UNIVERSITE DE YAOUNDE I
*****
FACULTE DES SCIENCES
****
DEPARTEMENT DE BIOCHIMIE
CENTRE DE RECHERCHE ET FORMATION DOCTORAL, SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT (CRFD-SVSE)

THE UNIVERSITY OF YAOUNDE I
*****
FACULTY OF SCIENCE
****
DEPARTMENT OF BIOCHEMISTRY
CENTER FOR RESEARCH AND GRADUATE STUDIES IN LIFE, HEALTH & ENVIRONMENTAL SCIENCES (CRFD-SVSE)

LABORATORY OF NUTRITION AND NUTRITIONAL BIOCHEMISTRY
LABORATOIRE DE NUTRITION ET BIOCHIMIE NUTRITIONNELLE

MOLECULAR PARASITOLOGY AND DISEASE VECTOR RESEARCH LABORATORY
THE BIOTECHNOLOGY CENTER
LABORATOIRE DE PARASITOLOGIE MOLECULAIRE ET DE RECHERCHE SUR LES MALADIES A TRANSMISSION VECTORIELLE, CENTRE DE BIOTECHNOLOGIE

RELATIONSHIP BETWEEN MALNUTRITION, ANAEMIA AND ANTI-MALARIAL IMMUNOGLOBULIN G (IgG) ANTIBODY RESPONSE IN MALARIA-INFECTED UNDER-TEN CHILDREN, IN THE NORTH REGION OF CAMEROON

THESIS

Presented in partial fulfillment of the requirements for the award of the Degree of Doctorat/Ph.D. in Biochemistry

Specialty: Nutrition

By

NOBELLE IKOMBE SAKWE
(M.Sc. in Biochemistry)
Registration Nº: 11R1291

Supervisors

BIGOGA JUDE
Professor
University of Yaoundé I

GOUADO INOCENT
Professor
University of Douala

Academic Year: 2021/2022

UNIVERSITE DE YAOUNDE I
*****
FACULTE DES SCIENCES
****
DEPARTEMENT DE BIOCHIMIE
CENTRE DE RECHERCHE ET FORMATION DOCTORAL, SCIENCE DE LA VIE, SANTE ET ENVIRONNEMENT (CRFD-SVSE)

THE UNIVERSITY OF YAOUNDE I
*****
FACULTY OF SCIENCE
****
DEPARTMENT OF BIOCHEMISTRY
CENTER FOR RESEARCH AND GRADUATE STUDIES IN LIFE, HEALTH & ENVIRONMENTAL SCIENCES (CRFD-SVSE)

LABORATORY OF NUTRITION AND NUTRITIONAL BIOCHEMISTRY
LABORATOIRE DE NUTRITION ET BIOCHIMIE NUTRITIONNELLE

MOLECULAR PARASITOLOGY AND DISEASE VECTOR RESEARCH LABORATORY
THE BIOTECHNOLOGY CENTER
LABORATOIRE DE PARASITOLOGIE MOLECULAIRE ET DE RECHERCHE SUR LES MALADIES A TRANSMISSION VECTORIELLE, CENTRE DE BIOTECHNOLOGIE

RELATIONSHIP BETWEEN MALNUTRITION, ANAEMIA AND ANTI-MALARIAL IMMUNOGLOBULIN G (IgG) ANTIBODY RESPONSE IN MALARIA-INFECTED UNDER-TEN CHILDREN, IN THE NORTH REGION OF CAMEROON

THESIS

Presented in partial fulfillment of the requirements for the award of the Degree of Doctorat/Ph.D. in Biochemistry

