel et al., 2002; Stein et al., 1991). Negative binomial and logistic regression models tested associations between main exposures and parasitic outcomes. Adjusted regression models controlled for factors that were significant in univariate and final, multivariate models. All regression models were derived using generalized linear models to generate log-linear models, and therefore effect estimates are presented as relative risk (RR) with a 95% confidence interval (CI) based on outputs. Statistical analyses were completed using STATA 13.1 (StataCorp. 2013. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP), with an alpha risk of 5%.

Sensitivity analyses

Missing data for mosquito net use and hygiene rating were 6% and 18%, respectively; therefore, models with and without these potential confounders were carried out. Malaria was tested in additional models as a binary variable, each HOME sub-scale was tested for potential associations with parasitic infection, and incidence of malaria in children before the onset of the study period was considered in additional models due to its impact on risk of future infection in children.

Ethical approval

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The institutional review boards of the Hospital Clinic of Barcelona, the Comité Consultatif de Déontologie et d'Éthique of the Institut de Recherche pour le Développement, the University of Abomey-Calavi, New York University (IRB#09-1253), and the Beninese Ethical Committee of the Faculté des Sciences de la Santé (FSS) approved all study protocols included in this paper. Written informed consent of women was obtained in the presence of a witness, with thumbprints provided if women could not read and/or write.

Results

Of the 1,005 pregnant women recruited into the MiPPAD clinical trial, 863 singleton children were born alive to these women [Figure I]. One-year post-partum, 747 mother-child pairs were followed up and assessed for maternal depressive symptoms and parent-child interactions. Our study included a sub-population of these children, 302 of whom were recruited for the TOLIMMUNPAL study to assess parasitic infection from 12-24 months of age and included in our analyses.

Socio-demographic characteristics of our population are displayed in Table I, along with characteristics of mother-child pairs not included in the 12-month prospective follow-up for parasitic infection. Mean maternal age of our population was 27 years, 39.4% of women had some education, and 63.3% of women were in monogamous marriages. Mean HOME score among women and children was 26.6. Using a cut-off score of 13, 12.9% of participating women showed symptoms of maternal depression according to the EPDS.

During the 12-month follow-up for parasitic infection in children, 84.1% of children had at least one malaria episode [Table II]. The average number of malaria episodes per child was 3, ranging from 0 to 14 episodes per child. Of the children assessed for helminth infection, 29.1% had at least one infection. At 12 and 18 months, the majority of helminth infection were caused by *Trichuris trichiura* species, (73.9%) and (51.3%) respectively. At 24 months, 73.6% of infections were caused by hookworm species.

Univariate analyses between potential confounders and outcomes are displayed in Table III. No maternal education, lower family possession score, normal birthweight, higher environmental risk of malaria, and previous history of malaria were risk factors for higher incidence of malaria episodes in children. Helminth infection in children was associated with

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higher maternal age, multiparae mothers, higher maternal BMI, and more people living in the household.

Symptoms of maternal depression were associated with poorer parent-child interactions measured by the HOME test RR 0.98 (95% CI 0.96, 0.99) [Table IV], after verifying linearity between the two variables. Higher EPDS score was associated with lower acceptance of child misbehavior RR 0.91 (95% CI 0.85, 0.99), lower parental involvement with the child at home RR 0.82 (95% CI 0.77, 0.87) and less variety of daily stimulation and social interaction provided for the child RR 0.93 (95% CI 0.88, 0.98). These associations remained in adjusted models.

Crude and adjusted negative binomial regression models did not reveal associations between maternal depressive symptoms and total malaria episodes in children RR 1.00 (95% CI 0.97, 1.03) and RR 1.00 (95% CI 0.98, 1.03), respectively; nor between maternal depressive symptoms and helminth infection RR 0.99 (95% CI 0.95, 1.04) and RR 1.00 (95% CI 0.95, 1.05), respectively [Table V]. Crude and adjusted logistic regression models between maternal depressive symptoms and parent-child interactions were also found to have no association with helminth infection in children. Mosquito-net use and hygiene rating were kept in final models, despite not being associated with outcomes and not significantly changing effect estimates of final models if excluded, due to being important protective factors against parasitic infection (ter Kuile et al., 2003; Vaz Nery et al., 2019).

Conclusions for Practice

Our hypothesis that women with more depressive symptoms and worse parent-child interactions would be associated with higher risk of parasitic infection in children was not confirmed within analyses. While maternal depressive symptoms are associated with worse parent-child interactions, this does not seem to impact risk of malaria and soil-transmitted helminth infection in children.

Our prospective cohort study involved repeated biological assessments for malaria and helminth infection, along with anthropological and neurodevelopmental assessments, which few African cohorts have done before. Two diagnostic techniques were used to assess malaria, with the thick blood smear test being the gold standard of malaria diagnosis according to the WHO. Another strength of this study is the consideration of several potential confounding factors identified in the literature, such as socio-economic status, household size, the use of mosquito bed nets, and hygiene habits, including hand-washing.

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A limitation of this study is the low power due to small sample size; malaria-helminth co-infection could not be analyzed in this study due to the low number of co-infections. Selection bias due to attrition could also be present; however, maternal depressive symptoms and parent-child interactions did not significantly differ between our population and the mother-child pairs excluded from TOLIMMUNPAL. Our population of 302 children were more iron deficient (47%) compared to excluded children (34.1%). Mothers in our population could have been depressed before one-year post-partum, however only parasitic infection diagnosed after administration of the EPDS was considered for temporality. Most infants in high malaria-transmission settings experience their first malaria episodes several months or one year after birth due to acquired immunity (Dobbs & Dent, 2016). Our study controlled for children who had malaria episodes before the onset of the study period, which increases their risk for subsequent malaria episodes.

About 13% of women in our population were identified as having symptoms of maternal depression one-year post-partum according to a cut-off score of 13, similar to studies from other SSA cohorts using the same cut-off (Ebeigbe & Akhigbe, 2008; Okronipa et al., 2012). Our analyses also support findings that show maternal depressive symptoms are associated with poor parent-child interactions after controlling for socio-economic risk factors. The link between post-partum depression and impaired mother-child interactions has been established in previous works (Stein et al., 1991), with many researchers hypothesizing that this association is a mediator for poor neurodevelopmental outcomes in children. One study found that treatment of post-partum depression in mothers improved the quality of mother-child interactions and of infant play (Goodman et al., 2008). Our study adds to this literature by further exploring how maternal depression may impact specific aspects of parent-child interactions measured by the HOME subscales. Our results show that maternal depressive symptoms negatively impact the way mothers discipline their children, physically interact with them, and provide variety in stimulation and social interactions outside the home. Stein et al reported that mothers with post-partum depression in their population provided less social stimulation for their children, compared to non-depressed mothers (Stein et al., 1991). Other studies have further concluded that poor physical interactions between depressed mothers and their children impair the intellectual stimulation needed by children for proper cognitive and emotional development at a young age (Azak & Raeder, 2013). Organization of the child's personal space and the learning materials in

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the house were not found to be hindered by maternal depressive symptoms. Within the pilot study, the HOME was also found to be associated to maternal education and to cognitive development measured by the Mullen Scales of Early Learning (MSEL) in children at 1 year of age (Koura et al., 2013), as seen in a previous study in Uganda (Bass et al., 2016). Each sub-scale of the HOME was individually tested for associations with parasitic infection in children; results mirrored those found between the HOME total score and parasitic infection, showing no association.

Few previous studies have explored the relationship between maternal depression and child morbidity (Guo et al., 2013; Rahman et al., 2007). Authors have hypothesized that socio-economic status and education level are common risk factors for both and could play important roles in this mechanism (Familiar et al., 2016), which is why family possession score of families and maternal education were included in our analyses. It has also been hypothesized that maternal depression, which can lead to hormonal abnormalities, could compromise the immune system and subsequent response to viral infection (Schuster et al., 2011). While this mechanism was not explored in this study in the context of parasitic infection, future studies could further elaborate on this pathway. Maternal depression can also make it difficult for mothers to perform daily tasks and chores (Rahman et al., 2007), including providing adequate care for their children. In terms of disease treatment and prevention, mothers with depression were found to have more medication nonadherence for treatment of Human Immunodeficiency Virus (HIV), compared to non-depressed mothers (Schuster et al., 2011).

If malaria or helminth infection was diagnosed in children, treatment was given to them during the visit to the health clinic, therefore potentially underestimating the effect of depressive symptoms and parent-child interactions on parasitic infection. The study by Guo et al, which found maternal depression to increase risk of febrile illness in children, collected the physical status of children at just two timepoints: 3 months and 12 months of age (Guo et al., 2013). Our results suggest that in the presence of more regular screening and treatment of parasitic infection in children, maternal depressive symptoms and parent-child interactions do not impact risk of subsequent infection.

Despite current prevention and treatment recommendations, our study shows a high proportion of malaria (84.1%) and soil-transmitted helminth (29.1%) infection at least once in children during the 12-month follow-up. While 75% of our population reported always sleeping

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under ITNs at night, there is a potential for social desirability bias which could overestimate the true frequency of ITN-use for malaria prevention in households. Of the children who were infected by helminths between 12-24 months, 87.6% of them were only infected once, indicating that there was very little chronic infection in children and that once children were treated, most remained uninfected. Previous observational and cluster randomized control trials have shown the efficacy of water, sanitation, and hygiene (WASH) interventions in reducing disease burden of soil-transmitted helminths (Vaz Nery et al., 2019), however results are equivocal (Clasen et al., 2014). Our univariate analyses do not show an association between hygiene habits and risk of soil-transmitted helminth infection in children.

The number of malaria episodes in children during the 12-month follow-up period was considered as our main outcome, instead of a binary outcome of malaria, because multiple malaria episodes are common in children living in high-transmission settings like Benin. Sensitivity analyses were completed using a binary outcome for malaria during the 12-month follow-up and no associations were found between exposures and malaria.

Maternal depression impacts how mothers are able to care and provide for their children; however, in the presence of close screening and treatment of parasitic infection these factors do not increase risk of parasitic infection in children. Our study considered maternal depressive symptoms and parent-child interactions separately in their role on child parasitic infection, however future studies could further explore the mediating factor of parent-child interactions in the association between maternal depression and child morbidity in larger prospective cohorts in order to have more statistical power. *P. falciparum* malaria and soil-transmitted helminth infection carry a large burden of disease in children in Benin, as seen by the large prevalence of infection in our population. Adequate screening, prevention, and treatment must be available for at risk groups such as children under the age of five.

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References

- Anderson, S. E., & Keim, S. A. (2016). Parent-Child Interaction, Self-Regulation, and Obesity Prevention in Early Childhood. *Current Obesity Reports*, 5(2), 192–200. <https://doi.org/10.1007/s13679-016-0208-9>
- Avan, B., Richter, L. M., Ramchandani, P. G., Norris, S. A., & Stein, A. (2010). Maternal postnatal depression and children's growth and behaviour during the early years of life: Exploring the interaction between physical and mental health. *Archives of Disease in Childhood*, 95(9), 690–695. <https://doi.org/10.1136/adc.2009.164848>
- Azak, S., & Raeder, S. (2013). Trajectories of parenting behavior and maternal depression. *Infant Behavior and Development*, 36(3), 391–402. <https://doi.org/10.1016/j.infbeh.2013.03.004>
- Bass, J. K., Nakasujja, N., Familiar-Lopez, I., Sikorskii, A., Murray, S. M., Opoka, R., Augustinavicius, J., & Boivin, M. J. (2016). Association of caregiver quality of care with neurocognitive outcomes in HIV-affected children aged 2-5 years in Uganda. *AIDS Care*, 28 Suppl 1, 76–83. <https://doi.org/10.1080/09540121.2016.1146215>
- President's Malaria Initiative. (n.d.). Where we work: Benin. Retrieved May 4, 2018, from <https://www.pmi.gov/where-we-work/benin>
- Bodeau-Livinec, F., Glorennec, P., Cot, M., Dumas, P., Durand, S., Massougbojji, A., Ayotte, P., & Le Bot, B. (2016). Elevated Blood Lead Levels in Infants and Mothers in Benin and Potential Sources of Exposure. *International Journal of Environmental Research and Public Health*, 13(3). <https://doi.org/10.3390/ijerph13030316>
- Clasen, T., Boisson, S., Routray, P., Torondel, B., Bell, M., Cumming, O., Ensink, J., Freeman, M., Jenkins, M., Odagiri, M., Ray, S., Sinha, A., Suar, M., & Schmidt, W.-P. (2014). Effectiveness of a rural sanitation programme on diarrhoea, soil-transmitted helminth infection, and child malnutrition in Odisha, India: A cluster-randomised trial. *The Lancet Global Health*, 2(11), e645–e653. [https://doi.org/10.1016/S2214-109X\(14\)70307-9](https://doi.org/10.1016/S2214-109X(14)70307-9)

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Cortinovis, I., Vella, V., & Ndiku, J. (1993). Construction of a socio-economic index to facilitate analysis of health data in developing countries. *Social Science & Medicine (1982)*, *36*(8), 1087–1097. [https://doi.org/10.1016/0277-9536\(93\)90127-p](https://doi.org/10.1016/0277-9536(93)90127-p)

Coughlin, S. S. (2012). Anxiety and Depression: Linkages with Viral Diseases. *Public Health Reviews*, *34*(2). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4175921/>

Dobbs, K. R., & Dent, A. E. (2016). Plasmodium malaria and antimalarial antibodies in the first year of life. *Parasitology*, *143*(2), 129–138. <https://doi.org/10.1017/S0031182015001626>

Ebeigbe, P. N., & Akhigbe, K. O. (2008). Incidence and associated risk factors of postpartum depression in a tertiary hospital in Nigeria. *The Nigerian Postgraduate Medical Journal*, *15*(1), 15–18.

Familiar, I., Murray, S., Ruisenor-Escudero, H., Sikorskii, A., Nakasujja, N., Boivin, M. J., Opoka, R., & Bass, J. K. (2016). Socio-demographic correlates of depression and anxiety among female caregivers living with HIV in rural Uganda. *AIDS Care*, *28*(12), 1541–1545. <https://doi.org/10.1080/09540121.2016.1191609>

González, R., Desai, M., Macete, E., Ouma, P., Kakolwa, M. A., Abdulla, S., Aponte, J. J., Bulu, H., Kabanyanyi, A. M., Katana, A., Maculuve, S., Mayor, A., Nhacolo, A., Otieno, K., Pahlavan, G., Rupérez, M., Sevene, E., Slutsker, L., Vala, A., ... Menéndez, C. (2014). Intermittent preventive treatment of malaria in pregnancy with mefloquine in HIV-infected women receiving cotrimoxazole prophylaxis: A multicenter randomized placebo-controlled trial. *PLoS Medicine*, *11*(9), e1001735. <https://doi.org/10.1371/journal.pmed.1001735>

Goodman, S. H., Broth, M. R., Hall, C. M., & Stowe, Z. N. (2008). Treatment of postpartum depression in mothers: Secondary benefits to the infants. *Infant Mental Health Journal*, *29*(5), 492–513. <https://doi.org/10.1002/imhj.20188>

Guo, N., Bindt, C., Te Bonle, M., Appiah-Poku, J., Hinz, R., Barthel, D., Koffi, M., Posdich, S., Deymann, S., Barkmann, C., Schlüter, L., Jaeger, A., Blay Nguah, S., Eberhardt, K. A., N’Goran, E., Tagbor, H., Ehrhardt, S., & International CDS Study Group. (2013). Association of antepartum and postpartum depression in Ghanaian and Ivorian women with febrile illness in their offspring:

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A prospective birth cohort study. *American Journal of Epidemiology*, 178(9), 1394–1402.
<https://doi.org/10.1093/aje/kwt142>

Ibikounle, M., Onzo-Aboki, A., Doritchamou, J., Tougoue, J.-J., Boko, P. M., Savassi, B. S., Siko, E. J., Dare, A., Batcho, W., Massougbdji, A., Kinde-Gazard, D. A., & Kabore, A. (2018). Results of the first mapping of soil-transmitted helminths in Benin: Evidence of countrywide hookworm predominance. *Plos Neglected Tropical Diseases*, 12(3), e0006241.
<https://doi.org/10.1371/journal.pntd.0006241>

Koura, K. G., Boivin, M. J., Davidson, L. L., Ouédraogo, S., Zoumenou, R., Alao, M. J., Garcia, A., Massougbdji, A., Cot, M., & Bodeau-Livinec, F. (2013). Usefulness of child development assessments for low-resource settings in francophone Africa. *Journal of Developmental and Behavioral Pediatrics: JDBP*, 34(7), 486–493. <https://doi.org/10.1097/DBP.0b013e31829d211c>

Liu, Y., Kaaya, S., Chai, J., McCoy, D. C., Surkan, P. J., Black, M. M., Sutter-Dallay, A.-L., Verdoux, H., & Smith-Fawzi, M. C. (2017). Maternal depressive symptoms and early childhood cognitive development: A meta-analysis. *Psychological Medicine*, 47(4), 680–689.
<https://doi.org/10.1017/S003329171600283X>

Lysaniuk, B., Ladsous, R., Tabeaud, M., Cottrell, G., Penetier, C., & Garcia, A. (2015). Types of homes and ways of life: A territorial analysis of the environmental determinants that factor into the proliferation of malaria vectors in the rural region of Allada in Benin. *Rural and Remote Health*, 15, 2696.

Mulu, A., Kassu, A., Legesse, M., Erko, B., Nigussie, D., Shimelis, T., Belyhun, Y., Moges, B., Ota, F., & Elias, D. (2014). Helminths and malaria co-infections are associated with elevated serum IgE. *Parasites & Vectors*, 7, 240. <https://doi.org/10.1186/1756-3305-7-240>

Okronipa, H. E. T., Marquis, G. S., Lartey, A., Brakohiapa, L., Perez-Escamilla, R., & Mazur, R. E. (2012). Postnatal Depression Symptoms are Associated with Increased Diarrhea among Infants of HIV-Positive Ghanaian Mothers. *AIDS and Behavior*, 16(8), 2216–2225.
<https://doi.org/10.1007/s10461-012-0153-x>

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Patel, V., Rodrigues, M., & DeSouza, N. (2002). Gender, poverty, and postnatal depression: A study of mothers in Goa, India. *The American Journal of Psychiatry*, *159*(1), 43–47.
<https://doi.org/10.1176/appi.ajp.159.1.43>

Rahman, A., Bunn, J., Lovel, H., & Creed, F. (2007). Maternal depression increases infant risk of diarrhoeal illness: --A cohort study. *Archives of Disease in Childhood*, *92*(1), 24–28.
<https://doi.org/10.1136/adc.2005.086579>

Sawyer, A., Ayers, S., & Smith, H. (2010). Pre- and postnatal psychological wellbeing in Africa: A systematic review. *Journal of Affective Disorders*, *123*(1), 17–29.
<https://doi.org/10.1016/j.jad.2009.06.027>

Schuster, R., Bornovalova, M., & Hunt, E. (2011). The Influence of Depression on the Progression of HIV: Direct and Indirect Effects. *Behavior Modification*.
<https://doi.org/10.1177/0145445511425231>

Stein, A., Gath, D. H., Bucher, J., Bond, A., Day, A., & Cooper, P. J. (1991). The relationship between post-natal depression and mother-child interaction. *The British Journal of Psychiatry: The Journal of Mental Science*, *158*, 46–52. <https://doi.org/10.1192/bjp.158.1.46>

Stewart, D. (2005). Depression during pregnancy. *Canadian Family Physician*, *51*(8), 1061–1063.

ter Kuile, F. O., Terlouw, D. J., Kariuki, S. K., Phillips-Howard, P. A., Mirel, L. B., Hawley, W. A., Friedman, J. F., Shi, Y. P., Kolczak, M. S., Lal, A. A., Vulule, J. M., & Nahlen, B. L. (2003). Impact of permethrin-treated bed nets on malaria, anemia, and growth in infants in an area of intense perennial malaria transmission in western Kenya. *The American Journal of Tropical Medicine and Hygiene*, *68*(4 Suppl), 68–77.

Vaz Nery, S., Pickering, A. J., Abate, E., Asmare, A., Barrett, L., Benjamin-Chung, J., Bundy, D. A. P., Clasen, T., Clements, A. C. A., Colford, J. M., Ercumen, A., Crowley, S., Cumming, O., Freeman, M. C., Haque, R., Mengistu, B., Oswald, W. E., Pullan, R. L., Oliveira, R. G., ... Brooker, S. J. (2019). The role of water, sanitation and hygiene interventions in reducing soil-transmitted

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helminths: Interpreting the evidence and identifying next steps. *Parasites & Vectors*, 12(1), 273.
<https://doi.org/10.1186/s13071-019-3532-6>

Villegas, L., McKay, K., Dennis, C.-L., & Ross, L. E. (2011). Postpartum depression among rural women from developed and developing countries: A systematic review. *The Journal of Rural Health: Official Journal of the American Rural Health Association and the National Rural Health Care Association*, 27(3), 278–288. <https://doi.org/10.1111/j.1748-0361.2010.00339.x>

Weinstock, M. (2005). The potential influence of maternal stress hormones on development and mental health of the offspring. *Brain, Behavior, and Immunity*, 19(4), 296–308.
<https://doi.org/10.1016/j.bbi.2004.09.006>

World Health Organization. (1994). *Bench aids for the diagnosis of intestinal parasites*. World Health Organization. <https://apps.who.int/iris/handle/10665/37323>

World Health Organization. (2012). *Soil-transmitted helminthiases: Eliminating as public health problem soil-transmitted helminthiases in children : progress report 2001-2010 and strategic plan 2011-2020*. World Health Organization. <https://apps.who.int/iris/handle/10665/44804>

World Health Organization. *Maternal mental health*. (n.d.). Retrieved February 27, 2020, from https://www.who.int/mental_health/maternal-child/maternal_mental_health/en/

World Health Organization. (n.d.) *Malaria Fact Sheet* [Fact sheet]. Retrieved May 9, 2019, from <https://www.who.int/news-room/fact-sheets/detail/malaria>

Tables

Table I. Socio-demographic characteristics of mother-child pairs included and excluded from prospective follow-up in TOLIMMUNPAL.

Parameter	Category	Included (N=302) N (%) or mean (SD) ^a	Excluded (N=442) N (%) or mean (SD)	p-value ^b
Maternal education	None	183/302 (60.6)	270/437 (61.8)	0.74
	Some	119/302 (39.4)	167/437 (38.2)	
Maternal age (years)		27.3 (6.1)	26.7 (5.6)	0.28
Maternal Body Mass Index (BMI)	Underweight	19/302 (6.3)	22/329 (6.7)	0.06
	Normal	248/302 (82.1)	247/329 (75.1)	
	Overweight/obese	35/302 (11.6)	60/329 (18.2)	
Family possession score quartile ^c	1 st	89/302 (29.5)	113/437 (25.9)	0.17
	2 nd	97/302 (32.1)	120/437 (27.5)	
	3 rd	93/302 (30.8)	166/437 (38.0)	
	4 th	23/302 (7.6)	38/437 (8.7)	
Household size (number of people)		5.0 (1.6)	4.9 (1.6)	0.60
Marital status	Monogamous	190/300 (63.3)	273/430 (63.5)	0.97
	Polygamous	110/300 (36.7)	157/430 (36.5)	
Parity	Primipara	47/302 (15.6)	69/329 (21.0)	0.08
	Multipara	255/302 (84.4)	260/329 (79.0)	
Child sex	Male	146/302 (48.3)	232/442 (52.5)	0.27
	Female	156/302 (51.7)	210/442 (47.5)	
Birthweight	Normal (≥ 2500 g)	277/300 (92.3)	283/320 (88.4)	0.10
	Low (< 2500 g)	23/300 (7.7)	37/320 (11.6)	
Gestational age (ballard score)		38.0 (2.8)	38.5 (3.5)	0.11
Child iron deficiency	No	157/296 (53.0)	241/366 (65.9)	0.001
	Yes	139/296 (47.0)	125/366 (34.1)	
HOME score		26.6 (2.4)	27.0 (2.2)	0.14
Depressive symptoms (EPDS)	<13	263/302 (87.1)	370/436 (84.9)	0.40
	≥ 13	39/302 (12.9)	66/436 (15.1)	

^a SD=Standard Deviation

^b Fisher-exact test for categorical variables, Wilcoxon rank-sum test for continuous variables

^c 1st quartile refers to group with fewest possessions (i.e. most deprived), 4th quartile refers to group with most possessions (i.e. least deprived)

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Table II. Malaria and soil-transmitted helminth infection in children from 12-24 months of age.

Infection	N (%)
Number of malaria episodes	
None	48/302 (15.9)
1	49/302 (16.2)
2	59/302 (19.5)
3+	146/302 (48.3)
Average number of episodes per child ^a	3 (0-14)
Average parasite density (parasites/ μ L) ^a	100,036 (0-869,071)
Malaria before 12 months	87/302 (29)
Number of helminth infections	
None	200/282 (70.9)
1	72/282 (25.5)
2	10/282 (3.6)
Helminth infection at 12 months	23/223 (10.3)
<i>Ankylostomes</i>	2/23 (8.7)
<i>Ascaris lumbricoides</i>	2/23 (8.7)
<i>Trichuris trichiura</i>	17/23 (73.9)
<i>Schistosoma mansoni</i>	1/23 (4.3)
Other (<i>Enterobius vermicularis</i>)	1/23 (4.3)
Helminth infection at 18 months	39/200 (19.5)
<i>Ankylostomes</i>	8/39 (20.5)
<i>Ascaris lumbricoides</i>	13/39 (33.3)
<i>Trichuris trichiura</i>	20/39 (51.3)
<i>Schistosoma mansoni</i>	0
Other	0
Helminth infection at 24 months	30/197 (15.2)
<i>Ankylostomes</i>	8/30 (26.8)
<i>Ascaris lumbricoides</i>	14/30 (46.8)
<i>Trichuris trichiura</i>	10/30 (33.4)
<i>Schistosoma mansoni</i>	0
Other	0

^a Mean (range)

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Table III. Univariate analyses between potential confounding factors and parasitic infection outcomes.

Variable	Missin g n (%)	Malaria between 12-24 months		Helminth infection between 12-24 months		
		Episodes (median)	P- value ^a	Not Infected n (%)	Infected n (%)	P- value ^b
Maternal age	0		0.32			0.05
<25		2		111 (76)	35 (24)	
>25		3		89 (65)	47 (35)	
Maternal education	0		0.08			0.82
None		3		119 (70)	50 (30)	
Some		2		81 (72)	32 (28)	
Maternal BMI	0		0.81			0.08
Underweight		3		15 (94)	1 (6)	
Normal		2		159 (69)	73 (31)	
Overweight/obese		2		26 (76)	26 (24)	
Family possession score (quartile)	0		0.21			0.55
1 st		2		66 (77)	20 (23)	
2 nd		3		62 (68)	29 (32)	
3 rd		2		28 (70)	12 (30)	
4 th		2		44 (68)	21 (32)	
Household size	0		0.43			0.08
<5		2.5		132 (75)	45 (25)	
>5		2		68 (65)	37 (35)	
Marital status	0		0.33			0.89
Monogamous		3		75 (71)	30 (29)	
Polygamous		2		125 (71)	52 (29)	
Parity	0		0.26			0.01
Primipara		2		38 (86)	6 (14)	
Multipara		3		162 (68)	76 (32)	
Child sex	0		0.86			0.70
Male		2		95 (70)	41 (30)	
Female		3		105 (72)	41 (28)	
Birthweight	2 (<1)		0.004			0.36
Normal (≥2500 g)		2		183 (70)	77 (30)	
Low (<2500 g)		1		16 (80)	4 (20)	
Gestational age			0.79			0.46
Term (≥37 weeks)		2		177 (70)	75 (30)	
Preterm (<37 weeks)		3		23 (77)	7 (23)	
Iron deficient	6 (2)		0.70			0.55
No		3		108 (73)	40 (27)	
Yes		2		92 (70)	40 (30)	

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Environmental risk ^c	5 (2)	0.12	0.04	--	--	--
Mosquito net use	19 (6)		0.85			--
<i>Never/Occasionally</i>		3			--	
<i>Frequently</i>		2			--	
<i>Always</i>		3			--	
Malaria before 1 year			0.01			--
<i>No</i>		2			--	
<i>Yes</i>		3			--	
Hygiene rating	54 (18)		--			0.78
<i>Inferior</i>		--		27 (73)	10 (27)	
<i>Average</i>		--		73 (68)	35 (32)	
<i>Superior</i>		--		64 (67)	32 (33)	

^a P-value of Wilcoxon rank-sum test (for two-category variables) or Kruskal-Wallis test (for more-than-two-category variables)

^b P-value of chi-squared test

^c Spearman's rank correlation coefficient for two continuous variables

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Table IV. Associations between maternal depression and parent-child interactions one-year post-partum.

	Maternal depression	
	<i>Model 1^a</i> N=302	<i>Model 2^b</i> N=302
HOME total score	0.98 (0.96, 0.99)	0.97 (0.95, 0.99)
Responsivity	0.99 (0.95, 1.02)	0.97 (0.93, 1.01)
Acceptance	0.91 (0.85, 0.99)	0.92 (0.85, 0.99)
Organization	1.03 (0.95, 1.11)	1.02 (0.94, 1.10)
Learning Materials	1.02 (0.97, 1.07)	1.01 (0.95, 1.07)
Involvement	0.82 (0.77, 0.87)	0.82 (0.77, 0.88)
Variety	0.93 (0.88, 0.98)	0.93 (0.88, 0.99)

^a Crude RR (95% CI)

^b Adjusted RR (95% CI) for socio-economic variables: family possession score, maternal education, marital status

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Table V. Associations between maternal depression and parent-child interactions exposures and child parasitic infection outcomes.

	Maternal depression RR (95% CI)	Parent-child interactions RR (95% CI)
Total malaria episodes		
Model 1 ^a	1.00 (0.97, 1.03)	0.97 (0.94, 1.01)
Model 2 ^b	1.00 (0.98, 1.03)	0.98 (0.94, 1.02)
Soil-transmitted helminth infection		
Model 1 ^c	0.99 (0.95, 1.04)	1.05 (0.96, 1.15)
Model 2 ^d	1.00 (0.95, 1.05)	1.02 (0.93, 1.12)

^a Crude model; N=302

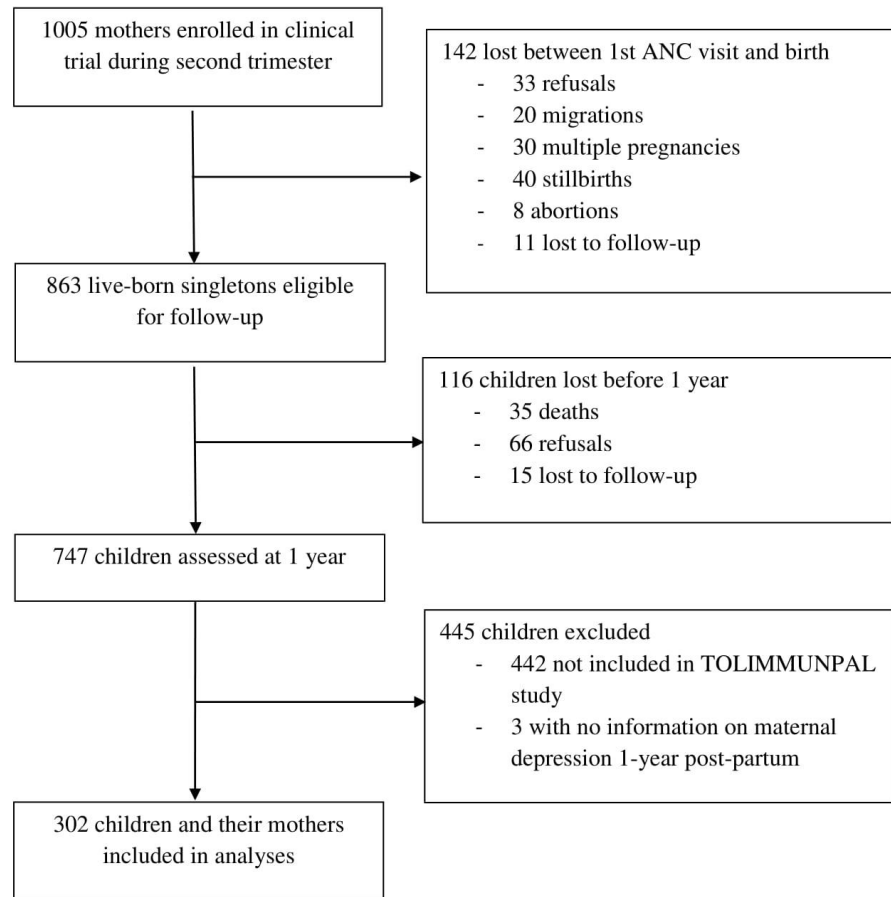
^b Adjusted for maternal education, family possession score, household size, environmental risk, low birthweight, mosquito-net use, and history of malaria; N=281

^c Crude model; N=282

^d Adjusted for maternal age and hygiene rating; N=250

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Figure Captions

Figure 1. Population flowchart of follow-up from pregnancy to one year of age in children.

Chapter 6. Blood lead level and risk of malaria infection

In this chapter, I present the fourth article of this thesis investigating the potential confounding role of blood lead levels from environmental exposure on risk of malaria infection. Previous work by Moya-Alvarez et al. (2016) demonstrated an inverse relationship between elevated blood lead levels ($\geq 50 \mu\text{g/L}$) and malaria risk in a sub-cohort of 203 12-month-old Beninese children in our population. We therefore sought to investigate whether or not this relationship would continue if the prospective follow-up of these same children from 12-24 months of age was taken into account, thus offering stronger evidence of this association. However, our analyses revealed that elevated blood lead levels in children at 12 months of age did not impact subsequent risk of malaria infection in a prospective follow-up from 12-24 months. While these analyses were completed within a sub-cohort of children for whom information on lead exposure and malaria infection was available, this study aided our overall understanding of risk factors for parasitic infections and provided evidence that the previously hypothesized inverse effect of lead exposure on malaria risk should not be taken into account in models investigating the relationship between parasitic infection in pregnancy and child development, even given the strong evidence linking lead exposure to impaired child development (254). This paper was published in *Plos One* as an original research article.

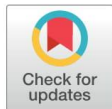
RESEARCH ARTICLE

Blood lead level in infants and subsequent risk of malaria: A prospective cohort study in Benin, Sub-Saharan Africa

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OPEN ACCESS

Citation: Garrison A, Khoshnood B, Courtin D, Milet J, Garcia A, Massougbojji A, et al. (2019) Blood lead level in infants and subsequent risk of malaria: A prospective cohort study in Benin, Sub-Saharan Africa. PLoS ONE 14(7): e0220023. <https://doi.org/10.1371/journal.pone.0220023>

Editor: Luzia Helena Carvalho, Instituto Rene Rachou, BRAZIL

Received: April 19, 2019

Accepted: July 8, 2019

Published: July 18, 2019

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Data Availability Statement: Relevant data on the TOVI and TOLIMMUNPAL studies are available as Supporting Information files. Data from the MiPPAD and APEC studies cannot be made available due to ethical and consent form restrictions. Access to data from the MiPPAD study may be available upon request through The MiPPAD Executive Committee (Dr. Raquel Gonzalez at +34-932-275-400 or raquel.gonzalez@crefib.cat).

Abstract

Lead and malaria both present significant health risks to children in Sub-Saharan Africa. Previous studies have shown that high blood lead levels in children act as a protective factor against subsequent malaria incidence. The main objective of this study was to investigate associations between blood lead level and malaria outcomes prospectively in Beninese children from 12 to 24 months of age. Two-hundred and four children were assessed for lead at 12 months and closely followed until 24 months for malaria; when symptoms and parasite density were also recorded. Univariate and multivariate negative binomial and linear regression models tested associations between blood lead level quartile and total episodes of malaria (total symptomatic and asymptomatic episodes) and parasite density, respectively. Median blood lead level among children measured at 12 months was 56.50 (4.81–578) µg/L. During the 12-month follow-up, 172 (84.31%) children had at least one malaria episode. Univariate and multivariate negative binomial and linear regressions did not reveal significant associations between blood lead level quartile and malaria outcomes. Iron deficiency was not found to be an effect modifier. Results from this prospective child-cohort study investigating associations between blood lead level and malaria did not confirm results from previous cross-sectional studies. Further research is needed to further explore this relationship and other co-morbidities due to malaria and lead.

Introduction

Lead (Pb), a toxic heavy metal with a blood half-life time between 30–40 days [1][2], is found in gasoline, paint, contaminated soil, ammunition, and water pipes [3] and can have permanent, harmful effects on the human body if ingested or inhaled [4]. Elevated levels of lead

Funding: The Eunice Kennedy Shriver National Institute of Child Health & Human Development (NIH/NICHD) funded the TOVI study, grant R21-HD060524. The MiPPAD trial (NCT00811421) was co-funded by the European and Developing Countries Clinical Trials Partnership (EDCTP-IP.07.31080.002). The “Anaemia in Pregnancy: Etiologies and Consequences” (APEC) study received funding from a grant from the Bill and Melinda Gates Foundation. The TOLIMMUNPAL project was funded by the French ANR (Agence Nationale de la Recherche) and coordinated by Dr André Garcia (IRD, UMR 216). Amanda Garrison received funding from PRINCEPS (Université Sorbonne Paris Cité) during the writing of this manuscript. 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 that no competing interests exist.

exposure (defined by the Centers for Disease Control (CDC) as a blood lead level of 50 µg/L or more [5]) have specific consequences on child health, including decreased Intelligence Quotient (IQ), decreased attention span, and increased antisocial behaviors [6][7][8]. Impacts of lead on child health and development are particularly pertinent in developing countries, such as Benin, where exposure to lead is still relatively high. A cohort study involving children and their mothers in Benin, Sub-Saharan Africa in 2016 identified high levels of lead exposure in the population, with 58% of children having blood lead levels greater than 50µg/L [9].

Malaria also presents significant health risks for children in Benin, where malaria caused primarily by the *Plasmodium falciparum* parasite is the leading cause of death in children under five years of age in the country and the main cause of morbidity in adults [10]. The incidence rate of malaria in Benin is 293 cases per 1,000 population at risk, with 100% of the population considered at risk of developing malaria [11]. In areas of the world with high transmission of malaria, exposed children develop an acquired clinical immunity after repeated infections and tend to exhibit more asymptomatic than symptomatic malaria episodes over time [12]. With children at higher risk of death from malaria in Benin, it is important to identify specific risk factors and potential co-exposures present in their environments, such as lead and other heavy metals that may impact their risk of disease.

Thus far, only cross-sectional investigations of associations between blood lead level and malaria have been conducted in child populations, one involving 653 Nigerian children ages 2 to 9 years [13] and one involving 203 children in Benin at 12 months of age [14]. Both studies observed significant negative associations between high blood lead levels and malaria risk in children. Based on these previous studies, we identified the need for a prospective analysis of associations between lead and malaria, while taking into consideration iron deficiency as a potential effect modifier. We also sought to investigate symptomatic and asymptomatic malaria episodes and parasite density in children as secondary outcomes.

The aim of our study was to analyze data in a cohort of Beninese children followed between the ages of 12 to 24 months in order to study the associations between blood lead levels taken between 10 to 13 months of age and subsequent malaria episodes up to 24 months of age. We hypothesized that children with high blood lead level (in higher blood lead level quartiles) at 12 months of age would be associated with fewer number of subsequent malaria episodes and lower parasite density between ages 12 to 24 months in Benin compared to children within the lowest blood lead level quartile.

Methods

Study population

This was an observational, prospective cohort study following children born to women enrolled in a randomized clinical trial (Malaria in Pregnancy Preventive Alternative Drugs, MiPPAD, NCT00811421) investigating two intervention therapies for malaria during pregnancy in the semi-rural area of Allada, 40 kilometers north of Cotonou, in Benin, Sub-Saharan Africa. Study protocol and inclusion criteria of participants for the clinical trial is explained elsewhere [15]. All singleton children born to mothers from the MiPPAD trial were invited to participate in a subsequent cross-sectional study (TOVI) at 12 months of age [16]. A sub-cohort of these children was then followed closely to measure malaria incidence and parasite density between 12 to 24 months of age within the TOLIMMUNPAL study. Participating children in our analyses included those who met the following inclusion criteria: had mothers who met the original inclusion criteria for the MiPPAD clinical trial, were assessed for blood lead level within the TOVI study between 10 to 13 months of age, and were followed for malaria incidence within the TOLIMMUNPAL study from 12 to 24 months of age.