Specialty: Nutrition

By

NOBELLE IKOMBE SAKWE
(M.Sc. in Biochemistry)
Registration N°: 11R1291

Supervisors

BIGOGA JUDE
Professor
University of Yaoundé I

GOUADO INOCENT
Professor
University of Douala

Academic Year: 2021/2022

UNIVERSITÉ DE YAOUNDE I

CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE, SANTE ET ENVIRONNEMENT

UNITE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE

DEPARTEMENT DE BIOCHIMIE

UNIVERSITY OF YAOUNDE I

CENTRE FOR RESEARCH AND TRAINING IN GRADUATE STUDIES IN LIFE, HEALTH AND ENVIRONMENTAL SCIENCES

RESEARCH AND DOCTORATE TRAINING UNIT IN LIFE SCIENCES

DEPARTMENT OF BIOCHEMISTRY

ATTESTATION DE CORRECTION

Nous soussignés : Pr MOUNDIPA FEWOU Paul, Président et Pr DJUIDJE Marceline NGOUNOUE, examinateur du jury, attestons que Mme. **Nobelle IKOMBE SAKWE**, matricule **11R1291** a effectué les corrections conformément aux exigences du jury de sa thèse de Doctorat/Ph.D en Biochimie, option Sciences de Aliments et Nutrition, soutenue le 13 Décembre 2021 à 08h30 dans la salle S01/02 de la Faculté des Sciences de l'Université de Yaoundé I sur le thème « **Relationship between malnutrition, anaemia and anti-malarial immunoglobulin G (IgG) antibody response in malaria-infected under-ten children, in the North Region of Cameroon».**

En foi de quoi la présente attestation lui est établie pour servir et valoir ce que de droit.

Yaoundé le 28 MARS 2022

Examinateur

Marceline Djuidje-Ngounoue, Ph.D.
Associate Professor, University of Yaoundé I
Fulbright Scholar Alumna, Yale University, USA

Président du jury

Moundipa Fewou Paul
Professeur
Enzymologie - Toxicologie

Chef de Département

Moundipa Fewou Paul
Professor
Enzymology-Toxicology

UNIVERSITE DE YAOUNDE I
UNIVERSITY OF YAOUNDE I
Département de Biochimie
Department of Biochemistry
Le Chef de Département
The Head of Department
FACULTE DES SCIENCES
FACULTY OF SCIENCES

UNIVERSITÉ DE YAOUNDÉ I **Faculté des Sciences** Division de la Programmation et du Suivi des Activités Académiques		THE UNIVERSITY OF YAOUNDE I **Faculty of Science** Division of Programming and Follow-up of Academic Affaires
LISTE DES ENSEIGNANTS PERMANENTS		**LIST OF PERMANENT TEACHING STAFF**

ANNÉE ACADEMIQUE 2021/2022

(Par Département et par Grade)

DATE D'ACTUALISATION 22 septembre2021

ADMINISTRATION

DOYEN : TCHOUANKEU Jean- Claude, *Maître de Conférences*
VICE-DOYEN / DPSAA :ATCHADE Alex de Théodore, *Maître de Conférences*
VICE-DOYEN / DSSE :NYEGUE Maximilienne Ascension, *Professeur*
VICE-DOYEN / DRC : ABOSSOLO Monique, *Maître de Conférences*
Chef Division Administrative et Financière : NDOYE FOE Marie C. F., *Maître de Conférences*
Chef Division des Affaires Académiques, de la Scolarité et de la Recherche DAASR :
AJEAGAH Gideon AGHAINDUM, *Professeur*

1- DÉPARTEMENT DE BIOCHIMIE (BC) (38)

N°	NOMS ET PRÉNOMS	GRADE	OBSERVATIONS
1	BIGOGA DAIGA Jude	Professeur	En poste
2	FEKAM BOYOM Fabrice	Professeur	En poste
3	FOKOU Elie	Professeur	En poste
4	KANSCI Germain	Professeur	En poste
5	MBACHAM FON Wilfried	Professeur	En poste
6	MOUNDIPA FEWOU Paul	Professeur	Chef de Département
7	NINTCHOM PENLAP V. épse BENG	Professeur	En poste
8	OBEN Julius ENYONG	Professeur	En poste
9	ACHU Merci BIH	Maître de Conférences	En poste
10	ATOGHO Barbara Mma	Maître de Conférences	En poste
11	AZANTSA KINGUE GABIN BORIS	Maître de Conférences	En poste
12	BELINGA née NDOYE FOE M. C. F.	Maître de Conférences	Chef DAF / FS
13	BOUDJEKO Thaddée	Maître de Conférences	En poste
14	DJUIDJE NGOUNOUE Marcelline	Maître de Conférences	En poste
15	EFFA NNOMO Pierre	Maître de Conférences	En poste
16	**EWANE Cécile Anne**	Maître de Conférences	En poste
17	MOFOR née TEUGWA Clotilde	Maître de Conférences	Inspecteur de Service MINESUP
18	NANA Louise épouse WAKAM	Maître de Conférences	En poste
19	NGONDI Judith Laure	Maître de Conférences	En poste
20	NGUEFACK Julienne	Maître de Conférences	En poste
21	NJAYOU Frédéric Nico	Maître de Conférences	En poste
22	TCHANA KOUATCHOUA Angèle	Maître de Conférences	En poste
23	AKINDEH MBUH NJI	Chargé de Cours	En poste
24	BEBEE Fadimatou	Chargée de Cours	En poste
25	BEBOY EDJENGUELE Sara Nathalie	Chargé de Cours	En poste

25	DAKOLE DABOY Charles	Chargé de Cours	En poste
26	DJUIKWO NKONGA Ruth Viviane	Chargée de Cours	En poste
27	DONGMO LEKAGNE Joseph Blaise	Chargé de Cours	En poste
28	FONKOUA Martin	Chargé de Cours	En poste
29	KOTUE KAPTUE Charles	Chargé de Cours	En poste
30	LUNGA Paul KEILAH	Chargé de Cours	En poste
31	MANANGA Marlyse Joséphine	Chargée de Cours	En poste
32	MBONG ANGIE M. Mary Anne	Chargée de Cours	En poste
33	Palmer MASUMBE NETONGO	Chargé de Cours	En poste
34	PECHANGOU NSANGOU Sylvain	Chargé de Cours	En poste

35	MBOUCHE FANMOE Marceline Joëlle	Assistante	En poste
36	OWONA AYISSI Vincent Brice	Assistant	En poste
37	WILFRIED ANGIE Abia	Assistante	En poste

2- DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE ANIMALES (BPA) (46)

1	AJEAGAH Gideon AGHAINDUM	Professeur	*DAARS/FS*
2	BILONG BILONG Charles-Félix	Professeur	Chef de Département
3	DIMO Théophile	Professeur	En Poste
4	DJIETO LORDON Champlain	Professeur	En Poste
5	**DZEUFIET DJOMENI Paul Désiré**	Professeur	En Poste
6	ESSOMBA née NTSAMA MBALA	Professeur	*Vice Doyen/FMSB/UYI*
7	FOMENA Abraham	Professeur	En Poste
8	KAMTCHOUING Pierre	Professeur	En poste
9	**KEKEUNOU Sévilor**	Professeur	En poste
10	NJAMEN Dieudonné	Professeur	En poste
11	NJIOKOU Flobert	Professeur	En Poste
12	NOLA Moïse	Professeur	En poste
13	TAN Paul VERNYUY	Professeur	En poste
14	TCHUEM TCHUENTE Louis Albert	Professeur	*Inspecteur de service* *Coord.Progr./MINSANTE*
15	ZEBAZE TOGOUET Serge Hubert	Professeur	*En poste*

16	BILANDA Danielle Claude	Maître de Conférences	En poste
17	DJIOGUE Séfirin	Maître de Conférences	En poste
18	JATSA BOUKENG Hermine épse MEGAPTCHE	Maître de Conférences	En Poste
19	**LEKEUFACK FOLEFACK Guy B.**	Maître de Conférences	En poste
20	MEGNEKOU Rosette	Maître de Conférences	En poste
21	MONY Ruth épse NTONE	Maître de Conférences	En Poste
22	NGUEGUIM TSOFACK Florence	Maître de Conférences	En poste
23	TOMBI Jeannette	Maître de Conférences	En poste