Exposure of interest

Blood lead level, the primary exposure of interest in this study, was measured once in children ages 10–13 months within the TOVI study. Eight mL of venous blood was collected from each child and 4mL put into a tube with dipotassium EDTA. An aliquot of EDTA blood was diluted 20-fold in ammonia 0.5% v/v and 0.1% v/v surfactant Triton-X and analyzed by inductively coupled plasma mass spectrometry (ICP-MS; Perkin Elmer Sciex Elan DRC II ICP-MS instrument) at the Centre de Toxicologie, Institut National de Santé Publique du Québec (Québec, Canada). The limit of detection for blood lead analysis was 0.2 µg/L [9]. Blood lead level was analyzed in quartiles due to the non-linear relationship between lead and malaria and in order to better interpret results.

Outcomes of interest

There were two primary outcomes of interest within this study, malaria and parasite density in children. Malaria was diagnosed in children from 12 to 24 months of age through a positive thick blood smear test and/or rapid diagnostic test (RDT) within the TOLIMMUNPAL study. A positive thick blood smear test, the gold standard of malaria diagnosis, involves the examination of blood samples using microscopy in order to diagnosis the presence of parasites [17,18]. Pan/Pf RDT (Parascreen), able to give results within 15 minutes of administration, identifies the presence of *Plasmodium*-specific histidine-rich protein-2 produced in the infected human blood [19]. Each month during the 12-month follow-up, participating children were visited by a nurse, who performed a thick blood smear test to diagnose malaria, regardless of symptoms, and an RDT if they presented with fever (temperature equal to or greater than 37.5°C). A nurse also visited children at home every 15 days, during which time a thick blood smear test and an RDT were administered if children had fever or history of fever in the previous 24 hours. Additionally, children and their families had access to free emergency care and were able to attend the clinic if symptoms occurred. During these visits, a thick blood smear test and an RDT were administered. Symptomatic malaria was defined as a positive thick blood smear and/or RDT and the presence of fever within three days of diagnosis. Parasite density (in parasites/µL) was measured in children positively diagnosed with malaria by a thick blood smear test, through the use of a multiplication factor applied to the average parasitemia/field [14].

Malaria was analyzed as the total number of malaria episodes, total number of symptomatic episodes, and total number of asymptomatic episodes during the 12-month follow-up. Parasite density was log-transformed and analyzed as mean logarithm parasite density per child.

Potential confounders

Potential confounders included in adjusted models were socioeconomic status (SES), maternal education, iron deficiency, mosquito-net use, malaria status before 12 months, maternity ward location, and environmental risk of infection. Information regarding maternal education, mosquito-net use, and maternity ward were collected via questionnaire given to mothers during follow-up. Serum ferritin concentrations were measured in children using an AxSym Immuno-Assay Analyzer (Abbott Laboratories, Abbott Park, IL) with a sample of 500 µL of serum. Iron deficiency was defined as a serum ferritin concentration less than 12 µg/L or as serum ferritin concentration of 12 to 70 µg/L in the presence of inflammation (CRP concentration > 5mg/L) [20]. In addition to being considered as a potential confounding factor, iron deficiency was also tested as an effect modifier. Malaria status before 12 months was defined as having at least one malaria episode before the lead assessment at 12 months of age.

TOLIMMUNPAL researchers collected information on mosquito density, as well as environmental (rainfall, soil type, nearby water sources, vegetation index) and biological data (number of inhabitants per room in household, use of bed nets and/or insecticides) in order to calculate a time- and space-dependent environmental risk of infection quantifying each child's exposure to malaria vectors using a predictive model [21].

Statistical analysis

Univariate analyses of associations between the primary exposure of interest, blood lead level, the primary outcomes of interest, total malaria episodes and mean logarithm parasite density, and potential confounders were performed. Confounders found to be associated to the primary exposure and/or outcomes ($p < 0.20$) were kept in final, adjusted models. Multivariate negative binomial regression models were run to examine associations between blood lead level and number of malaria episodes, including number of symptomatic and asymptomatic malaria episodes separately. Linear regression models tested associations between blood lead level and mean logarithm parasite density measured in children.

Additionally, characteristics of children lost to follow-up (i.e. not present at 24 months) were compared to those present for the entire follow-up using t-tests, Wilcoxon-rank sum tests, and Fisher exact tests when appropriate. Two sensitivity analyses were conducted: (1) regression analyses after removal of children potentially exposed to lead through contaminated paint chips, and (2) regression analyses including only malaria episodes diagnosed within first 6 months after lead assessment, due to the 30-day half life of lead. Statistical analyses were completed using STATA 14.2 (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP) for Windows.

Ethical consideration

All studies from which data were used for the purpose of this study were approved by the following bodies: the Hospital Clinic of Barcelona (Spain), the Comité Consultatif de Déontologie et d'Éthique of the Institut de Recherche pour le Développement (France) [15], the University of Abomey-Calavi in Benin and New York University in the United States (IRB#09–1253) [9], and the Beninese Ethical Committee of the Faculté des Sciences de la Santé (FSS).

Results

The follow-up of children prior to and during the TOLIMMUNPAL study is illustrated in Fig 1. Of the 204 children eligible for our study for which data was available on lead assessment at 12 months of age and malaria follow-up, 170 (83.33%) were present at 24 months of age. All regression analyses included all children and took into account the differing follow-up times of children during the 12-month period.

Demographic and clinical characteristics of children are shown in Table 1. Median blood lead level among children was 56.50 (± 56.83) $\mu\text{g/L}$. During follow-up, 172 (84.31%) children had at least one malaria episode, with 40 (19.61%) having one episode, 41 (20.10%) having two episodes, and 91 (44.61%) having more than two malaria episodes during the 12 months. Of all occurring malaria episodes, 69.45% of them were symptomatic episodes. Median parasite density per child was 1,674.40 ($\pm 6,396.53$) parasites/ μL .

Trends in malaria incidence and prevalence and parasite density were examined in monthly intervals during the follow-up period to further describe our population (Fig 2). At 12 months of age, 19% of children were positively diagnosed with malaria, 12% had symptomatic malaria and 9% had asymptomatic malaria. After 12 months, malaria incidence decreased slightly each month until 20 months of age when incidence increased. By the end of the study period,

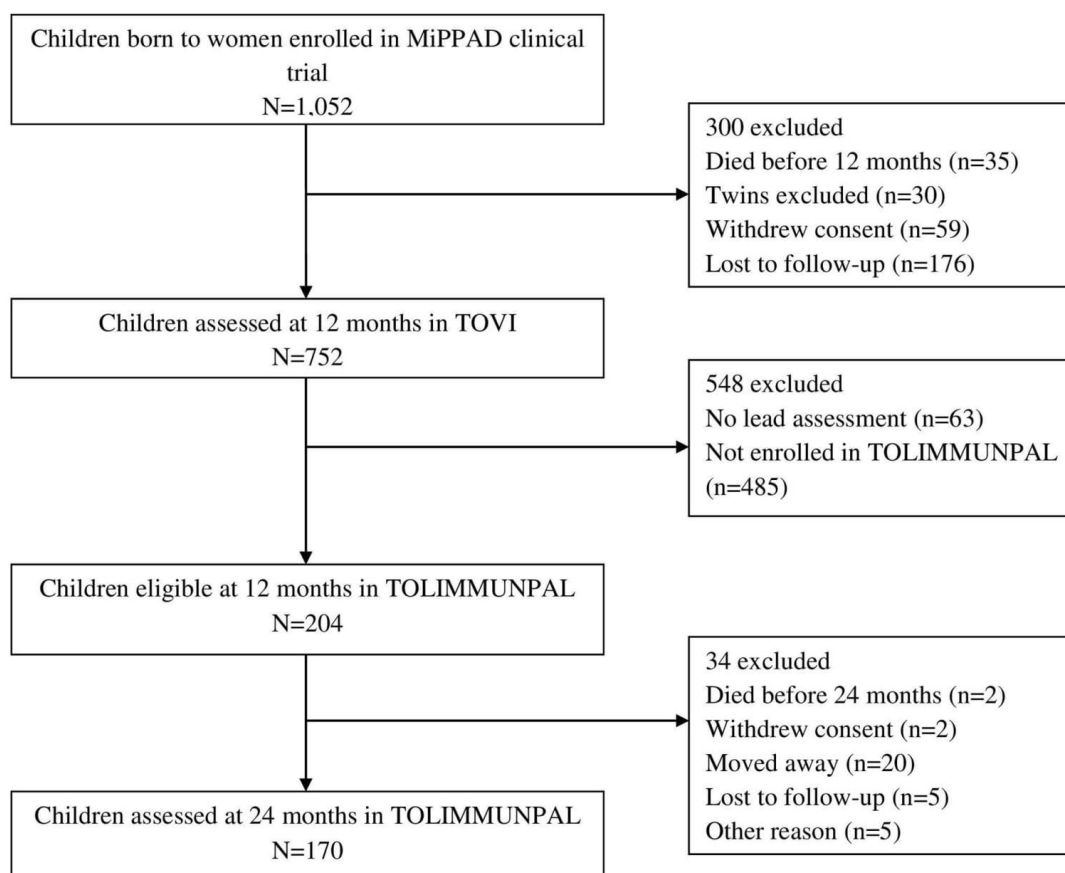


Fig 1. Population follow-up. Follow-up of participating children from birth to 24 months of age.

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roughly 29% of children were diagnosed with malaria; with 16% having symptomatic malaria and 12% having asymptomatic malaria. Mean parasite density in children was highest at 12 months (8,148 parasites/ μ L), although malaria prevalence was relatively low at this age.

Univariate analyses were carried out between potential confounders and outcome variables (total malaria episodes and parasite density) and kept in final models if found significant ($p < 0.20$) (Tables 2 and 3). No significant associations were found in univariate analyses between blood lead level quartile and any malaria outcomes. Children born to mothers with a primary education were found to have lower incidence rate ratio of total symptomatic episodes [0.81 (0.65, 0.99)] and lower mean logarithm parasite density [-0.15 (-0.30, -0.01)] compared to children born to mothers without at least a primary education. Increased environmental risk was also found to be significantly associated to higher incidence rate ratios for total malaria episodes [2.89 (1.44, 5.80)], total symptomatic episodes [1.95 (1.07, 3.54)], and higher

Table 1. Demographic and clinical characteristics of participating children (N = 204).

Parameter	Category	Mean (\pm SD) ¹ or n (%)
Sex	Female	104 (50.98%)
	Male	100 (49.02%)
Age at lead assessment (months)		11.98 (\pm 0.36)
Blood lead level (μ g/L)		56.50 (\pm 56.83)
Ferritin concentration (μ g/L)		20.15 (\pm 98.73)
Inflammation (CRP > 5mg/L)	Yes	99 (48.53%)
	No	105 (51.47%)
Iron deficiency (ferritin < 12 μ g/L or 12–70 μ g/L if CRP > 5mg/L)	Yes	95 (47.98%)
	No	103 (52.02%)
Socioeconomic status	Lowest	88 (44%)
	Medium	67 (33.50%)
	Highest	45 (22.50%)
Maternal education	Some	86 (42.80%)
	None	115 (57.20%)
Maternity ward location	Attogon	51 (25.37%)
	Sékou	150 (74.63%)
Use of mosquito nets in house	Rare	4 (2.12%)
	Occasional	15 (7.94%)
	Frequent	24 (12.70%)
	Always	146 (77.25%)
Environmental risk		0.04 (\pm 0.17)
Malaria status before 12 months	No malaria before 12 months	152 (74.51%)
	At least one malaria episode before 12 months	52 (25.49%)
Malaria status of children during follow-up	No malaria	32 (15.69%)
	One episode	40 (19.61%)
	Two episodes	41 (20.10%)
	More than two episodes	91 (44.61%)
Total malaria episodes	Symptomatic	407 (69.45%)
	Asymptomatic	179 (30.55%)
Parasite density per child (parasites/ μ L)		1,674 (\pm 6,396)

¹Median (\pm SD) shown for variables with skewed distribution.

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mean logarithm parasite density [0.53 (0.09, 0.97)]. Children examined at the maternity ward in Sékou were also found to have significantly higher incidence rate ratios of total malaria

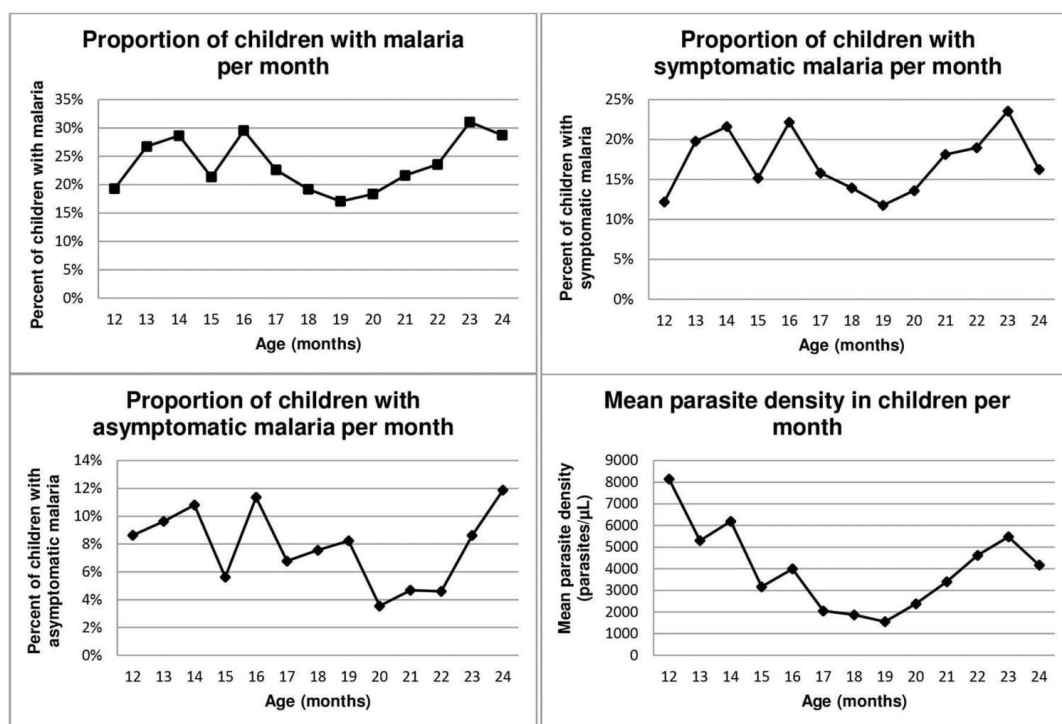


Fig 2. Proportion of children diagnosed with malaria and parasite densities each month of follow-up.

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episodes [1.36 (1.03, 1.81)], total symptomatic episodes [1.37 (1.05, 1.78)], and higher mean logarithm parasite density [0.17 (0.01, 0.34)]. Neither socioeconomic status nor iron deficiency were found to be significantly associated to either outcome, but were kept in final models due to their previously reported associations with malaria. Iron deficiency was also found to be strongly positively associated with lead ($p < 0.01$) in univariate analyses.

Multivariate negative binomial regression models were carried out between blood lead level quartile and total number of malaria episodes, total number of symptomatic episodes, and total number of asymptomatic episodes while adjusting for potential confounding factors (Table 4). Higher blood lead level quartiles were not found to be significantly associated with any outcome in multivariate analyses, as hypothesized, although children in the 4th (highest) blood lead level quartile had the lowest incidence rate ratios for total malaria episodes [0.94 (0.68, 1.30)], total symptomatic episodes [0.92 (0.68, 1.24)], and total asymptomatic episodes [0.62 (0.29, 1.31)] compared to children in the 1st (lowest) quartile.

Multivariate linear regression analyses between blood lead level quartile and mean logarithm parasite density found reduced coefficient estimates for parasite density in children within the 2nd [-0.02 (-0.18, 0.13)], 3rd [-0.03 (-0.19, 0.13)], and 4th [-0.04 (-0.20, 0.12)] quartiles, however no associations were found to be statistically significant (Table 5).

Table 2. Univariate negative binomial regression between potential confounders and malaria outcomes[†].

Parameter	Category	Total malaria episodes IRR [‡] (95% CI)	P-value	Total symptomatic episodes IRR (95% CI)	P-value	Total asymptomatic episodes IRR (95% CI)	P-value
Blood lead level quartile	1 st [Ref]	1	0.80	1	0.62	1	0.86
	2 nd	0.89 (0.65, 1.24)		1.00 (0.75, 1.33)		0.85 (0.42, 1.70)	
	3 rd	0.99 (0.71, 1.36)		1.06 (0.80, 1.41)		0.80 (0.40, 1.59)	
	4 th	0.87 (0.62, 1.22)		0.88 (0.66, 1.19)		0.69 (0.34, 1.41)	
Iron deficient	No [Ref]	1	0.80	1	0.99	1	0.41
	Yes	1.03 (0.82, 1.30)		0.99 (0.81, 1.22)		1.23 (0.75, 2.03)	
Maternal education	None [Ref]	1	0.09	1	<0.01	1	0.71
	Some	0.81 (0.64, 1.03)		0.81 (0.65, 0.99)		1.21 (0.74, 2.00)	
Socioeconomic status quartile	1 st [Ref]	1	0.47	1	0.71	1	0.48
	2 nd	0.89 (0.66, 1.19)		0.94 (0.72, 1.22)		0.80 (0.43, 1.49)	
	3 rd	0.76 (0.54, 1.11)		0.86 (0.62, 1.19)		0.69 (0.32, 1.50)	
	4 th	0.81 (0.58, 1.12)		1.00 (0.75, 1.33)		0.63 (0.31, 1.29)	
Mosquito Net Use	Rarely [Ref]	1	0.30	1	0.25	1	0.39
	Occasionally	0.59 (0.27, 1.29)		0.88 (0.46, 1.71)		0.22 (0.04, 1.18)	
	Frequently	0.66 (0.31, 1.42)		0.82 (0.43, 1.56)		0.41 (0.09, 1.94)	
	Always	0.55 (0.28, 1.10)		0.76 (0.42, 1.36)		0.44 (0.11, 1.80)	
Malaria before 12 months	No [Ref]	1	0.56	1	0.50	1	0.64
	Yes	1.08 (0.83, 1.40)		0.98 (0.78, 1.23)		1.03 (0.59, 1.79)	
Environmental risk		2.89 (1.44, 5.80)	<0.01	1.95 (1.07, 3.54)	<0.01	2.78 (0.58, 13.36)	0.13
Maternity ward	Attogon [Ref]	1	0.03	1	<0.01	1	0.79
	Sékou	1.36 (1.03, 1.81)		1.37 (1.05, 1.78)		1.02 (0.56, 1.85)	

[†]Sample sizes for children differ among characteristics due to missing values.

[‡]IRR = Incidence Rate Ratio

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Secondary analyses were stratified by iron deficiency and an interaction term for iron deficiency was tested for significance to reveal potential effect modification; however, no significant effect modification was identified and the interaction term was not kept in final, multivariate models.

Sensitivity analysis removing children with potential acute post-natal exposure to lead through ingestion of fallen paint chips (n = 23) did not reveal significantly different associations between blood lead level quartile and malaria outcomes in negative binomial and linear regression models (S2 Table). Sensitivity analyses including only malaria episodes that occurred in the 6 months followed lead assessment (ages 12–18 months) were conducted (S3 Table). While these analyses revealed lower effect estimates in associations between blood lead level and malaria outcomes, results were not statistically different from analyses including all malaria outcomes from the 12-month study period.

Table 3. Univariate linear regression between potential confounders and mean logarithm parasite density[†].