24	ALENE Désirée Chantal	Chargée de Cours	En poste
25	ATSAMO Albert Donatien	Chargé de Cours	En poste

26	BELLET EDIMO Oscar Roger	Chargé de Cours	En poste
27	DONFACK Mireille	Chargée de Cours	En poste
28	ETEME ENAMA Serge	Chargé de Cours	En poste
29	GOUNOUE KAMKUMO Raceline	Chargée de Cours	En poste
30	KANDEDA KAVAYE Antoine	Chargé de Cours	En poste
31	MAHOB Raymond Joseph	Chargé de Cours	En poste
32	MBENOUN MASSE Paul Serge	Chargé de Cours	En poste
33	MOUNGANG LucianeMarlyse	Chargée de Cours	En poste
34	MVEYO NDANKEU Yves Patrick	Chargé de Cours	En poste
35	NGOUATEU KENFACK Omer Bébé	Chargé de Cours	En poste
36	NGUEMBOK	Chargé de Cours	En poste
37	NJUA Clarisse Yafi	Chargée de Cours	Chef Div. UBA
38	NOAH EWOTI Olive Vivien	Chargée de Cours	En poste
39	TADU Zephyrin	Chargé de Cours	En poste
40	TAMSA ARFAO Antoine	Chargé de Cours	En poste
41	YEDE	Chargé de Cours	En poste

42	BASSOCK BAYIHA Etienne Didier	Assistant	En poste
43	ESSAMA MBIDA Désirée Sandrine	Assistante	En poste
44	KOGA MANG DOBARA	Assistant	En poste
45	LEME BANOCK Lucie	Assistante	En poste
46	YOUNOUSSA LAME	Assistant	En poste

3- DÉPARTEMENT DE BIOLOGIE ET PHYSIOLOGIE VÉGÉTALES (BPV) (33)

1	AMBANG Zachée	Professeur	Chef Division/UYII
2	BELL Joseph Martin	Professeur	En poste
3	DJOCGOUE Pierre François	Professeur	En poste
4	**MBOLO Marie**	Professeur	En poste
5	MOSSEBO Dominique Claude	Professeur	En poste
6	YOUMBI Emmanuel	Professeur	Chef de Département
7	ZAPFACK Louis	Professeur	En poste

8	ANGONI Hyacinthe	Maître de Conférences	En poste
9	BIYE Elvire Hortense	Maître de Conférences	En poste
10	KENGNE NOUMSI Ives Magloire	Maître de Conférences	En poste
11	MALA Armand William	Maître de Conférences	En poste
12	MBARGA BINDZI Marie Alain	Maître de Conférences	CT/ MINESUP
13	NDONGO BEKOLO	Maître de Conférences	*CE / MINRESI*
14	NGODO MELINGUI Jean Baptiste	Maître de Conférences	En poste
15	NGONKEU MAGAPTCHE Eddy L.	Maître de Conférences	En poste
16	TONFACK Libert Brice	Maître de Conférences	En poste
17	TSOATA Esaïe	Maître de Conférences	En poste

18	DJEUANI Astride Carole	Chargé de Cours	En poste
19	GOMANDJE Christelle	Chargée de Cours	En poste
20	MAFFO MAFFO Nicole Liliane	Chargé de Cours	En poste
21	MAHBOU SOMO TOUKAM. Gabriel	Chargé de Cours	En poste

22	NGALLE Hermine BILLE	Chargée de Cours	En poste
23	NGOUO Lucas Vincent	Chargé de Cours	En poste
24	NNANGA MEBENGA Ruth Laure	Chargé de Cours	En poste
25	NOUKEU KOUAKAM Armelle	Chargé de Cours	En poste
26	ONANA JEAN MICHEL	Chargé de Cours	En poste

27	GODSWILL NTSOMBAH NTSEFONG	Assistant	En poste
28	KABELONG BANAHO Louis-Paul-Roger	Assistant	En poste
29	KONO Léon Dieudonné	Assistant	En poste
30	LIBALAH Moses BAKONCK	Assistant	En poste
31	LIKENG-LI-NGUE Benoit C	Assistant	En poste
32	TAEDOUNG Evariste Hermann	Assistant	En poste
33	TEMEGNE NONO Carine	Assistant	En poste

4- DÉPARTEMENT DE CHIMIE INORGANIQUE (CI) (33)