Parameter	Category	Parasite density (mean logarithm) Coefficient (95% CI)	P-value
Blood lead level quartile	1 st [Ref]	0	0.25
	2 nd	-0.19 (-0.39, 0.02)	
	3 rd	-0.16 (-0.37, 0.05)	
	4 th	-0.18 (-0.39, 0.03)	
Iron deficient	No [Ref]	0	0.45
	Yes	-0.06 (-0.20, 0.09)	
Maternal education	None [Ref]	0	0.04
	Some	-0.15 (-0.30, -0.01)	
Socioeconomic status quartile	1 st [Ref]	0	0.23
	2 nd	-0.08 (-0.27, 1.11)	
	3 rd	-0.20 (-0.42, 0.02)	
	4 th	-0.17 (-0.38, 0.03)	
Mosquito Net Use	Rarely [Ref]	0	0.15
	Occasionally	-0.32 (-0.76, 0.11)	
	Frequently	-0.14 (-0.55, 0.28)	
	Always	-0.29 (-0.68, 0.10)	
Malaria before 12 months	No [Ref]	0	0.15
	Yes	0.12 (-0.04, 0.29)	
Environmental risk		0.53 (0.09, 0.97)	0.02
Maternity ward	Attogon [Ref]	0	0.05
	Sékou	0.17 (0.01, 0.34)	

[†]Sample sizes for children differ among characteristics due to missing values.

<https://doi.org/10.1371/journal.pone.0220023.t003>

Discussion

Children in higher blood lead level quartiles were not found to have significantly lower rates of malaria or lower parasite densities compared to children in the lowest blood lead level quartile, as hypothesized. Our prospective findings do not support the cross-sectional analyses by

Table 4. Multivariate negative binomial regression models for associations between blood lead level quartile and malaria outcomes[†].

Blood lead level quartile	Total malaria episodes (N = 183) IRR (95% CI)	P-value	Total symptomatic episodes (N = 166) IRR (95% CI)	P-value	Total asymptomatic episodes (N = 166) IRR (95% CI)	P-value
1 st [Ref]	1		1		1	
2 nd	1.00 (0.73, 1.36)	1.00	1.08 (0.81, 1.43)	0.60	0.78 (0.38, 1.57)	0.51
3 rd	1.02 (0.74, 1.40)	0.92	1.06 (0.79, 1.40)	0.42	0.78 (0.38, 1.63)	0.68
4 th	0.94 (0.68, 1.30)	0.70	0.92 (0.68, 1.24)	0.82	0.62 (0.29, 1.31)	0.29

[†]Adjusted for iron deficiency, maternal education, socioeconomic status, mosquito net use, environmental risk, and maternity ward location

<https://doi.org/10.1371/journal.pone.0220023.t004>

Table 5. Multivariate linear regression for associations between blood lead level quartile and mean logarithm parasite density[†].

Blood lead level quartile	Parasite density (mean logarithm) (N = 183) Coefficient (95% CI)	P-value
1 st [Ref]	0	
2 nd	-0.02 (-0.18, 0.13)	0.76
3 rd	-0.03 (-0.19, 0.13)	0.69
4 th	-0.04 (-0.20, 0.12)	0.65

[†]Adjusted for iron deficiency, maternal education, socioeconomic status, mosquito net use, environmental risk, malaria status before 12 months, and maternity ward location.

<https://doi.org/10.1371/journal.pone.0220023.t005>

Moya-Alvarez et al (2016) which found significant negative associations between elevated blood lead level and malaria incidence and parasite density at 12 months of age in Beninese children.

The ingestion of lead-based paint chips by children is an acute post-natal exposure pathway that varies with age when children begin to exhibit hand-to-mouth behaviors and are more likely to pick up contaminated paint chips off the ground in their homes and ingest them [22]. For this reason, sensitivity analyses were conducted excluding these children exposed to lead via paint chips (n = 23), as this type of exposure could have led to higher and more variable blood lead levels during the 12-month follow-up, thus potentially under-estimating the level of lead exposure after 12 months in our population. However, these sensitivity analyses did not reveal different results when excluding these children.

Limitations of this study include the small sample size, missing information for certain confounding factors which resulted in the loss of these children in analyses, and the fact that blood lead level was measured only once in children at the onset of the study period. Fluctuations in blood lead levels could have occurred, however since they were not measured at multiple intervals during the 12-month study period, it is difficult to ascertain their exact fluctuations and effects on malaria outcomes over time. The half-life of lead in blood is estimated between 30–40 days, although lead accumulated in bone may have a half-life of several decades [2] which can act as a source of lead transfer from mother to fetus during pregnancy [23]. Sensitivity analyses were conducted to include only malaria episodes and parasite densities measured in the first 6 months after lead assessment to account for blood-lead half-life; however these analyses did not reveal significantly different results from analyses including all malaria outcomes during the entire follow-up. Demographic characteristics of children present and absent at 24 months of age were compared to assess any potential differences in these populations (S1 Table). Median blood lead level was significantly higher in children lost to follow-up (68.55 µg/L [17.00–260.00]) compared to those not lost (45.20 µg/L [36.00–73.4]). Use of mosquito nets in households was also significantly different between children present and absent at the final visit (p<0.04), with children absent at the 24-month visit less likely to have always used mosquito nets during the follow-up, compared to children present at the final visit. Therefore, children lost to follow-up were more exposed and more at risk of developing malaria, meaning that analyses within this paper potentially underestimate associations between blood lead level and subsequent malaria episodes in our population.

Based on the strong negative associations found between blood lead level and malaria, including parasite density, in this population of children cross-sectionally at 12 months of age [14], we expected to see similar results over a prospective period of time. However, lead no longer seemed to have a significant protective impact on malaria incidence after 12 months of age

in children. Numerous studies within malaria-endemic regions of the world have exhibited identified iron deficiency as a protective factor against malaria [24–26]. However, we found no significant associations between iron deficiency status in children and malaria outcomes in univariate analyses to support these previous findings. These results could be due to the nature of the prospective follow-up of this study, during which time children received iron supplements to treat iron-deficiency anemia. Iron deficiency was only assessed at 12 months of age. In total, follow-up in the study may have resulted in lowering iron deficiency and malaria as explained earlier between 12 and 24 months of age.

The mechanism between blood lead level and malaria is not well known, and currently very little literature exists on this topic. Nriagu et al proposed three biologically plausible potential mechanisms to explain their observed negative associations between blood lead level and malaria in children (2008). Lead, which is absorbed and stored within red blood cells (RBCs), could inhibit the life cycle of the *Plasmodium* parasite which lives and replicates within RBCs as well. Lead poisoning has been shown to inhibit protein synthesis and certain cellular processes [27,28], and its accumulation in RBCs could lead to poor utilization of iron by *Plasmodium* during development [29]. Another hypothesis is that the phenotype-specific immune response to lead poisoning in the body, which leads to the production of T-helper 2 (Th2) cells, may contribute to a T-helper 1 (Th1)/Th2 balance and thus promote protection against malaria [30,31]. Iron deficiency could also play a role in the mechanism between lead and malaria, as studies have shown iron deficiency to be associated with increased lead uptake [29] and lower risk of malaria in high-transmission areas [25,32]. Our analyses attempted to explore this potential mechanism by testing iron deficiency as an effect modifier in analyses, however iron deficiency was not found to be significant and thus the association was not further investigated.

Between 6–12 months of age, children are no longer protected by immunity passed on to them through their mothers' antibodies and they begin experiencing their first malaria episodes. Malaria incidence fluctuated from month to month during the follow-up period as children were infected with malaria and were subsequently treated within the confines of the study. Of interest is the observation that mean parasite density in children was highest at 12 months, although malaria prevalence was relatively low at this point (18%). As children were more actively screened at and after 12 months and treated for malaria if positively diagnosed, *Plasmodium* concentrations generally declined in children after 12 months. Subsequently, the proportion of children with malaria decreased. This may be due to a large proportion of children who were treated after active screening around 12 months of age. Towards the end of the study period, the incidence of asymptomatic malaria episodes increased and the incidence of symptomatic episodes decreased between 23–24 months of age, which reflects the acquired immunity typically observed in children born into high malaria transmission settings. Therefore, malaria between 12 and 24 months was highly affected by the nature of the follow-up for this study, which potentially biases results found within our analyses towards the null. This may explain why this present study with a longitudinal follow-up does not confirm results found at 12 months of age.

The high blood lead levels measured within this population of infants make a case for the need for more efficient lead exposure identification and control in Benin. Iron deficiency leads to increased lead absorption, as indicated in the literature and in our study, further emphasizing the need for effective strategies to prevent and treat iron deficiency in vulnerable populations. Many populations in Sub-Saharan Africa are exposed to several co-morbidities, such as anemia, lead poisoning, malaria, helminthes, and malnutrition. These co-morbidities should be more closely evaluated and their impacts on one another investigated in order to estimate

the true burden of disease in these nations and improve existing prevention and treatment strategies.

Conclusions

In conclusion, our prospective study does not confirm previous cross-sectional findings on associations between blood lead level and malaria in children. Iron deficiency is shown to be strongly positively correlated to increased lead absorption, suggesting that prevention and treatment of iron deficiency should be in accordance with strategies to reduce lead exposure in vulnerable populations. Malaria prevalence remains high in Benin, with the highest mortality rates in children under 5 years of age. Due to the numerous co-morbidities faced by populations in developing countries, further research should investigate interactions between these co-morbidities and further explore the existing mechanisms between lead and malaria in order to improve prevention and treatment strategies.

Supporting information

S1 Table. Demographic characteristics of children present and absent at the final systematic visit at 24 months of age.

(DOCX)

S2 Table. (A) Multivariate negative binomial regression results excluding children potentially exposed to lead by ingestion of fallen paint chips. (B) Multivariate linear regression results excluding children potentially exposed to lead by ingestion of fallen paint chips.

(DOCX)

S3 Table. (A) Multivariate negative binomial regression results for first 6 months after lead assessment. (B) Multivariate linear regression results for first 6 months after lead assessment.

(DOCX)

S1 File. TOVI database used in analyses.

(PDF)

S2 File. TOLIMMUNPAL database used in analyses.

(PDF)

Acknowledgments

We thank all mothers and children who participated in this study, as well as the staff of the three health centers in Benin (Allada, Attogon, Sékou).

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References

1. Mason LH, Harp JP, Han DY. Pb Neurotoxicity: Neuropsychological Effects of Lead Toxicity [Internet]. BioMed Research International. 2014 [cited 2018 Nov 14]. Available from: <https://www.hindawi.com/journals/bmri/2014/840547/>
2. Todd AC, Wetmur JG, Moline JM, Godbold JH, Levin SM, Landrigan PJ. Unraveling the chronic toxicity of lead: an essential priority for environmental health. *Environ Health Perspect*. 1996 Mar; 104(Suppl 1):141–6.
3. Akkus C, Ozdenerol E. Exploring Childhood Lead Exposure through GIS: A Review of the Recent Literature. *Int J Environ Res Public Health*. 2014 Jun; 11(6):6314–34. <https://doi.org/10.3390/ijerph110606314> PMID: 24945189
4. Wani AL, Ara A, Usmani JA. Lead toxicity: a review. *Interdiscip Toxicol*. 2015 Jun; 8(2):55–64. <https://doi.org/10.1515/intox-2015-0009> PMID: 27486361
5. Betts KS. CDC Updates Guidelines for Children's Lead Exposure. *Environ Health Perspect*. 2012 Jul 1; 120(7):a268–a268. <https://doi.org/10.1289/ehp.120-a268> PMID: 22759595
6. Huang S, Hu H, Sánchez BN, Peterson KE, Ettinger AS, Lamadrid-Figueroa H, et al. Childhood Blood Lead Levels and Symptoms of Attention Deficit Hyperactivity Disorder (ADHD): A Cross-Sectional Study of Mexican Children. *Environ Health Perspect*. 2016 Jun 1; 124(6):868–74. <https://doi.org/10.1289/ehp.1510067> PMID: 26645203
7. Miranda ML, Kim D, Galeano MAO, Paul CJ, Hull AP, Morgan SP. The Relationship between Early Childhood Blood Lead Levels and Performance on End-of-Grade Tests. *Environ Health Perspect*. 2007 Aug; 115(8):1242–7. <https://doi.org/10.1289/ehp.9994> PMID: 17687454
8. Zhang N, Baker HW, Tufts M, Raymond RE, Salihi H, Elliott MR. Early Childhood Lead Exposure and Academic Achievement: Evidence From Detroit Public Schools, 2008–2010. *Am J Public Health*. 2013 Mar; 103(3):e72–7. <https://doi.org/10.2105/AJPH.2012.301164> PMID: 23327265
9. Bodeau-Livinec F, Glorennec P, Cot M, Dumas P, Durand S, Massougbodji A, et al. Elevated Blood Lead Levels in Infants and Mothers in Benin and Potential Sources of Exposure. *Int J Environ Res Public Health* [Internet]. 2016 Mar; 13(3). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4808979/>
10. President's Malaria Initiative. Benin. [Internet]. [cited 2018 May 4]. Available from: <https://www.pmi.gov/where-we-work/benin>
11. World Health Organization. World Health Statistics 2017: Monitoring health for the SDGs [Internet]. WHO. [cited 2019 Feb 26]. Available from: http://www.who.int/gho/publications/world_health_statistics/2017/en/
12. Gupta S, Snow RW, Donnelly CA, Marsh K, Newbold C. Immunity to non-cerebral severe malaria is acquired after one or two infections. *Nat Med*. 1999 Mar; 5(3):340–3. <https://doi.org/10.1038/6560> PMID: 10086393
13. Nriagu J, Afeiche M, Linder A, Arowolo T, Ana G, Sridhar MKC, et al. Lead poisoning associated with malaria in children of urban areas of Nigeria. *Int J Hyg Environ Health*. 2008 Oct; 211(5–6):591–605. <https://doi.org/10.1016/j.ijheh.2008.05.001> PMID: 18599348
14. Moya-Alvarez V, Mireku MO, Ayotte P, Cot M, Bodeau-Livinec F. Elevated Blood Lead Levels Are Associated with Reduced Risk of Malaria in Beninese Infants. *PLOS ONE*. 2016 Feb 11; 11(2): e0149049. <https://doi.org/10.1371/journal.pone.0149049> PMID: 26866471

15. González R, Mombo-Ngoma G, Ouédraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multi-centre Randomized Controlled Trial. *PLOS Med*. 2014 Sep 23; 11(9):e1001733. <https://doi.org/10.1371/journal.pmed.1001733> PMID: 25247709
16. Mireku MO, Davidson LL, Koura GK, Ouédraogo S, Boivin MJ, Xiong X, et al. Prenatal Hemoglobin Levels and Early Cognitive and Motor Functions of One-Year-Old Children. *Pediatrics*. 2015 Jul; 136(1): e76–83. <https://doi.org/10.1542/peds.2015-0491> PMID: 26055847
17. Tjitra E, Suprianto S, Dyer M, Currie BJ, Anstey NM. Field Evaluation of the ICT Malaria P.f/P.v Immunochromatographic Test for Detection of *Plasmodium falciparum* and *Plasmodium vivax* in Patients with a Presumptive Clinical Diagnosis of Malaria in Eastern Indonesia. *J Clin Microbiol*. 1999 Aug; 37(8):2412–7. PMID: 10405377
18. World Health Organization. Roll Back Malaria & United States. New perspectives: malaria diagnosis: report of a joint WHO/USAID informal consultation, 25–27 October 1999. 2000 [cited 2019 Jun 3]; Available from: <https://apps.who.int/iris/handle/10665/66321>
19. Mouatcho JC, Goldring JPD. Malaria rapid diagnostic tests: challenges and prospects. *J Med Microbiol*. 2013; 62(10):1491–505.
20. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr*. 2010 Sep; 92(3):546–55. <https://doi.org/10.3945/ajcn.2010.29284> PMID: 20610634
21. Lysaniuk B, Ladsous R, Tabeaud M, Cottrell G, Penetier C, Garcia A. Types of homes and ways of life: a territorial analysis of the environmental determinants that factor into the proliferation of malaria vectors in the rural region of Allada in Benin. *Rural Remote Health*. 2015; 15:2696. PMID: 25766636
22. AuYeung W, Canales RA, Beamer P, Ferguson AC, Leckie JO. Young Children's Mouthing Behavior: An Observational Study via Videotaping in a Primarily Outdoor Residential Setting. *J Child Health*. 2005 Jan 1; 2(3–4):271–95.
23. Gulson BL, Mizon KJ, Korsch MJ, Palmer JM, Donnelly JB. Mobilization of lead from human bone tissue during pregnancy and lactation—a summary of long-term research. *Sci Total Environ*. 2003 Feb 15; 303(1):79–104.
24. Moya-Alvarez V, Bodeau-Livinec F, Cot M. Iron and malaria: a dangerous liaison? *Nutr Rev*. 2016; 74(10):612–23. <https://doi.org/10.1093/nutrit/nuw021> PMID: 27566983
25. Spottiswoode N, Duffy PE, Drakesmith H. Iron, anemia and hepcidin in malaria. *Front Pharmacol [Internet]*. 2014 May 30; 5. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4039013/>
26. Adam I. Anemia, Iron Supplementation and Susceptibility to *Plasmodium falciparum* Malaria. *EBioMedicine*. 2016 Dec 1; 14:13–4. <https://doi.org/10.1016/j.ebiom.2016.11.036> PMID: 27919755
27. Graziano JH, Slavkovic V, Factor-Litvak P, Popovac D, Ahmedi X, Mehmeti A. Depressed serum erythropoietin in pregnant women with elevated blood lead. *Arch Environ Health*. 1991 Dec; 46(6):347–50. <https://doi.org/10.1080/00039896.1991.9934401> PMID: 1772259
28. de Souza ID, de Andrade AS, Dalmolin RJS. Lead-interacting proteins and their implication in lead poisoning. *Crit Rev Toxicol*. 2018 May 28; 48(5):375–86. <https://doi.org/10.1080/10408444.2018.1429387> PMID: 29431551
29. Kwong WT, Friello P, Semba RD. Interactions between iron deficiency and lead poisoning: epidemiology and pathogenesis. *Sci Total Environ*. 2004 Sep 1; 330(1–3):21–37. <https://doi.org/10.1016/j.scitotenv.2004.03.017> PMID: 15325155
30. Bayoumi RAL. Does the mechanism of protection from falciparum malaria by red cell genetic disorders involve a switch to a balanced TH1/TH2 cytokine production mode? *Med Hypotheses*. 1997 Jan 1; 48(1):11–7. PMID: 9049983
31. Angulo I, Fresno M. Cytokines in the Pathogenesis of and Protection against Malaria. *Clin Diagn Lab Immunol*. 2002 Nov; 9(6):1145–52. <https://doi.org/10.1128/CDLI.9.6.1145-1152.2002> PMID: 12414742
32. Moya-Alvarez V, Cottrell G, Ouédraogo S, Accrombessi M, Massougbdgi A, Cot M. High Iron Levels Are Associated with Increased Malaria Risk in Infants during the First Year of Life in Benin. *Am J Trop Med Hyg*. 2017 Aug 2; 97(2):497–503. <https://doi.org/10.4269/ajtmh.16-0001> PMID: 28722565

Chapter 7. Discussion

This chapter details and interprets the main findings of each objective presented in preceding chapters, as well as discusses the major strengths and limitations of the work done for this thesis as a whole, in an effort to not repeat elements of the discussions found in each individual article. This chapter also presents the implications of these findings across public health domains and suggests further research that is needed on this and related subjects.

Main findings

Multiple linear regression analyses analyzed the impact of SHIP and MIP on child neurocognitive and behavioral development. Our analyses revealed that SHIP diagnosed at each ANV and at least once during pregnancy, as well as hookworm density during pregnancy were not found to be associated with neurocognitive development, motor function, or attention/hyperactivity disorder in children at six years of age. However, SHIP was associated with impaired emotional development. Helminth infection at the 2nd ANV/delivery and at any point in pregnancy were associated with higher internalizing scores, indicating more emotional problems. Mediation analyses revealed that inflammation measured by CRP in pregnancy was not found to be a significant mediating factor of this association, as hypothesized. Regarding MIP, we found that infection at least once during pregnancy diagnosed by microscopy, placental malaria diagnosed by qPCR, and high parasite density at delivery were associated with impaired gross motor development scores measured by the MSEL in children at one year. MIP diagnosed by microscopy and high parasite density at the 2nd ANV negatively impacted NVI scores of the KABC-II, and analyses show a trend that infections occurring later in pregnancy more negatively impact KABC-II scores than earlier infections.

Parasitic infection during pregnancy is known to increase the risk of infection in childhood (84,148), which in turn can impair their neurocognitive development according to observational studies (198,200). Therefore, this thesis investigated other hypothesized factors that could influence parasitic infection in childhood, that are well-established risk factors of impaired neurocognitive development (184,195), and contribute to the main findings of this thesis.

We conducted a quantitative analysis of the impact of maternal post-partum depression measured by the EPDS on parasitic infection in childhood. This quantitative analysis is being done in tandem with a qualitative analysis, which has unfortunately been re-scheduled several times over the course of this thesis due to ongoing political unrest in Benin in 2019 and the coronavirus pandemic in 2020. A summary of the qualitative study beginning in Allada, Benin

in June 2021 is included in Appendix 1. Briefly, the aim of this mixed-methods study is to investigate the experiences, barriers, and challenges pregnant women have in taking preventive treatments for anemia, malaria, and soil-transmitted helminth infection during antenatal care follow-up, including an in-depth analysis of the impact of maternal depression on pre- and post-natal preventive care. The quantitative analysis of this research question looked more closely at the link between maternal depression, parent-child interactions, and parasitic infection. One previous study had found an association between maternal depression and increased risk of diarrheal disease in children (15), hypothesizing that depressed women have more difficulty caring for themselves and their children, including noticing when illness occurs and providing timely treatment, thus leading to greater risk of infection. Our regression analyses did not find maternal depression one-year post-partum to impact risk of parasitic infection in children. However, our analyses did confirm that maternal depression was associated with lower quality and quantity of parent-child interactions as measured by the HOME, as seen in previous research (32), with many hypothesizing that this association is a mediator for poor neurodevelopmental outcomes in children.

Recently, two cross-sectional cohort studies revealed an inverse relationship between blood lead level and risk of malaria infection in African cohorts. These studies, conducted in children, hypothesized that absorption of lead into red blood cells could inhibit the life cycle of the *P. falciparum* parasite, which also occurs in red blood cells, and therefore “protect” against malaria infection (14,255). In response to this previous research, as well as a study showing high blood lead levels (50 µg/L or more according to the CDC) in our population of mothers and children in Benin (20), we studied whether or not we would see this same inverse association between blood lead level and malaria infection in our population prospectively over the course of a 12-month follow-up of a sub-cohort of children for whom blood lead level and repeated malaria incidence was available. Median blood lead level among the 204 children in our analyses was 56.50 (±56.83) µg/L, above the CDC intervention threshold. Our analyses found that blood lead level measured at one year of age in this population of children was not associated with increased risk of malaria episodes between 12-24 months of age, even after adjusting for other relevant risk factors. The previous significant association found by Moya-Alvarez et al. (2016) could be due to the fact that at 12 months of age, children in malaria-endemic countries begin to lose *P.falciparum* immunity acquired from their mothers and they experience their first episodes of malaria and have higher parasite densities in their red blood cells. These results suggested to us that in the context of routine infection screening and treatment, as recommended by the WHO, environmental exposure from lead does not seem to

impact long-term risk of malaria infection in children, and therefore this exposure was not taken into account as a potential risk factor for infection in pregnant women in our primary analyses either.

Strengths & Limitations of thesis

Each article of this thesis details the strengths and limitations for each objective and the analyses used within them. Overall, the studies conducted within this thesis are the first of their kind that explore the long-term neurocognitive consequences of parasitic infection in pregnancy, thus adding significantly to existing epidemiological research. Perhaps the greatest strength of this work is the extensive follow-up of participants in the Allada mother-child cohort, a rarity in SSA populations. The quality and validity of biological, neurocognitive, and anthropometric assessments used during follow-up contribute to this strength.

In addition to the limitations of the analyses for each objective, there are some overarching limitations to what we were able to accomplish within the confines of this thesis. One such limitation is the fact that we did not include maternal or infant iron deficiency/anemia in many analyses due to its complex and multifactorial nature. This thesis sought to investigate the impact of parasitic infection in pregnancy independently from maternal and infant anemia, and further studies are currently underway to provide more insight into the risk factors of anemia and how it impacts child neurocognitive development in this population. Secondly, the objective studying the association between SHIP and neurocognitive and behavioral development was done in the presence of regular helminth prophylaxis and treatment during pregnancy and childhood, therefore our study was unable to investigate whether or not prophylaxis could attenuate or even reverse the negative effects of SHIP or child helminth infection, as seen in some previous studies. The impact of anti-helminthic prophylaxis treatments has been widely studied; however, results remain conflicting on how they may affect neurocognitive development in children.

Epidemiological implications

The findings of this thesis have implications in epidemiology, society, environmental science, and health policy, providing an interdisciplinary perspective on parasitic infection and its consequences in vulnerable populations. By viewing public health issues through this interdisciplinary lens, we as researchers, health professionals, and public health stakeholders can be more prepared to tackle these issues on an international, cooperative scale.

One hypothesized mechanism to explain the adverse neurocognitive and behavioral outcomes due to maternal infection is the effect of inflammation during pregnancy on fetal neurodevelopmental abnormalities and risk of psychiatric disorders. Maternal infection in

pregnancy has been shown to activate the mothers' inflammatory immune response, which in turn can lead to increased risk of behavioral problems and disorders in offspring, including autism spectrum disorder and depression (25,26). One previous study specifically found that elevated markers of inflammation in pregnant women were associated with more internalizing and externalizing behavioral symptoms in their offspring later in childhood (28). However, these studies either overlooked inflammation due to parasitic infection entirely or used a composite value for inflammation due to differing types of maternal infection during pregnancy, without independently studying specific parasitic infections. One existing laboratory-based study provides evidence of a causal link between experimental malaria infection in pregnancy and impaired neurocognitive development in the fetus through activation of the inflammatory complement system (13,27). Results from this thesis suggest that the general inflammation marker of CRP does not mediate the relationship between SHIP and impaired behavioral development in children. Other cytokines and markers specific to STH and malaria infection should be used when investigating the mediating role of inflammation in these associations, since maternal inflammation measured by CRP in our population could have been the result of maternal infection or co-morbidities other than SHIP.

Societal implications

Impaired neurocognitive development of younger generations can have population-level consequences, particularly in SSA countries with less developed economic and administrative systems. Additionally, populations living in these countries are often exposed to several additional factors that influence child development over the course of life, including anemia, malnutrition, conflict, poverty, and low social support systems. The findings of this thesis show that intervention to improve neurocognitive outcomes in surviving offspring can begin *in utero*, potentially mitigating the long-term impacts of prenatal parasitic infection.

In 2015, the United Nations General Assembly set the 2030 Sustainable Development Goals (SDGs), a set of 17 global goals designed to achieve a better and more sustainable future (256). As part of these goals, improving maternal and child morbidity from infectious diseases and addressing factors that could inhibit child and economic development remain high priorities. Addressing the social inequalities between nations and the specific challenges faced by developing children in SSA countries is a pivotal step in achieving these goals. While the SDGs do not mention specific infectious diseases that need public attention, the WHO has deemed soil-transmitted helminth infections to be a part of their list of Neglected Tropical Diseases (NTDs), a list of diseases that disproportionately effect poor populations, receive little to no public funding for research and intervention, receive little media attention, and impact

large proportions of these under-served populations. Therefore, innovative research, such as the work done within this thesis, on NTDs is important to shed light on the extended burden they bring to millions of individuals around the world.

Data collection and reporting on developmental disabilities and impairments in African populations is greatly needed in order to better understand their health burden (257). To date, there are no standardized, comprehensive developmental diagnostic tools for Beninese and other SSA children. The existing tools used in these populations come from Western societies and norms, and are often adapted to African populations and have their construct validity verified prior to their use (22). This gap in knowledge related to developmental norms, definitions, and educational and clinical treatment protocols in SSA countries further hinders these populations and their overall development as societies. Prospective cohorts such as the one included in this thesis are vital for further research efforts to provide more comprehensive data on neurodevelopment in African populations and their implications for society and public health. Further research on this subject is greatly needed in African cohorts, and in fact one study 11-years post-partum is underway in the Beninese population included in this thesis to better interpret results from neurocognitive assessments and to use these results to understand the clinical representations of impairments in this population.

Environmental implications

This thesis provides evidence that exposure to lead, a neurotoxic heavy metal, is still a public health concern in low-resources settings like Benin, even though exposure to lead via paint and gasoline has been eradicated in high-income countries for the most part (29). It is critical to provide regular surveillance for lead exposure in this population since ammunition used to hunt bushmeat, dust particulates inside homes, and contaminated water pipes have all been found to contain significant amounts of lead in previous studies (30,241). There is also a public health interest in studying how these persistent environmental exposures impact populations already vulnerable to several other risk factors of disease and impaired cognitive functioning.

Policy implications

The close follow-up of women during antenatal care in the clinical trial allowed researchers to provide WHO and study protocol recommended prevention and treatment medications for maternal anemia, soil-transmitted helminth infection, and malaria infection. Even in the presence of this close antenatal follow-up, prevalence of parasitic infection was still in congruence with expected estimates (1). At the time of the clinical trial, only two doses of IPTp-SP were recommended to prevent malaria in high-transmission areas of SSA, like Benin.

We saw in our follow-up that there was a higher prevalence of soil-transmitted helminth infection and malaria infection in pregnant women at delivery compared to the 2nd ANV, suggesting inadequate preventive treatment for these women who are chronically exposed to the parasites causing these infections. Research on the timing of maternal infections during pregnancy and its impact on fetal health remains equivocal (6,7). One prospective mother-child cohort study found that in the presence of IPTp-SP and active malaria screening and treatment throughout pregnancy, maternal infection was not associated with adverse birth outcomes (6). Our analyses show a trend between late-term parasitic infection, particularly malaria infection, and lower neurocognitive and behavioral scores in children at one and six years. In 2013, after the clinical trial, the WHO published a policy brief recommending at least three doses of IPTp-SP during prenatal care in malaria-endemic countries. Studies on the impacts of prenatal malarial infection on child developmental outcomes are needed in order to know the impact this protocol may have on associations found within this thesis and to add to the limited research on the timing of malarial infection in pregnancy on adverse birth outcomes in the presence of more regular screening and treatment.

Our analyses provide evidence that submicroscopic, asymptomatic malarial infections pose a high risk to pregnant women in malaria-endemic countries like Benin, and sufficient IPTp and close follow-up during antenatal care is vital to address its risks on fetal development. The diagnosis of placental malaria using qPCR techniques, which are more time- and resource-intensive to analyze in low-income settings like Benin, revealed that women with these infections at time of delivery were more likely to have offspring with impaired neurocognition. These submicroscopic, asymptomatic infections are left untreated, due to often undetectable parasitic density of these infections through gold standard diagnostic methods and the inability to diagnose infection based on visible symptoms, and therefore could reflect more chronic infections of women. Diagnostic methods that are quick, reliable, affordable, and able to detect low parasite density thresholds (which is of special importance in endemic populations where acquired immunity is active) and close antenatal care follow-up, are imperative in order to provide adequate and timely treatment of infections and curb potential long-term consequences.

The qualitative study that will explore mothers' experiences, barriers, and attitudes toward infection prevention and treatment will also provide insight into the real-life translation of and adherence to pre- and post-natal prophylaxis treatments and the barriers that women and children face that could be addressed by policy adaptations. Previous qualitative studies interested in women's experience with IPTp have recommended increased community healthcare professional training to address questions and concerns of women during antenatal

care visits (258), alternative sources of information regarding IPTp and its risks (259), and better availability and access to free-of-charge antenatal services, particularly in rural areas (260).

Conclusion and Perspectives

It is imperative to mention that advances in clinical practices and international public health policy implementation in recent decades has allowed for vast improvements in terms of morbidity and mortality from parasitic infection in SSA countries. However, parasitic infection in endemic areas of the world, primarily low- and middle-income countries, still carries a large burden of disease in vulnerable groups and has long-term consequences for survivors. Understanding these long-term consequences using an interdisciplinary approach in public health allows a multitude of innovative initiatives and interventions to emerge. Public health issues today call for global and synergistic approaches to tackle them (33), therefore participation from stakeholders in various disciplines allows for adequate health intervention analysis and action. While maternal morbidity from parasitic infection has improved over the years, there is still much intervention to be done in order to improve health outcomes and quality of life for both mother and fetus throughout the life course.

This thesis provides novel evidence of these long-term consequences of parasitic infection in pregnancy on child development. Our studies showed that even in the presence of prevention and treatment strategies for parasitic infection during antenatal care, SHIP and MIP are associated with impaired child behavioral and neurocognitive development at one and six years of age. Our analyses revealed that later term infections were more deleterious, even in statistically non-significant results. The numerous sensitivity analyses, including multiple imputation of missing values, mediation analyses, and regression analyses including various additional potential confounding factors, confirm the primary results found for each objective and show the robustness of our results.

Future studies in large, prospective SSA cohorts are needed in order to confirm the results seen in this thesis and should explore the mediating role of anemia and maternal inflammation more in depth with proper markers. Further studies should also continue to explore the mediating relationship of parasitic infection during childhood on the relationship between infection during pregnancy and child development. The qualitative study being carried out in tandem with this thesis will complement the quantitative results on the impacts of maternal depression on child parasitic infection and provide valuable data to better understand the experiences and barriers to adequate preventive treatment for parasitic infection during pregnancy. Morbidity and mortality from malaria has decreased over time with improved prevention, surveillance, and treatment efforts; however, the detection and treatment of asymptomatic infections remains of public health concern.

As previously mentioned, the clinical manifestation and diagnosis of neurocognitive impairments in African populations are not well-documented, and further studies should seek to provide country-specific norms and definitions for developmental diseases and disabilities. Prevention of parasitic infection during pregnancy using IPTp and helminth prophylaxis, surveillance and active detection of cases through close antenatal follow-up, and treatment of malaria and helminth infection cases in pregnancy are needed in order to reduce the burden of these infections in vulnerable populations. Another future area of interest for researchers is the impact of insecticides, in the form of long-lasting insecticide-treated bed nets, spirals, and insecticide sprays, on long-term child neurocognitive development, as these are effective methods of vector control but their long-term impacts on exposed individuals remain widely unknown.

References

1. Soil-transmitted helminth infections [Internet]. [cited 2021 Apr 13]. Available from: <https://www.who.int/news-room/fact-sheets/detail/soil-transmitted-helminth-infections>
2. Fact sheet about Malaria [Internet]. World Health Organization. [cited 2018 Apr 30]. Available from: <http://www.who.int/news-room/fact-sheets/detail/malaria>
3. Alelign T, Degarege A, Erko B. Soil-Transmitted Helminth Infections and Associated Risk Factors among Schoolchildren in Durbete Town, Northwestern Ethiopia. *J Parasitol Res* [Internet]. 2015;2015. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4487929/>
4. Cottrell G, Moussiliou A, Luty AJF, Cot M, Fievet N, Massougbdji A, et al. Submicroscopic Plasmodium falciparum Infections Are Associated With Maternal Anemia, Premature Births, and Low Birth Weight. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2015 May 15;60(10):1481–8.
5. Dreyfuss ML, Msamanga GI, Spiegelman D, Hunter DJ, Urassa EJ, Hertzmark E, et al. Determinants of low birth weight among HIV-infected pregnant women in Tanzania. *Am J Clin Nutr*. 2001 Dec;74(6):814–26.
6. Kalilani-Phiri L, Thesing PC, Nyirenda OM, Mawindo P, Madanitsa M, Membe G, et al. Timing of Malaria Infection during Pregnancy Has Characteristic Maternal, Infant and Placental Outcomes. *PLOS ONE*. 2013 Sep 18;8(9):e74643.
7. Mahande AM, Mahande MJ. Prevalence of parasitic infections and associations with pregnancy complications and outcomes in northern Tanzania: a registry-based cross-sectional study. *BMC Infect Dis*. 2016 Feb 13;16:78.
8. WHO | Deworming in pregnant women [Internet]. WHO. [cited 2019 May 10]. Available from: http://www.who.int/elena/titles/deworming_pregnancy/en/
9. Badirou A, Barikissou Georgia D, Sètonджи Géraud Roméo P, Modeste Luc K, Edgard Marius O. Adherence to Intermittent Preventive Treatment of Malaria in Pregnancy with Sulfadoxine–Pyrimethamine and Associated Factors: A Cross-Sectional Survey in Benin’s Public Hospitals. *Open Public Health J* [Internet]. 2018 Jan 31 [cited 2021 Apr 13];11(1). Available from: <https://benthamopen.com/FULLTEXT/TOPHJ-11-28>
10. Global Malaria Programme [Internet]. [cited 2021 Apr 13]. Available from: <https://www.who.int/teams/health-product-and-policy-standards/standards-and-specifications/vaccines-quality/vaccine-pq/global-malaria-programme>
11. Nicinski D. Soil Transmitted Helminths [Internet]. Neglected Tropical Disease Program. [cited 2021 Apr 13]. Available from: <https://www.neglecteddiseases.gov/usaid-targeted-diseases/soil-transmitted-helminths/>
12. Mireku MO, Boivin MJ, Davidson LL, Ouédraogo S, Koura GK, Alao MJ, et al. Impact of helminth infection during pregnancy on cognitive and motor functions of one-year-old children. *PLoS Negl Trop Dis*. 2015 Mar;9(3):e0003463.

13. McDonald CR, Cahill LS, Ho KT, Yang J, Kim H, Silver KL, et al. Experimental Malaria in Pregnancy Induces Neurocognitive Injury in Uninfected Offspring via a C5a-C5a Receptor Dependent Pathway. *PLoS Pathog.* 2015 Sep;11(9):e1005140.
14. Moya-Alvarez V, Mireku MO, Ayotte P, Cot M, Bodeau-Livinec F. Elevated Blood Lead Levels Are Associated with Reduced Risk of Malaria in Beninese Infants. *PLOS ONE.* 2016 Feb 11;11(2):e0149049.
15. Rahman A, Bunn J, Lovel H, Creed F. Maternal depression increases infant risk of diarrhoeal illness: --a cohort study. *Arch Dis Child.* 2007 Jan;92(1):24–8.
16. González R, Mombo-Ngoma G, Ouédraogo S, Kakolwa MA, Abdulla S, Accrombessi M, et al. Intermittent Preventive Treatment of Malaria in Pregnancy with Mefloquine in HIV-Negative Women: A Multicentre Randomized Controlled Trial. *PLOS Med.* 2014 Sep 23;11(9):e1001733.
17. Ouédraogo S, Accrombessi M, Diallo I, Codo R, Ouattara A, Ouédraogo L, et al. Placental impression smears is a good indicator of placental malaria in sub-Saharan Africa. *Pan Afr Med J [Internet].* 2019 Sep 16 [cited 2020 Nov 23];34(30). Available from: <https://www.panafrican-med-journal.com/content/article/34/30/full/>
18. Organization WH. Bench aids for the diagnosis of intestinal parasites [Internet]. World Health Organization; 1994 [cited 2021 Mar 19]. Available from: <https://apps.who.int/iris/handle/10665/37323>
19. Mayor A, Bardají A, Macete E, Nhampossa T, Fonseca AM, González R, et al. Changing Trends in *P. falciparum* Burden, Immunity, and Disease in Pregnancy [Internet]. <http://dx.doi.org/10.1056/NEJMoa1406459>. Massachusetts Medical Society; 2015 [cited 2021 Mar 19]. Available from: <https://www.nejm.org/doi/10.1056/NEJMoa1406459>
20. Mullen-Scales-ed-KS-AK.pdf [Internet]. [cited 2021 Mar 22]. Available from: <https://txautism.net/assets/uploads/docs/Mullen-Scales-ed-KS-AK.pdf>
21. Boivin MJ, Giordani B. Neuropsychological assessment of African children: evidence for a universal brain/behavior omnibus within a coconstructivist paradigm. In: Chiao JY, editor. *Progress in Brain Research [Internet].* Elsevier; 2009. p. 113–35. (Cultural Neuroscience: Cultural Influences on Brain Function; vol. 178). Available from: <http://www.sciencedirect.com/science/article/pii/S0079612309178081>
22. Koura KG, Boivin MJ, Davidson LL, Ouédraogo S, Zoumenou R, Alao MJ, et al. Usefulness of child development assessments for low-resource settings in francophone Africa. *J Dev Behav Pediatr JDBP.* 2013 Sep;34(7):486–93.
23. Huynh B-T, Fievet N, Gbaguidi G, Dechavanne S, Borgella S, Guézo-Mévo B, et al. Influence of the timing of malaria infection during pregnancy on birth weight and on maternal anemia in Benin. *Am J Trop Med Hyg.* 2011 Aug;85(2):214–20.
24. Uneke CJ. Impact of Placental *Plasmodium falciparum* Malaria on Pregnancy and Perinatal Outcome in Sub-Saharan Africa. *Yale J Biol Med.* 2007 Sep;80(3):95–103.