1	AGWARA ONDOH Moïse	Professeur	*Chef de Département*
2	**DJOUFAC WOUMFO Emmanuel**	Professeur	En poste
3	Florence UFI CHINJE épouse MELO	Professeur	*Recteur Univ.Ngaoundere*
4	GHOGOMU Paul MINGO	Professeur	*Ministre Chargé deMiss.PR*
5	NANSEU Njiki Charles Péguy	Professeur	En poste
6	NDIFON Peter TEKE	Professeur	*CT MINRESI*
7	NDIKONTAR Maurice KOR	Professeur	*Vice-Doyen Univ. Bamenda*
8	NENWA Justin	Professeur	En poste
9	NGAMENI Emmanuel	Professeur	*DOYEN FS UDs*
10	NGOMO Horace MANGA	Professeur	*Vice Chancelor/UB*

11	**ACAYANKA Elie**	Maître de Conférences	En poste
12	BABALE née DJAM DOUDOU	Maître de Conférences	*Chargée Mission P.R.*
13	EMADACK Alphonse	Maître de Conférences	En poste
14	KAMGANG YOUBI Georges	Maître de Conférences	En poste
15	KEMMEGNE MBOUGUEM Jean C.	Maître de Conférences	En poste
16	KONG SAKEO	Maître de Conférences	En poste
17	NDI NSAMI Julius	Maître de Conférences	En poste
18	NJIOMOU C. épse DJANGANG	Maître de Conférences	En poste
19	NJOYA Dayirou	Maître de Conférences	En poste
20	**TCHAKOUTE KOUAMO Hervé**	Maître de Conférences	En poste

21	BELIBI BELIBI Placide Désiré	Chargé de Cours	CS/ ENS Bertoua
22	CHEUMANI YONA Arnaud M.	Chargé de Cours	En poste
23	KENNE DEDZO GUSTAVE	Chargé de Cours	En poste
24	KOUOTOU DAOUDA	Chargé de Cours	En poste
25	MAKON Thomas Beauregard	Chargé de Cours	En poste
26	MBEY Jean Aime	Chargé de Cours	En poste
27	NCHIMI NONO KATIA	Chargé de Cours	En poste

28	NEBA nee NDOSIRI Bridget NDOYE	Chargée de Cours	CT/ MINFEM
29	NYAMEN Linda Dyorisse	Chargée de Cours	En poste
30	PABOUDAM GBAMBIE A.	Chargée de Cours	En poste

31	NJANKWA NJABONG N. Eric	Assistant	En poste
32	PATOUOSSA ISSOFA	Assistant	En poste
33	SIEWE Jean Mermoz	Assistant	En Poste

5- DÉPARTEMENT DE CHIMIE ORGANIQUE (CO) (34)			
1	DONGO Etienne	Professeur	Vice-Doyen/FSE/UYI
2	GHOGOMU TIH Robert Ralph	Professeur	Dir. IBAF/UDA
3	NGOUELA Silvère Augustin	Professeur	Chef de Département UDS
4	NYASSE Barthélemy	Professeur	En poste
5	PEGNYEMB Dieudonné Emmanuel	Professeur	*Directeur/ MINESUP/* Chef de Département
6	WANDJI Jean	Professeur	En poste

7	Alex de Théodore ATCHADE	Maître de Conférences	Vice-Doyen / DPSAA
8	**AMBASSA Pantaléon**	Maître de Conférences	En poste
9	EYONG Kenneth OBEN	Maître de Conférences	En poste
10	FOLEFOC Gabriel NGOSONG	Maître de Conférences	En poste
11	FOTSO WABO Ghislain	Maître de Conférences	En poste
12	KEUMEDJIO Félix	Maître de Conférences	En poste
13	KEUMOGNE Marguerite	Maître de Conférences	En poste
14	KOUAM Jacques	Maître de Conférences	En poste
15	MBAZOA née DJAMA Céline	Maître de Conférences	En poste
16	MKOUNGA Pierre	Maître de Conférences	En poste
17	**MVOT AKAK CARINE**	Maître de Conférences	En poste
18	NGO MBING Joséphine	Maître de Conférences	Sous/Direct. MINERESI
19	NGONO BIKOBO Dominique Serge	Maître de Conférences	**C.E/** MINESUP
20	NOTE LOUGBOT Olivier Placide	Maître de Conférences	C.S/ MINESUP
21	NOUNGOUE TCHAMO Diderot	Maître de Conférences	En poste
22	TABOPDA KUATE Turibio	Maître de Conférences	En poste
23	**TAGATSING FOTSING Maurice**	Maître de Conférences	En poste
24	TCHOUANKEU Jean-Claude	Maître de Conférences	*Doyen /FS/ UYI*
25	TIH née NGO BILONG E. Anastasie	Maître de Conférences	En poste
26	YANKEP Emmanuel	Maître de Conférences	En poste
27	**ZONDEGOUMBA Ernestine**	Maître de Conférences	En poste