25. Meyer U, Feldon J, Fatemi SH. In-vivo rodent models for the experimental investigation of prenatal immune activation effects in neurodevelopmental brain disorders. *Neurosci Biobehav Rev.* 2009 Jul 1;33(7):1061–79.
26. Shi L, Tu N, Patterson PH. Maternal influenza infection is likely to alter fetal brain development indirectly: the virus is not detected in the fetus. *Int J Dev Neurosci.* 2005 Apr 1;23(2):299–305.
27. Weckman AM, Ngai M, Wright J, McDonald CR, Kain KC. The Impact of Infection in Pregnancy on Placental Vascular Development and Adverse Birth Outcomes. *Front Microbiol* [Internet]. 2019 Aug 22 [cited 2020 Feb 17];10. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6713994/>
28. Mac Giollabhui N, Breen EC, Murphy SK, Maxwell SD, Cohn BA, Krigbaum NY, et al. Maternal inflammation during pregnancy and offspring psychiatric symptoms in childhood: Timing and sex matter. *J Psychiatr Res.* 2019;111:96–103.
29. Akkus C, Ozdenerol E. Exploring Childhood Lead Exposure through GIS: A Review of the Recent Literature. *Int J Environ Res Public Health.* 2014 Jun;11(6):6314–34.
30. Ahmadi S, Le Bot B, Zoumenou R, Durand S, Fiévet N, Ayotte P, et al. Follow-Up of Elevated Blood Lead Levels and Sources in a Cohort of Children in Benin. *Int J Environ Res Public Health.* 2020 Nov 23;17(22).
31. Lysaniuk B, Ladsous R, Tabeaud M, Cottrell G, Pannetier C, Garcia A. Types of homes and ways of life: a territorial analysis of the environmental determinants that factor into the proliferation of malaria vectors in the rural region of Allada in Benin. *Rural Remote Health.* 2015;15:2696.
32. Stein A, Gath DH, Bucher J, Bond A, Day A, Cooper PJ. The relationship between post-natal depression and mother-child interaction. *Br J Psychiatry J Ment Sci.* 1991 Jan;158:46–52.
33. Kivits J, Ricci L, Minary L. Interdisciplinary research in public health: the ‘why’ and the ‘how.’ *J Epidemiol Community Health.* 2019 Dec 1;73(12):1061–2.
34. Murray CJL, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet Lond Engl.* 2012 Dec 15;380(9859):2197–223.
35. Khan D, Fernando P, Cicvaric A, Berger A, Pollak A, Monje FJ, et al. Long-term effects of maternal immune activation on depression-like behavior in the mouse. *Transl Psychiatry.* 2014 Feb;4(2):e363–e363.
36. Pullan RL, Smith JL, Jasrasaria R, Brooker SJ. Global numbers of infection and disease burden of soil transmitted helminth infections in 2010. *Parasit Vectors.* 2014 Jan 21;7:37.
37. Organization WH. Soil-transmitted helminthiases : eliminating as public health problem soil-transmitted helminthiases in children : progress report 2001-2010 and

- strategic plan 2011-2020 [Internet]. World Health Organization; 2012 [cited 2019 May 10]. Available from: <https://apps.who.int/iris/handle/10665/44804>
38. Dawaki S, Al-Mekhlafi HM, Ithoi I. The burden and epidemiology of polyparasitism among rural communities in Kano State, Nigeria. *Trans R Soc Trop Med Hyg*. 2019 Apr 1;113(4):169–82.
 39. Neglected tropical diseases [Internet]. [cited 2021 May 3]. Available from: <https://www.who.int/news-room/q-a-detail/neglected-tropical-diseases>
 40. Vos T, Barber RM, Bell B, Bertozzi-Villa A, Biryukov S, Bolliger I, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 301 acute and chronic diseases and injuries in 188 countries, 1990–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*. 2015 Aug 22;386(9995):743–800.
 41. Prevention C-C for DC and. CDC - Ascariasis - Biology [Internet]. 2019 [cited 2021 Mar 30]. Available from: <https://www.cdc.gov/parasites/ascariasis/biology.html>
 42. Mireku MO, Davidson LL, Zoumenou R, Massougboji A, Cot M, Bodeau-Livinec F. Consequences of prenatal geophagy for maternal prenatal health, risk of childhood geophagy and child psychomotor development. *Trop Med Int Health TM IH*. 2018 Aug;23(8):841–9.
 43. Viswanath A, Yarrarapu SNS, Williams M. Trichuris Trichiura. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 [cited 2021 Mar 30]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK507843/>
 44. CDC - DPDx - Intestinal Hookworm [Internet]. 2019 [cited 2021 Mar 30]. Available from: <https://www.cdc.gov/dpdx/hookworm/index.html>
 45. Wei K-Y, Yan Q, Tang B, Yang S-M, Zhang P-B, Deng M-M, et al. Hookworm Infection: A Neglected Cause of Overt Obscure Gastrointestinal Bleeding. *Korean J Parasitol*. 2017 Aug;55(4):391–8.
 46. Mutombo PN, Man NWY, Nejsun P, Ricketson R, Gordon CA, Robertson G, et al. Diagnosis and drug resistance of human soil-transmitted helminth infections: A public health perspective. *Adv Parasitol*. 2019;104:247–326.
 47. Katz N, Chaves A, Pellegrino J. A simple device for quantitative stool thick-smear technique in Schistosomiasis Mansoni. *Rev Inst Med Trop São Paulo*. 1971 Nov 30;14:397–400.
 48. Keller L, Patel C, Welsche S, Schindler T, Hürlimann E, Keiser J. Performance of the Kato-Katz method and real time polymerase chain reaction for the diagnosis of soil-transmitted helminthiasis in the framework of a randomised controlled trial: treatment efficacy and day-to-day variation. *Parasit Vectors*. 2020 Oct 15;13(1):517.
 49. Water, Sanitation and Hygiene (WASH) [Internet]. [cited 2021 May 3]. Available from: <https://www.unicef.org/wash>

50. Strunz EC, Addiss DG, Stocks ME, Ogden S, Utzinger J, Freeman MC. Water, Sanitation, Hygiene, and Soil-Transmitted Helminth Infection: A Systematic Review and Meta-Analysis. *PLOS Med.* 2014 Mar 25;11(3):e1001620.
51. WHO | Deworming in children [Internet]. WHO. World Health Organization; [cited 2021 May 4]. Available from: <http://www.who.int/elena/titles/deworming/en/>
52. WHO | Deworming in non-pregnant adolescent girls and women of reproductive age [Internet]. WHO. World Health Organization; [cited 2021 May 4]. Available from: <http://www.who.int/elena/titles/deworming-women/en/>
53. Ziegelbauer K, Speich B, Mäusezahl D, Bos R, Keiser J, Utzinger J. Effect of Sanitation on Soil-Transmitted Helminth Infection: Systematic Review and Meta-Analysis. *PLoS Med* [Internet]. 2012 Jan 24;9(1). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3265535/>
54. Derso A, Nibret E, Munshea A. Prevalence of intestinal parasitic infections and associated risk factors among pregnant women attending antenatal care center at Felege Hiwot Referral Hospital, northwest Ethiopia. *BMC Infect Dis.* 2016 Sep 30;16(1):530.
55. Ouédraogo S, Koura GK, Accrombessi MMK, Bodeau-Livinec F, Massougbodji A, Cot M. Maternal anemia at first antenatal visit: prevalence and risk factors in a malaria-endemic area in Benin. *Am J Trop Med Hyg.* 2012 Sep;87(3):418–24.
56. Adegnika AA, Agnandji ST, Chai SK, Ramharter M, Breitling L, Kendjo E, et al. Increased prevalence of intestinal helminth infection during pregnancy in a Sub-Saharan African community. *Wien Klin Wochenschr.* 2007;119(23–24):712–6.
57. Eijk AM van, Lindblade KA, Odhiambo F, Peterson E, Rosen DH, Karanja D, et al. Geohelminth Infections among Pregnant Women in Rural Western Kenya; a Cross-Sectional Study. *PLoS Negl Trop Dis.* 2009 Jan 27;3(1):e370.
58. Ojurongbe O, Okorie PN, Opatokun RL, Ojurongbe TA, Mabayoje VO, Olowe OA, et al. Prevalence and associated factors of *Plasmodium falciparum* and soil transmitted helminth infections among pregnant women in Osun state, Nigeria. *Afr Health Sci.* 2018 Sep;18(3):542–51.
59. Woodburn PW, Muhangi L, Hillier S, Ndibazza J, Namujju PB, Kizza M, et al. Risk factors for helminth, malaria, and HIV infection in pregnancy in Entebbe, Uganda. *PLoS Negl Trop Dis.* 2009 Jun 30;3(6):e473.
60. Mpairwe H, Tweyongyere R, Elliott A. Pregnancy and helminth infections. *Parasite Immunol.* 2014 Aug;36(8):328–37.
61. Wekesa AW, Mulambalah CS, Muleke CI, Odhiambo R. Intestinal Helminth Infections in Pregnant Women Attending Antenatal Clinic at Kitale District Hospital, Kenya [Internet]. *Journal of Parasitology Research.* 2014 [cited 2019 May 7]. Available from: <https://www.hindawi.com/journals/jpr/2014/823923/>
62. Anderson AS, Trumble BC, Hové C, Kraft TS, Kaplan H, Gurven M, et al. Old friends and friendly fire: Pregnancy, hookworm infection, and anemia among tropical horticulturalists. *Am J Hum Biol.* 2020;32(2):e23337.

63. Kurtis JD, Higashi A, Wu H-W, Gundogan F, McDonald EA, Sharma S, et al. Maternal Schistosomiasis japonica is associated with maternal, placental, and fetal inflammation. *Infect Immun*. 2011 Mar;79(3):1254–61.
64. Nurdia DS, Sumarni S, Suyoko null, Hakim M, Winkvist A. Impact of intestinal helminth infection on anemia and iron status during pregnancy: a community based study in Indonesia. *Southeast Asian J Trop Med Public Health*. 2001 Mar;32(1):14–22.
65. Sun D, McLeod A, Gandhi S, Malinowski AK, Shehata N. Anemia in Pregnancy: A Pragmatic Approach. *Obstet Gynecol Surv*. 2017 Dec;72(12):730–7.
66. Brooker S, Clements AC, Hotez PJ, Hay SI, Tatem AJ, Bundy DA, et al. The co-distribution of Plasmodium falciparum and hookworm among African schoolchildren. *Malar J*. 2006 Nov 3;5(1):99.
67. Brooker S, Akhwale W, Pullan R, Estambale B, Clarke SE, Snow RW, et al. Epidemiology of plasmodium-helminth co-infection in Africa: populations at risk, potential impact on anemia, and prospects for combining control. *Am J Trop Med Hyg*. 2007 Dec;77(6 Suppl):88–98.
68. Adegnika AA, Kremsner PG. Epidemiology of malaria and helminth interaction: a review from 2001 to 2011. *Curr Opin HIV AIDS*. 2012 May;7(3):221–4.
69. Degarege A, Legesse M, Medhin G, Anmut A, Erko B. Malaria and related outcomes in patients with intestinal helminths: a cross-sectional study. *BMC Infect Dis*. 2012 Nov 9;12:291.
70. Shapiro AE, Tukahebwa EM, Kasten J, Clarke SE, Magnussen P, Olsen A, et al. Epidemiology of helminth infections and their relationship to clinical malaria in southwest Uganda. *Trans R Soc Trop Med Hyg*. 2005 Jan;99(1):18–24.
71. Naing C, Whittaker MA, Nyunt-Wai V, Reid SA, Wong SF, Mak JW, et al. Malaria and soil-transmitted intestinal helminth co-infection and its effect on anemia: a meta-analysis. *Trans R Soc Trop Med Hyg*. 2013 Nov 1;107(11):672–83.
72. Stephenson LS, Latham MC, Ottesen EA. Malnutrition and parasitic helminth infections. *Parasitology*. 2000;121 Suppl:S23-38.
73. Arinola GO, Morenikeji OA, Akinwande KS, Alade AO, Olateru-Olagbegi O, Alabi PE, et al. Serum Micronutrients in Helminth-infected Pregnant Women and Children: Suggestions for Differential Supplementation During Anti-helminthic Treatment. *Ann Glob Health*. 2015 Oct;81(5):705–10.
74. Bleker LS, de Rooij SR, Roseboom TJ. Malnutrition and depression in pregnancy and associations with child behaviour and cognitive function: a review of recent evidence on unique and joint effects 1. *Can J Physiol Pharmacol*. 2019 Mar;97(3):158–73.
75. Passerini L, Casey GJ, Biggs BA, Cong DT, Phu LB, Phuc TQ, et al. Increased Birth Weight Associated with Regular Pre-Pregnancy Deworming and Weekly Iron-Folic Acid Supplementation for Vietnamese Women. *PLoS Negl Trop Dis*. 2012 Apr 3;6(4):e1608.

76. Friedman JF, Mital P, Kanzaria HK, Olds GR, Kurtis JD. Schistosomiasis and pregnancy. *Trends Parasitol.* 2007 Apr;23(4):159–64.
77. Boel M, Carrara VI, Rijken M, Proux S, Nacher M, Pimanpanarak M, et al. Complex Interactions between Soil-Transmitted Helminths and Malaria in Pregnant Women on the Thai-Burmese Border. *PLoS Negl Trop Dis* [Internet]. 2010 Nov 16 [cited 2021 May 17];4(11). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2982827/>
78. Christian P, Khattry SK, West KP. Antenatal anthelmintic treatment, birthweight, and infant survival in rural Nepal. *Lancet Lond Engl.* 2004 Sep 11;364(9438):981–3.
79. de Silva NR, Sirisena JL, Gunasekera DP, Ismail MM, de Silva HJ. Effect of mebendazole therapy during pregnancy on birth outcome. *Lancet Lond Engl.* 1999 Apr 3;353(9159):1145–9.
80. Torlesse H, Hodges M. Albendazole therapy and reduced decline in haemoglobin concentration during pregnancy (Sierra Leone). *Trans R Soc Trop Med Hyg.* 2001 Apr;95(2):195–201.
81. Larocque R, Casapia M, Gotuzzo E, MacLean JD, Soto JC, Rahme E, et al. A double-blind randomized controlled trial of antenatal mebendazole to reduce low birthweight in a hookworm-endemic area of Peru. *Trop Med Int Health TM IH.* 2006 Oct;11(10):1485–95.
82. Ndibazza J, Muhangi L, Akishule D, Kiggundu M, Ameke C, Oweka J, et al. Effects of deworming during pregnancy on maternal and perinatal outcomes in Entebbe, Uganda: a randomized controlled trial. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2010 Feb 15;50(4):531–40.
83. Ndyomugenyi R, Kabatereine N, Olsen A, Magnussen P. Efficacy of ivermectin and albendazole alone and in combination for treatment of soil-transmitted helminths in pregnancy and adverse events: a randomized open label controlled intervention trial in Masindi district, western Uganda. *Am J Trop Med Hyg.* 2008 Dec;79(6):856–63.
84. Petersen E. Protozoan and helminth infections in pregnancy. Short-term and long-term implications of transmission of infection from mother to foetus. *Parasitology.* 2007 Dec;134(13):1855–62.
85. Maizels RM, Balic A, Gomez-Escobar N, Nair M, Taylor MD, Allen JE. Helminth parasites--masters of regulation. *Immunol Rev.* 2004 Oct;201:89–116.
86. Ndibazza J, Webb EL, Lule S, Mpairwe H, Akello M, Oduru G, et al. Associations Between Maternal Helminth and Malaria Infections in Pregnancy and Clinical Malaria in the Offspring: A Birth Cohort in Entebbe, Uganda. *J Infect Dis.* 2013 Dec 15;208(12):2007–16.
87. da Paz VRF, Sequeira D, Pyrrho A. Infection by *Schistosoma mansoni* during pregnancy: Effects on offspring immunity. *Life Sci.* 2017 Sep 15;185:46–52.
88. Elliott AM, Kizza M, Quigley MA, Ndibazza J, Nampijja M, Muhangi L, et al. The impact of helminths on the response to immunization and on the incidence

- of infection and disease in childhood in Uganda: design of a randomized, double-blind, placebo-controlled, factorial trial of deworming interventions delivered in pregnancy and early childhood [ISRCTN32849447]. *Clin Trials Lond Engl*. 2007;4(1):42–57.
89. Dickson R, Awasthi S, Williamson P, Demellweek C, Garner P. Effects of treatment for intestinal helminth infection on growth and cognitive performance in children: systematic review of randomised trials. *BMJ*. 2000 Jun 24;320(7251):1697–701.
 90. EZEAMAMA AE, FRIEDMAN JF, ACOSTA LP, BELLINGER DC, LANGDON GC, MANALO DL, et al. HELMINTH INFECTION AND COGNITIVE IMPAIRMENT AMONG FILIPINO CHILDREN. *Am J Trop Med Hyg*. 2005 May;72(5):540–8.
 91. Nampijja M, Apule B, Lule S, Akurut H, Muhangi L, Webb EL, et al. Effects of Maternal Worm Infections and Anthelmintic Treatment during Pregnancy on Infant Motor and Neurocognitive Functioning. *J Int Neuropsychol Soc*. 2012 Nov;18(6):1019–30.
 92. Smith SEP, Li J, Garbett K, Mirnics K, Patterson PH. Maternal Immune Activation Alters Fetal Brain Development through Interleukin-6. *J Neurosci*. 2007 Oct 3;27(40):10695–702.
 93. Chang S, Zeng L, Brouwer ID, Kok FJ, Yan H. Effect of iron deficiency anemia in pregnancy on child mental development in rural China. *Pediatrics*. 2013 Mar;131(3):e755-763.
 94. Le Doaré K, Bland R, Newell M-L. Neurodevelopment in children born to HIV-infected mothers by infection and treatment status. *Pediatrics*. 2012 Nov;130(5):e1326-1344.
 95. Murphy SK, Fineberg AM, Maxwell SD, Alloy LB, Zimmermann L, Krigbaum NY, et al. Maternal infection and stress during pregnancy and depressive symptoms in adolescent offspring. *Psychiatry Res*. 2017;257:102–10.
 96. Lau R, Chris RB, Phuong MS, Khatib A, Kopalakrishnan S, Bhasker S, et al. Treatment of soil-transmitted helminth infections in pregnancy: a systematic review and meta-analysis of maternal outcomes. *J Travel Med*. 2020 Mar 13;27(2).
 97. Imhoff-Kunsch B, Briggs V. Anthelmintics in pregnancy and maternal, newborn and child health. *Paediatr Perinat Epidemiol*. 2012 Jul;26 Suppl 1:223–38.
 98. Bloland PB, Williams HA, Population NRC (US) C on, Program on Forced Migration and Health at the Mailman School of Public Health CU. *Epidemiology of Malaria [Internet]. Malaria Control during Mass Population Movements and Natural Disasters*. National Academies Press (US); 2002 [cited 2021 May 18]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK221152/>
 99. World malaria report 2020 [Internet]. [cited 2021 May 18]. Available from: <https://www.who.int/publications-detail-redirect/9789240015791>

100. Prevention C-C for DC and. CDC - Malaria - About Malaria - Where Malaria Occurs [Internet]. 2020 [cited 2021 May 18]. Available from: <https://www.cdc.gov/malaria/about/distribution.html>
101. Prevention C-C for DC and. CDC - Malaria - About Malaria - Biology [Internet]. 2020 [cited 2021 May 18]. Available from: <https://www.cdc.gov/malaria/about/biology/index.html>
102. Florens L, Washburn MP, Raine JD, Anthony RM, Grainger M, Haynes JD, et al. A proteomic view of the Plasmodium falciparum life cycle. *Nature*. 2002 Oct 3;419(6906):520–6.
103. Vaughan AM, Aly ASI, Kappe SHI. Malaria parasite pre-erythrocytic stage infection: Gliding and Hiding. *Cell Host Microbe*. 2008 Sep 11;4(3):209–18.
104. Tangpukdee N, Duangdee C, Wilairatana P, Krudsood S. Malaria Diagnosis: A Brief Review. *Korean J Parasitol*. 2009 Jun;47(2):93–102.
105. Tarimo DS, Minjas JN, Bygbjerg IC. Malaria diagnosis and treatment under the strategy of the integrated management of childhood illness (IMCI): relevance of laboratory support from the rapid immunochromatographic tests of ICT Malaria P.f/P.v and OptiMal. *Ann Trop Med Parasitol*. 2001 Jul;95(5):437–44.
106. Prevention C-C for DC and. CDC - Malaria - Diagnosis & Treatment (United States) - Diagnosis (U.S.) [Internet]. 2019 [cited 2019 Jun 3]. Available from: https://www.cdc.gov/malaria/diagnosis_treatment/diagnostic_tools.html
107. Prevention C-C for DC and. CDC - Malaria - Diagnosis & Treatment (United States) - Diagnosis (U.S.) [Internet]. 2020 [cited 2021 May 18]. Available from: https://www.cdc.gov/malaria/diagnosis_treatment/diagnostic_tools.html
108. Prevention C-C for DC and. CDC - Malaria - Malaria Worldwide - How Can Malaria Cases and Deaths Be Reduced? - Insecticide-Treated Bed Nets [Internet]. 2019 [cited 2021 May 18]. Available from: https://www.cdc.gov/malaria/malaria_worldwide/reduction/itn.html
109. Gamble C, Ekwaru PJ, Garner P, Kuile FO ter. Insecticide-Treated Nets for the Prevention of Malaria in Pregnancy: A Systematic Review of Randomised Controlled Trials. *PLOS Med*. 2007 Mar 27;4(3):e107.
110. WHO | Insecticide-treated nets to reduce the risk of malaria in pregnant women [Internet]. WHO. World Health Organization; [cited 2021 May 18]. Available from: http://www.who.int/elena/titles/bednets_malaria_pregnancy/en/
111. Choi L, Pryce J, Garner P. Indoor residual spraying for preventing malaria in communities using insecticide-treated nets. *Cochrane Database Syst Rev* [Internet]. 2019 May 23 [cited 2021 May 18];2019(5). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6532761/>
112. Kleinschmidt I, Bradley J, Knox TB, Mnzava AP, Kafy HT, Mbogo C, et al. Implications of insecticide resistance for malaria vector control with long-lasting

- insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study. *Lancet Infect Dis*. 2018 Jun;18(6):640–9.
113. Anyanwu EC, Ehiri JE, Kanu I, Merrick J. Health effects of long-term exposure to insecticide-treated mosquito nets in the control of malaria in endemic regions, revised. *ScientificWorldJournal*. 2006 Dec 15;6:1631–41.
 114. Lu G, Traoré C, Meissner P, Kouyaté B, Kynast-Wolf G, Beiersmann C, et al. Safety of insecticide-treated mosquito nets for infants and their mothers: randomized controlled community trial in Burkina Faso. *Malar J*. 2015 Dec 30;14(1):527.
 115. WHO | Malaria in children under five [Internet]. WHO. World Health Organization; [cited 2021 May 18]. Available from: http://www.who.int/malaria/areas/high_risk_groups/children/en/
 116. WHO Guidelines for malaria [Internet]. [cited 2021 May 18]. Available from: <https://www.who.int/publications-detail-redirect/WHO-UCN-GMP-2021.01>
 117. RTS,S malaria vaccine reaches more than 650 000 children in Ghana, Kenya and Malawi through groundbreaking pilot programme [Internet]. [cited 2021 May 19]. Available from: <https://www.who.int/news/item/20-04-2021-rt-s-malaria-vaccine-reaches-more-than-650-000-children-in-ghana-kenya-and-malawi-through-groundbreaking-pilot-programme>
 118. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoia K, Brabin B, et al. Epidemiology and burden of malaria in pregnancy. *Lancet Infect Dis*. 2007 Feb;7(2):93–104.
 119. Dellicour S, Tatem AJ, Guerra CA, Snow RW, ter Kuile FO. Quantifying the number of pregnancies at risk of malaria in 2007: a demographic study. *PLoS Med*. 2010 Jan 26;7(1):e1000221.
 120. Rogerson SJ, Hviid L, Duffy PE, Leke RF, Taylor DW. Malaria in pregnancy: pathogenesis and immunity. *Lancet Infect Dis*. 2007 Feb 1;7(2):105–17.
 121. Walker-Abbey A, Djokam RRT, Eno A, Leke RFG, Titanji VPK, Fogako J, et al. MALARIA IN PREGNANT CAMEROONIAN WOMEN: THE EFFECT OF AGE AND GRAVIDITY ON SUBMICROSCOPIC AND MIXED-SPECIES INFECTIONS AND MULTIPLE PARASITE GENOTYPES. *Am J Trop Med Hyg*. 2005 Mar 1;72(3):229–35.
 122. Ayisi JG, Branch OH, Rafi-Janajreh A, van Eijk AM, ter Kuile FO, Rosen DH, et al. Does Infection with Human Immunodeficiency Virus Affect the Antibody Responses to Plasmodium falciparum Antigenic Determinants in Asymptomatic Pregnant Women? *J Infect*. 2003 Apr 1;46(3):164–72.
 123. Accrombessi M, Yovo E, Fievet N, Cottrell G, Agbota G, Gartner A, et al. Effects of Malaria in the First Trimester of Pregnancy on Poor Maternal and Birth Outcomes in Benin. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 2019 Sep 27;69(8):1385–93.

124. Ndam NT, Mbuba E, González R, Cisteró P, Kariuki S, Sevene E, et al. Resisting and tolerating *P. falciparum* in pregnancy under different malaria transmission intensities. *BMC Med.* 2017 17;15(1):130.
125. Ngai M, Weckman AM, Erice C, McDonald CR, Cahill LS, Sled JG, et al. Malaria in Pregnancy and Adverse Birth Outcomes: New Mechanisms and Therapeutic Opportunities. *Trends Parasitol.* 2020 Feb 1;36(2):127–37.
126. Desai M, Hill J, Fernandes S, Walker P, Pell C, Gutman J, et al. Prevention of malaria in pregnancy. *Lancet Infect Dis.* 2018 Apr;18(4):e119–32.
127. Bauserman M, Conroy AL, North K, Patterson J, Bose C, Meshnick S. An overview of malaria in pregnancy. *Semin Perinatol.* 2019 Aug 1;43(5):282–90.
128. WHO | Worldwide prevalence of anaemia 1993-2005 [Internet]. WHO. World Health Organization; [cited 2021 May 19]. Available from: http://www.who.int/nutrition/publications/micronutrients/anaemia_iron_deficiency/9789241596657/en/
129. Ekvall H. Malaria and anemia. *Curr Opin Hematol.* 2003 Mar;10(2):108–14.
130. Muehlenbachs A, Fried M, McGready R, Harrington WE, Mutabingwa TK, Nosten F, et al. A novel histological grading scheme for placental malaria applied in areas of high and low malaria transmission. *J Infect Dis.* 2010 Nov 15;202(10):1608–16.
131. Shulman CE, Marshall T, Dorman EK, Bulmer JN, Cutts F, Peshu N, et al. Malaria in pregnancy: adverse effects on haemoglobin levels and birthweight in primigravidae and multigravidae. *Trop Med Int Health TM IH.* 2001 Oct;6(10):770–8.
132. Uneke CJ. Impact of placental *Plasmodium falciparum* malaria on pregnancy and perinatal outcome in sub-Saharan Africa: part III: placental malaria, maternal health, and public health. *Yale J Biol Med.* 2008 Mar;81(1):1–7.
133. Organization WH. Iron deficiency anemia. Assessment, prevention, and control. *Guide Programme Manag.* 2001;47–62.
134. Moya-Alvarez V, Bodeau-Livinec F, Cot M. Iron and malaria: a dangerous liaison? *Nutr Rev.* 2016;74(10):612–23.
135. Nyakeriga AM, Troye-Blomberg M, Dorfman JR, Alexander ND, Bäck R, Kortok M, et al. Iron deficiency and malaria among children living on the coast of Kenya. *J Infect Dis.* 2004 Aug 1;190(3):439–47.
136. Menéndez C, Romagosa C, Ismail MR, Carrilho C, Saute F, Osman N, et al. An Autopsy Study of Maternal Mortality in Mozambique: The Contribution of Infectious Diseases. *PLOS Med.* 2008 Feb 19;5(2):e44.
137. Rogerson SJ. Management of malaria in pregnancy. *Indian J Med Res.* 2017 Sep;146(3):328–33.

138. Moore KA, Simpson JA, Scoullar MJL, McGready R, Fowkes FJI. Quantification of the association between malaria in pregnancy and stillbirth: a systematic review and meta-analysis. *Lancet Glob Health*. 2017 Nov;5(11):e1101–12.
139. Steketee RW, Nahlen BL, Parise ME, Menendez C. The burden of malaria in pregnancy in malaria-endemic areas. *Am J Trop Med Hyg*. 2001 Feb;64(1-2 Suppl):28–35.
140. Guyatt HL, Snow RW. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. *Clin Microbiol Rev*. 2004 Oct;17(4):760–9, table of contents.
141. Guyatt HL, Snow RW. Impact of malaria during pregnancy on low birth weight in sub-Saharan Africa. *Clin Microbiol Rev*. 2004 Oct;17(4):760–9, table of contents.
142. Cates JE, Unger HW, Briand V, Fievet N, Valea I, Tinto H, et al. Malaria, malnutrition, and birthweight: A meta-analysis using individual participant data. *PLOS Med*. 2017 Aug 8;14(8):e1002373.
143. Moeller SL, Nyengaard JR, Larsen LG, Nielsen K, Bygbjerg IC, Msemo OA, et al. Malaria in Early Pregnancy and the Development of the Placental Vasculature. *J Infect Dis*. 2019 Sep 26;220(9):1425–34.
144. Fried M, Kurtis JD, Swihart B, Pond-Tor S, Barry A, Sidibe Y, et al. Systemic Inflammatory Response to Malaria During Pregnancy Is Associated With Pregnancy Loss and Preterm Delivery. *Clin Infect Dis*. 2017 Oct 30;65(10):1729–35.
145. Elphinstone RE, Weckman AM, McDonald CR, Tran V, Zhong K, Madanitsa M, et al. Early malaria infection, dysregulation of angiogenesis, metabolism and inflammation across pregnancy, and risk of preterm birth in Malawi: A cohort study. *PLOS Med*. 2019 Oct 1;16(10):e1002914.
146. Moya-Alvarez V, Abellana R, Cot M. Pregnancy-associated malaria and malaria in infants: an old problem with present consequences. *Malar J*. 2014 Jul 11;13:271.
147. Hulbert TV. Congenital malaria in the United States: report of a case and review. *Clin Infect Dis Off Publ Infect Dis Soc Am*. 1992 Apr;14(4):922–6.
148. Malhotra I, Dent A, Mungai P, Wamachi A, Ouma JH, Narum DL, et al. Can prenatal malaria exposure produce an immune tolerant phenotype? A prospective birth cohort study in Kenya. *PLoS Med*. 2009 Jul;6(7):e1000116.
149. Feeney ME. The immune response to malaria in utero. *Immunol Rev*. 2020 Jan;293(1):216–29.
150. Gay F, Zougbedé S, N'dilimabaka N, Rebollo A, Mazier D, Moreno A. Cerebral malaria: what is known and what is on research. *Rev Neurol (Paris)*. 2012 Mar;168(3):239–56.
151. Accrombessi M, Ouédraogo S, Agbota GC, Gonzalez R, Massougbojji A, Menéndez C, et al. Malaria in Pregnancy Is a Predictor of Infant Haemoglobin Concentrations during the First Year of Life in Benin, West Africa. *PloS One*. 2015;10(6):e0129510.

152. Conroy A, Serghides L, Finney C, Owino SO, Kumar S, Gowda DC, et al. C5a Enhances Dysregulated Inflammatory and Angiogenic Responses to Malaria In Vitro: Potential Implications for Placental Malaria. *PLOS ONE*. 2009 Mar 24;4(3):e4953.
153. National Academies of Sciences E, Division H and M, Practice B on PH and PH, Approach C on AN and S-BS from PTECDAHE, Negussie Y, Geller A, et al. *Healthy Development from Conception Through Early Childhood* [Internet]. *Vibrant and Healthy Kids: Aligning Science, Practice, and Policy to Advance Health Equity*. National Academies Press (US); 2019 [cited 2021 May 24]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK551480/>
154. Normal Brain Development – The Alcohol Pharmacology Education Partnership [Internet]. [cited 2021 May 24]. Available from: <https://sites.duke.edu/apep/module-5-alcohol-and-babies/explore-more/normal-brain-development/>
155. Flavell JH. *The developmental psychology of Jean Piaget*. Princeton, NJ, US: D Van Nostrand; 1963. xvi, 472 p. (The developmental psychology of Jean Piaget).
156. National Research Council (US) and Institute of Medicine (US) Committee on Integrating the Science of Early Childhood Development. *From Neurons to Neighborhoods: The Science of Early Childhood Development* [Internet]. Shonkoff JP, Phillips DA, editors. Washington (DC): National Academies Press (US); 2000 [cited 2021 May 24]. Available from: <http://www.ncbi.nlm.nih.gov/books/NBK225557/>
157. Bedford H, Walton S, Ahn J. Measures of Child Development: A review. :104.
158. Boggs D, Milner KM, Chandna J, Black M, Cavallera V, Dua T, et al. Rating early child development outcome measurement tools for routine health programme use. *Arch Dis Child*. 2019 Apr 1;104(Suppl 1):S22–33.
159. Mandy M, Nyirenda M. Developmental Origins of Health and Disease: the relevance to developing nations. *Int Health*. 2018 Mar;10(2):66–70.
160. Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B, et al. Developmental potential in the first 5 years for children in developing countries. *Lancet Lond Engl*. 2007 Jan 6;369(9555):60–70.
161. Rosenzweig MR, Wolpin KI. Are There Increasing Returns to the Intergenerational Production of Human Capital? Maternal Schooling and Child Intellectual Achievement. *J Hum Resour*. 1994;29(2):670–93.
162. Boyle MH, Racine Y, Georgiades K, Snelling D, Hong S, Omariba W, et al. The influence of economic development level, household wealth and maternal education on child health in the developing world. *Soc Sci Med*. 2006 Oct 1;63(8):2242–54.
163. UNESCO Digital Library [Internet]. [cited 2021 May 24]. Available from: <https://unesdoc.unesco.org/ark:/48223/pf0000232205>
164. Plotnin R, Rutter M. Child Development, Molecular Genetics, and What to Do with Genes Once They Are Found. *Child Dev*. 1998;69(4):1223–42.

165. Twite MD, Stenquist S, Ing RJ. Williams syndrome. *Paediatr Anaesth*. 2019 May;29(5):483–90.
166. Angulo MA, Butler MG, Cataletto ME. Prader-Willi syndrome: a review of clinical, genetic, and endocrine findings. *J Endocrinol Invest*. 2015;38:1249–63.
167. Vorstman JAS, Ophoff RA. Genetic causes of developmental disorders. *Curr Opin Neurol*. 2013 Apr;26(2):128–36.
168. MSc HM, Pitchford NJ, Hagger MS, Marlow N. Development of Executive Function and Attention in Preterm Children: A Systematic Review. *Dev Neuropsychol*. 2009 Jul 1;34(4):393–421.
169. Guerra CC, Barros MC de M, Goulart AL, Fernandes LV, Kopelman BI, Santos AM dos. Premature infants with birth weights of 1500–1999 g exhibit considerable delays in several developmental areas. *Acta Paediatr*. 2014;103(1):e1–6.
170. Aylward GP. Neurodevelopmental Outcomes of Infants Born Prematurely. *J Dev Behav Pediatr*. 2014 Aug;35(6):394–407.
171. Caravale B, Tozzi C, Albino G, Vicari S. Cognitive development in low risk preterm infants at 3–4 years of life. *Arch Dis Child - Fetal Neonatal Ed*. 2005 Nov 1;90(6):F474–9.
172. de Kieviet JF, Piek JP, Aarnoudse-Moens CS, Oosterlaan J. Motor Development in Very Preterm and Very Low-Birth-Weight Children From Birth to Adolescence: A Meta-analysis. *JAMA*. 2009 Nov 25;302(20):2235–42.
173. Nosarti C, Giouroukou E, Micali N, Rifkin L, Morris RG, Murray RM. Impaired executive functioning in young adults born very preterm. *J Int Neuropsychol Soc*. 2007 Jul;13(4):571–81.
174. Oudgenoeg-Paz O, Mulder H, Jongmans MJ, van der Ham IJM, Van der Stigchel S. The link between motor and cognitive development in children born preterm and/or with low birth weight: A review of current evidence. *Neurosci Biobehav Rev*. 2017 Sep 1;80:382–93.
175. Leung MP, Thompson B, Black J, Dai S, Alsweller JM. The effects of preterm birth on visual development. *Clin Exp Optom*. 2018 Jan;101(1):4–12.
176. Iskusnykh IY, Buddington RK, Chizhikov VV. Preterm birth disrupts cerebellar development by affecting granule cell proliferation program and Bergmann glia. *Exp Neurol*. 2018 Aug;306:209–21.
177. Bonnar K, Fraser D. Extrauterine Growth Restriction in Low Birth Weight Infants. *Neonatal Netw*. 2019 Jan 1;38(1):27–33.
178. Sangtawesin V, Singarj Y, Kanjanapattanakul W. Growth and development of very low birth weight infants aged 18-24 months at Queen Sirikit National Institute of Child Health. *J Med Assoc Thai Chotmaihet Thangphaet*. 2011 Aug;94 Suppl 3:S101-106.

179. Cunha AB, Miquelote AF, Santos DCC. Motor affordance at home for infants living in poverty: A feasibility study. *Infant Behav Dev.* 2018 May 1;51:52–9.
180. Pereira KR, Valentini NC, Saccani R. Brazilian infant motor and cognitive development: Longitudinal influence of risk factors. *Pediatr Int.* 2016;58(12):1297–306.
181. Caçola PM, Gabbard C, Montebelo MIL, Santos DCC. The new affordances in the home environment for motor development - infant scale (AHEMD-IS): Versions in English and Portuguese languages. *Braz J Phys Ther.* 2015 Sep 1;19:507–25.
182. Church AT, Katigbak MS. Home environment, nutritional status and maternal intelligence as determinants of intellectual development in rural Philippine preschool children. *Intelligence.* 1991 Mar;15(1):49–78.
183. Stewart D. Depression during pregnancy. *Can Fam Physician.* 2005 Aug 10;51(8):1061–3.
184. Liu Y, Kaaya S, Chai J, McCoy DC, Surkan PJ, Black MM, et al. Maternal depressive symptoms and early childhood cognitive development: a meta-analysis. *Psychol Med.* 2017 Mar;47(4):680–9.
185. Azak S, Raeder S. Trajectories of parenting behavior and maternal depression. *Infant Behav Dev.* 2013 Jun 1;36(3):391–402.
186. Bass JK, Nakasujja N, Familiar-Lopez I, Sikorskii A, Murray SM, Opoka R, et al. Association of caregiver quality of care with neurocognitive outcomes in HIV-affected children aged 2-5 years in Uganda. *AIDS Care.* 2016;28 Suppl 1:76–83.
187. Guo N, Bindt C, Te Bonle M, Appiah-Poku J, Hinz R, Barthel D, et al. Association of antepartum and postpartum depression in Ghanaian and Ivorian women with febrile illness in their offspring: a prospective birth cohort study. *Am J Epidemiol.* 2013 Nov 1;178(9):1394–402.
188. Kadawathagedara M, Lauzon-Guillain B de, Botton J. Environmental contaminants and child's growth. *J Dev Orig Health Dis.* 2018 Dec;9(6):632–41.
189. de Assis Araujo MS, Figueiredo ND, Camara VM, Froes Asmus CIR. Maternal-child exposure to metals during pregnancy in Rio de Janeiro city, Brazil: The Rio Birth Cohort Study of Environmental Exposure and Childhood Development (PIPA project). *Environ Res.* 2020 Apr;183:109155.
190. Hertz-Picciotto I, Sass JB, Engel S, Bennett DH, Bradman A, Eskenazi B, et al. Organophosphate exposures during pregnancy and child neurodevelopment: Recommendations for essential policy reforms. *PLoS Med.* 2018 Oct;15(10):e1002671.
191. González-Alzaga B, Lacasaña M, Aguilar-Garduño C, Rodríguez-Barranco M, Ballester F, Rebagliato M, et al. A systematic review of neurodevelopmental effects of prenatal and postnatal organophosphate pesticide exposure. *Toxicol Lett.* 2014 Oct 15;230(2):104–21.