28	KAMTO Eutrophe Le Doux	Chargé de Cours	En poste
29	NGNINTEDO Dominique	Chargé de Cours	En poste
30	NGOMO Orléans	Chargée de Cours	En poste
31	OUAHOUO WACHE Blandine M.	Chargée de Cours	En poste
32	SIELINOU TEDJON Valérie	Chargé de Cours	En poste

33	MESSI Angélique Nicolas	Assistant	En poste

34	TSEMEUGNE Joseph	Assistant	En poste

6- DÉPARTEMENT D'INFORMATIQUE (IN) (25)

1	ATSA ETOUNDI Roger	Professeur	*Chef Div.MINESUP*
2	FOUDA NDJODO Marcel Laurent	Professeur	*Chef Dpt ENS/Chef IGA.MINESUP*

3	NDOUNDAM Réné	Maître de Conférences	En poste

4	ABESSOLO ALO'O Gislain	Chargé de Cours	En poste
5	AMINOU Halidou	Chargé de Cours	*Chef de Département*
6	DJAM Xaviera YOUH - KIMBI	Chargé de Cours	En Poste
7	**DOMGA KOMGUEM Rodrigue**	Chargé de Cours	En poste
8	EBELE Serge Alain	Chargé de Cours	En poste
9	KOUOKAM KOUOKAM E. A.	Chargé de Cours	En poste
10	MELATAGIA YONTA Paulin	Chargé de Cours	En poste
11	MONTHE DJIADEU Valery M.	Chargé de Cours	En poste
12	MOTO MPONG Serge Alain	Chargé de Cours	En poste
13	OLLE OLLE Daniel Claude Delort	Chargé de Cours	Directeur adjoint Enset. Ebolowa
14	TAPAMO Hyppolite	Chargé de Cours	En poste
15	TINDO Gilbert	Chargé de Cours	En poste
16	TSOPZE Norbert	Chargé de Cours	En poste
17	WAKU KOUAMOU Jules	Chargé de Cours	En poste

18	BAYEM Jacques Narcisse	Assistant	En poste
19	EKODECK Stéphane Gaël Raymond	Assistant	En poste
20	HAMZA Adamou	Assistant	En poste
21	JIOMEKONG AZANZI Fidel	Assistant	En poste
22	MAKEMBE. S . Oswald	Assistant	En poste
23	MESSI NGUELE Thomas	Assistant	En poste
24	MEYEMDOU Nadège Sylvianne	Assistante	En poste
25	NKONDOCK. MI. BAHANACK.N.	Assistant	En poste

7- DÉPARTEMENT DE MATHÉMATIQUES (MA) (30)

1	**AYISSI Raoult Domingo**	Professeur	Chef de Département
2	EMVUDU WONO Yves S.	Professeur	*Inspecteur MINESUP*

3	**KIANPI Maurice**	Maître de Conférences	En poste
4	**MBANG Joseph**	Maître de Conférences	En poste
5	MBEHOU Mohamed	Maître de Conférences	En poste
6	**MBELE BIDIMA Martin Ledoux**	Maître de Conférences	En poste
7	NKUIMI JUGNIA Célestin	Maître de Conférences	En poste
8	NOUNDJEU Pierre	Maître de Conférences	*Chef service des programmes & Diplômes/FS/UYI*
9	TCHAPNDA NJABO Sophonie B.	Maître de Conférences	Directeur/AIMS Rwanda
10	**TCHOUNDJA Edgar Landry**	Maître de Conférences	En poste