192. Koureas M, Tsakalof A, Tsatsakis A, Hadjichristodoulou C. Systematic review of biomonitoring studies to determine the association between exposure to organophosphorus and pyrethroid insecticides and human health outcomes. *Toxicol Lett.* 2012 Apr 25;210(2):155–68.
193. Blood Lead Levels in Children | Lead | CDC [Internet]. 2021 [cited 2021 Mar 23]. Available from: <https://www.cdc.gov/ncetd/lead/prevention/blood-lead-levels.htm>
194. Dórea JG. Environmental exposure to low-level lead (Pb) co-occurring with other neurotoxicants in early life and neurodevelopment of children. *Environ Res.* 2019 Oct;177:108641.
195. Tatsuta N, Nakai K, Kasanuma Y, Iwai-Shimada M, Sakamoto M, Murata K, et al. Prenatal and postnatal lead exposures and intellectual development among 12-year-old Japanese children. *Environ Res.* 2020 Oct;189:109844.
196. Shah S, Jeong KS, Park H, Hong Y-C, Kim Y, Kim B, et al. Environmental pollutants affecting children's growth and development: Collective results from the MOCEH study, a multi-centric prospective birth cohort in Korea. *Environ Int.* 2020 Apr;137:105547.
197. Mitro SD, Johnson T, Zota AR. Cumulative Chemical Exposures During Pregnancy and Early Development. *Curr Environ Health Rep.* 2015 Dec;2(4):367–78.
198. Kuong K, Fiorentino M, Perignon M, Chamnan C, Berger J, Sinuon M, et al. Cognitive Performance and Iron Status are Negatively Associated with Hookworm Infection in Cambodian Schoolchildren. *Am J Trop Med Hyg.* 2016 Oct 5;95(4):856–63.
199. Pabalan N, Singian E, Tabangay L, Jarjanazi H, Boivin MJ, Ezeamama AE. Soil-transmitted helminth infection, loss of education and cognitive impairment in school-aged children: A systematic review and meta-analysis. *PLoS Negl Trop Dis.* 2018;12(1):e0005523.
200. Boivin MJ. Effects of early cerebral malaria on cognitive ability in Senegalese children. *J Dev Behav Pediatr JDBP.* 2002 Oct;23(5):353–64.
201. Fernando SD, Rodrigo C, Rajapakse S. The “hidden” burden of malaria: cognitive impairment following infection. *Malar J.* 2010 Dec 20;9:366.
202. Kariuki SM, Abubakar A, Newton CRJC, Kihara M. Impairment of executive function in Kenyan children exposed to severe falciparum malaria with neurological involvement. *Malar J.* 2014 Sep 16;13:365.
203. Williams PL, Jesson J. Growth and pubertal development in HIV-infected adolescents. *Curr Opin HIV AIDS.* 2018 May;13(3):179–86.
204. Chandna J, Ntozini R, Evans C, Kandawasvika G, Chasekwa B, Majo F, et al. Effects of improved complementary feeding and improved water, sanitation and hygiene on early child development among HIV-exposed children: substudy of a cluster randomised trial in rural Zimbabwe. *BMJ Glob Health.* 2020;5(1):e001718.

205. Struyf T, Dube Q, Cromwell EA, Sheahan AD, Heyderman RS, Van Rie A. The effect of HIV infection and exposure on cognitive development in the first two years of life in Malawi. *Eur J Paediatr Neurol EJPN Off J Eur Paediatr Neurol Soc.* 2020 Mar;25:157–64.
206. Kaiser L, Allen LH, American Dietetic Association. Position of the American Dietetic Association: nutrition and lifestyle for a healthy pregnancy outcome. *J Am Diet Assoc.* 2008 Mar;108(3):553–61.
207. Abu-Saad K, Fraser D. Maternal nutrition and birth outcomes. *Epidemiol Rev.* 2010;32:5–25.
208. Millward DJ. Nutrition, infection and stunting: the roles of deficiencies of individual nutrients and foods, and of inflammation, as determinants of reduced linear growth of children. *Nutr Res Rev.* 2017 Jun;30(1):50–72.
209. Muktabhant B, Lawrie TA, Lumbiganon P, Laopaiboon M. Diet or exercise, or both, for preventing excessive weight gain in pregnancy. *Cochrane Database Syst Rev.* 2015 Jun 15;(6):CD007145.
210. Symington EA, Baumgartner J, Malan L, Zandberg L, Ricci C, Smuts CM. Nutrition during pregnancy and early development (NuPED) in urban South Africa: a study protocol for a prospective cohort. *BMC Pregnancy Childbirth [Internet].* 2018 Jul 24 [cited 2021 May 25];18. Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6056931/>
211. John CC, Black MM, Nelson CA. Neurodevelopment: The Impact of Nutrition and Inflammation During Early to Middle Childhood in Low-Resource Settings. *Pediatrics.* 2017 Apr;139.
212. Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, et al. Maternal and child undernutrition and overweight in low-income and middle-income countries. *Lancet Lond Engl.* 2013 Aug 3;382(9890):427–51.
213. Means RT. Iron Deficiency and Iron Deficiency Anemia: Implications and Impact in Pregnancy, Fetal Development, and Early Childhood Parameters. *Nutrients [Internet].* 2020 Feb 11 [cited 2021 May 25];12(2). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7071168/>
214. Janbek J, Sarki M, Specht IO, Heitmann BL. A systematic literature review of the relation between iron status/anemia in pregnancy and offspring neurodevelopment. *Eur J Clin Nutr.* 2019 Dec;73(12):1561–78.
215. Sania A, Sudfeld CR, Danaei G, Fink G, McCoy DC, Zhu Z, et al. Early life risk factors of motor, cognitive and language development: a pooled analysis of studies from low/middle-income countries. *BMJ Open.* 2019 Oct 1;9(10):e026449.
216. Zavaleta N, Astete-Robilliard L. [Effect of anemia on child development: long-term consequences]. *Rev Peru Med Exp Salud Publica.* 2017 Dec;34(4):716–22.

217. Allali S, Brousse V, Sacri A-S, Chalumeau M, de Montalembert M. Anemia in children: prevalence, causes, diagnostic work-up, and long-term consequences. *Expert Rev Hematol.* 2017;10(11):1023–8.
218. O. ter Kuile F, van Eijk AM, Filler SJ. Effect of Sulfadoxine-Pyrimethamine Resistance on the Efficacy of Intermittent Preventive Therapy for Malaria Control During Pregnancy A Systematic Review. *JAMA.* 2007 Jun 20;297(23):2603–16.
219. Sevene E, González R, Menéndez C. Current knowledge and challenges of antimalarial drugs for treatment and prevention in pregnancy. *Expert Opin Pharmacother.* 2010 Jun 1;11(8):1277–93.
220. McGready R, Cho T, Hkirijaroen L, Simpson J, Chongsuphajaisiddhi T, White NJ, et al. Quinine and mefloquine in the treatment of multidrug-resistant *Plasmodium falciparum* malaria in pregnancy. *Ann Trop Med Parasitol.* 1998 Sep 1;92(6):643–53.
221. ter Kuile FO, Nosten F, Luxemburger C, Kyle D, Teja-Isavatharm P, Phaipun L, et al. Mefloquine treatment of acute falciparum malaria: a prospective study of non-serious adverse effects in 3673 patients. *Bull World Health Organ.* 1995;73(5):631–42.
222. Briand V, Bottero J, Noël H, Masse V, Cordel H, Guerra J, et al. Intermittent treatment for the prevention of malaria during pregnancy in Benin: a randomized, open-label equivalence trial comparing sulfadoxine-pyrimethamine with mefloquine. *J Infect Dis.* 2009 Sep 15;200(6):991–1001.
223. Denoeud-Ndam L, Clément M-C, Briand V, Akakpo J, Agossou VK, Atadokpédé F, et al. Tolerability of Mefloquine Intermittent Preventive Treatment for Malaria in HIV-Infected Pregnant Women in Benin. *JAIDS J Acquir Immune Defic Syndr.* 2012 Sep 1;61(1):64–72.
224. Cahier Village RGPH4 2013.pdf [Internet]. [cited 2021 Mar 18]. Available from: <https://insae.bj/images/docs/insae-statistiques/demographiques/population/Effectifs%20de%20la%20population%20des%20villages%20et%20quartiers%20de%20ville%20du%20benin/Cahier%20Village%20RGPH4%202013.pdf>
225. Pojda JA, Kelley L, Nutrition AC on CS on. Low birthweight : In UN ACC Sub-Committee on Nutrition,; 2000 [cited 2021 Mar 18]. Available from: <http://digitallibrary.un.org/record/430400>
226. WHO Expert Committee on Physical Status : the Use and Interpretation of Anthropometry (1993 : Geneva S, Organization WH. Physical status : the use of and interpretation of anthropometry , report of a WHO expert committee [Internet]. World Health Organization; 1995 [cited 2021 Mar 18]. Available from: <https://apps.who.int/iris/handle/10665/37003>
227. Ballard JL, Khoury JC, Wedig K, Wang L, Eilers-Walsman BL, Lipp R. New Ballard Score, expanded to include extremely premature infants. *J Pediatr.* 1991 Sep;119(3):417–23.

228. Bodeau-Livinec F, Briand V, Berger J, Xiong X, Massougbodji A, Day KP, et al. Maternal anemia in Benin: prevalence, risk factors, and association with low birth weight. *Am J Trop Med Hyg.* 2011 Sep;85(3):414–20.
229. Ouédraogo S, Koura GK, Bodeau-Livinec F, Accrombessi MMK, Massougbodji A, Cot M. Maternal anemia in pregnancy: assessing the effect of routine preventive measures in a malaria-endemic area. *Am J Trop Med Hyg.* 2013 Feb;88(2):292–300.
230. Rahman MM, Abe SK, Rahman MS, Kanda M, Narita S, Bilano V, et al. Maternal anemia and risk of adverse birth and health outcomes in low- and middle-income countries: systematic review and meta-analysis^{1,2}. *Am J Clin Nutr.* 2016 Feb 1;103(2):495–504.
231. Gonzales GF, Steenland K, Tapia V. Maternal hemoglobin level and fetal outcome at low and high altitudes. *Am J Physiol Regul Integr Comp Physiol.* 2009 Nov;297(5):R1477-1485.
232. Mireku MO, Davidson LL, Boivin MJ, Zoumenou R, Massougbodji A, Cot M, et al. Prenatal Iron Deficiency, Neonatal Ferritin, and Infant Cognitive Function. *Pediatrics.* 2016;138(6).
233. Cox JL, Holden JM, Sagovsky R. Detection of postnatal depression. Development of the 10-item Edinburgh Postnatal Depression Scale. *Br J Psychiatry J Ment Sci.* 1987 Jun;150:782–6.
234. Bradley RH. Constructing and Adapting Causal and Formative Measures of Family Settings: The HOME Inventory as Illustration. *J Fam Theory Rev.* 2015 Dec 1;7(4):381–414.
235. Totsika V, Sylva K. The Home Observation for Measurement of the Environment Revisited. *Child Adolesc Ment Health.* 2004;9(1):25–35.
236. Bodeau-Livinec F, Glorennec P, Cot M, Dumas P, Durand S, Massougbodji A, et al. Elevated Blood Lead Levels in Infants and Mothers in Benin and Potential Sources of Exposure. *Int J Environ Res Public Health* [Internet]. 2016 Mar;13(3). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4808979/>
237. Doolan DL, Dobaño C, Baird JK. Acquired immunity to malaria. *Clin Microbiol Rev.* 2009 Jan;22(1):13–36, Table of Contents.
238. Pinkevych M, Petravic J, Chelimo K, Kazura JW, Moormann AM, Davenport MP. The Dynamics of Naturally Acquired Immunity to Plasmodium falciparum Infection. *PLoS Comput Biol* [Internet]. 2012 Oct 18 [cited 2021 Mar 23];8(10). Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3475668/>
239. Thurnham DI, McCabe LD, Haldar S, Wieringa FT, Northrop-Clewes CA, McCabe GP. Adjusting plasma ferritin concentrations to remove the effects of subclinical inflammation in the assessment of iron deficiency: a meta-analysis. *Am J Clin Nutr.* 2010 Sep;92(3):546–55.

240. Suchdev PS, Williams AM, Mei Z, Flores-Ayala R, Pasricha S-R, Rogers LM, et al. Assessment of iron status in settings of inflammation: challenges and potential approaches. *Am J Clin Nutr.* 2017 Dec 1;106(suppl_6):1626S-1633S.
241. Ahmadi S, Maman S, Zoumenou R, Massougbojji A, Cot M, Glorennec P, et al. Hunting, Sale, and Consumption of Bushmeat Killed by Lead-Based Ammunition in Benin. *Int J Environ Res Public Health.* 2018 Jun 1;15(6).
242. Wani AL, Ara A, Usmani JA. Lead toxicity: a review. *Interdiscip Toxicol.* 2015 Jun;8(2):55–64.
243. Growth reference 5-19 years - Weight-for-age (5-10 years) [Internet]. [cited 2021 Mar 25]. Available from: <https://www.who.int/tools/growth-reference-data-for-5to19-years/indicators/weight-for-age-5to10-years>
244. Bangirana P, Seggane-Musisi null, Allebeck P, Giordani B, John CC, Opoka OR, et al. A preliminary examination of the construct validity of the KABC-II in Ugandan children with a history of cerebral malaria. *Afr Health Sci.* 2009 Sep;9(3):186–92.
245. Bodeau-Livinec F, Davidson LL, Zoumenou R, Massougbojji A, Cot M, Boivin MJ. Neurocognitive testing in West African children 3-6 years of age: Challenges and implications for data analyses. *Brain Res Bull.* 2018 Apr 6;
246. Chernoff MC, Laughton B, Ratswana M, Familiar I, Fairlie L, Vhembo T, et al. Validity of Neuropsychological Testing in Young African Children Affected by HIV. *J Pediatr Infect Dis.* 2018 Sep;13(3):185–201.
247. Goodman R. The Strengths and Difficulties Questionnaire: A Research Note. *J Child Psychol Psychiatry.* 1997;38(5):581–6.
248. al-Haddad BJS, Jacobsson B, Chabra S, Modzelewska D, Olson EM, Bernier R, et al. Long-term Risk of Neuropsychiatric Disease After Exposure to Infection In Utero. *JAMA Psychiatry.* 2019 Jun 1;76(6):594–602.
249. Garrison A, Boivin M, Khoshnood B, Courtin D, Alao J, Mireku M, et al. Soil-transmitted helminth infection in pregnancy and long-term child neurocognitive and behavioral development: A prospective mother-child cohort in Benin. *PLoS Negl Trop Dis.* 2021 Mar 19;15(3):e0009260.
250. Singla DR, Kumbakumba E, Aboud FE. Effects of a parenting intervention to address maternal psychological wellbeing and child development and growth in rural Uganda: a community-based, cluster-randomised trial. *Lancet Glob Health.* 2015 Aug 1;3(8):e458–69.
251. Schwarz NG, Adegnika AA, Breitling LP, Gabor J, Agnandji ST, Newman RD, et al. Placental malaria increases malaria risk in the first 30 months of life. *Clin Infect Dis Off Publ Infect Dis Soc Am.* 2008 Oct 15;47(8):1017–25.
252. Holding PA, Kitsao-Wekulo PK. Describing the burden of malaria on child development: what should we be measuring and how should we be measuring it? *Am J Trop Med Hyg.* 2004 Aug;71(2 Suppl):71–9.

253. Kihara M, Carter JA, Newton CRJC. The effect of Plasmodium falciparum on cognition: a systematic review. *Trop Med Int Health TM IH*. 2006 Apr;11(4):386–97.
254. Zhang N, Baker HW, Tufts M, Raymond RE, Salihu H, Elliott MR. Early Childhood Lead Exposure and Academic Achievement: Evidence From Detroit Public Schools, 2008–2010. *Am J Public Health*. 2013 Mar;103(3):e72–7.
255. Nriagu J, Afeiche M, Linder A, Arowolo T, Ana G, Sridhar MKC, et al. Lead poisoning associated with malaria in children of urban areas of Nigeria. *Int J Hyg Environ Health*. 2008 Oct;211(5–6):591–605.
256. THE 17 GOALS | Sustainable Development [Internet]. [cited 2021 Apr 13]. Available from: <https://sdgs.un.org/goals>
257. Alao MJ, Ayivi B, Lacombe D. Management of Children with Intellectual and Developmental Disability in an African Setting [Internet]. *Latest Findings in Intellectual and Developmental Disabilities Research*. IntechOpen; 2012 [cited 2021 Jun 7]. Available from: <https://www.intechopen.com/books/latest-findings-in-intellectual-and-developmental-disabilities-research/management-of-children-with-intellectual-and-developmental-disability-in-an-african-setting>
258. Rassi C, Graham K, King R, Ssekitooleko J, Mufubenga P, Gudoi SS. Assessing demand-side barriers to uptake of intermittent preventive treatment for malaria in pregnancy: a qualitative study in two regions of Uganda. *Malar J*. 2016 Nov 4;15(1):530.
259. Arnaldo P, Cambe MI, Magaço A, Chicumbe S, Rovira-Vallbona E, Rosanas-Urgell A, et al. Access to and use of preventive intermittent treatment for Malaria during pregnancy: A qualitative study in the Chókwè district, Southern Mozambique. *PloS One*. 2019;14(1):e0203740.
260. Hill J, Kayentao K, Achieng F, Diarra S, Dellicour S, Diawara SI, et al. Access and use of interventions to prevent and treat malaria among pregnant women in Kenya and Mali: a qualitative study. *PloS One*. 2015;10(3):e0119848.

Appendices

Appendix 1. Summary of qualitative study PAIR.



Summary of PAIR Project:

"Treatment and prevention of parasitic infections and iron deficiency: a qualitative study on experiences, barriers, and the role of maternal depression among pregnant women in Benin"

In a previous project in the Allada area (TOVI project), we found high exposure of pregnant women and children to malaria, helminths and iron deficiency. We therefore propose to study the perceptions, uses and barriers to the use of malaria treatments, iron supplements and helminth treatments. We propose a qualitative study of the knowledge, experiences, perceptions, and barriers that women and their children face regarding preventive and treatment strategies for anemia and parasitic infections. This will allow for better interpretation of the findings and data collected from children at age six in the EXPLORE project.

Primary Objective:

1. To describe women's knowledge and perceptions of prevention and treatment of anemia and parasitic infections for themselves and their children.

Secondary Objectives:

1. To explore factors that influence adherence to these preventive strategies, including maternal depression.
2. To identify feasible strategies in Benin to deliver the drugs, adapt the best schedule and duration of prevention and treatment of anemia and parasitic infections.

The interview will be conducted by Amanda Garrison, PhD student, and Romeo Zoumenou, psychologist. The interview will last approximately one hour. The interview guide asks women about their knowledge, previous experiences, perceived barriers, and recommendations for improving prevention and treatment strategies for anemia and parasitic infections during pregnancy and childhood. The interviews will be conducted in the local language, Fon, and will be recorded and transcribed into French and English for analysis. The data analysis will have four steps: 1) Content reading; 2) Coding; 3) Data reduction and 4) Data display. No statistical analysis will be performed as this is a qualitative study.