11	AGHOUKENG JIOFACK Jean Gérard	Chargé de Cours	Chef Cellule MINPLAMAT
12	CHENDJOU Gilbert	Chargé de Cours	En poste
13	DJIADEU NGAHA Michel	Chargé de Cours	En poste
14	DOUANLA YONTA Herman	Chargé de Cours	En poste
15	FOMEKONG Christophe	Chargé de Cours	En poste
16	KIKI Maxime Armand	Chargé de Cours	En poste
17	MBAKOP Guy Merlin	Chargé de Cours	En poste
18	MENGUE MENGUE David Joe	Chargé de Cours	En poste
19	NGUEFACK Bernard	Chargé de Cours	En poste
20	NIMPA PEFOUKEU Romain	Chargée de Cours	En poste
21	POLA DOUNDOU Emmanuel	Chargé de Cours	En poste
22	TAKAM SOH Patrice	Chargé de Cours	En poste
23	TCHANGANG Roger Duclos	Chargé de Cours	En poste
24	TETSADJIO TCHILEPECK M. E.	Chargé de Cours	En poste
25	TIAYA TSAGUE N. Anne-Marie	Chargée de Cours	En poste

26	BITYE MVONDO Esther Claudine	Assistante	En poste
27	MBATAKOU Salomon Joseph	Assistant	En poste
28	MBIAKOP Hilaire George	Assistant	En poste
29	MEFENZA NOUNTU Thiery	Assistant	En poste
30	TCHEUTIA Daniel Duviol	Assistant	En poste

8- DÉPARTEMENT DE MICROBIOLOGIE (MIB) (18)			
1	ESSIA NGANG Jean Justin	Professeur	*Chef de Département*
2	**NYEGUE Maximilienne Ascension**	Professeur	*VICE-DOYEN / DSSE*
3	**NWAGA Dieudonné M.**	Professeur	En poste
4	**ASSAM ASSAM Jean Paul**	Maître de Conférences	En poste
5	BOYOMO ONANA	Maître de Conférences	En poste
6	RIWOM Sara Honorine	Maître de Conférences	En poste
7	SADO KAMDEM Sylvain Leroy	Maître de Conférences	En poste
8	BODA Maurice	Chargé de Cours	En poste
9	BOUGNOM Blaise Pascal	Chargé de Cours	En poste
10	ESSONO OBOUGOU Germain G.	Chargé de Cours	En poste
11	NJIKI BIKOÏ Jacky	Chargée de Cours	En poste
12	TCHIKOUA Roger	Chargé de Cours	En poste
13	ESSONO Damien Marie	Assistant	En poste
14	LAMYE Glory MOH	Assistant	En poste
15	MEYIN A EBONG Solange	Assistante	En poste
16	NKOUDOU ZE Nardis	Assistant	En poste
17	SAKE NGANE Carole Stéphanie	Assistante	En poste
18	TOBOLBAÏ Richard	Assistant	En poste

9. DEPARTEMENT DE PYSIQUE(PHY) (40)			
1	BEN- BOLIE Germain Hubert	Professeur	En poste
2	DJUIDJE KENMOE épouse ALOYEM	Professeur	En poste
3	EKOBENA FOUDA Henri Paul	Professeur	*Vice-Recteur. UN*
4	ESSIMBI ZOBO Bernard	Professeur	En poste
5	KOFANE Timoléon Crépin	Professeur	En poste
6	NANA ENGO Serge Guy	Professeur	En poste
7	**NANA NBENDJO Blaise**	Professeur	En poste
8	NDJAKA Jean Marie Bienvenu	Professeur	Chef de Département
9	NJANDJOCK NOUCK Philippe	Professeur	En poste
10	NOUAYOU Robert	Professeur	En poste
11	PEMHA Elkana	Professeur	En poste
12	TABOD Charles TABOD	Professeur	Doyen FS Univ/Bda
13	TCHAWOUA Clément	Professeur	En poste
14	WOAFO Paul	Professeur	En poste
15	ZEKENG Serge Sylvain	Professeur	En poste

16	BIYA MOTTO Frédéric	Maître de Conférences	DG/HYDRO Mekin
17	BODO Bertrand	Maître de Conférences	En poste
18	**ENYEGUE A NYAM épse BELINGA**	Maître de Conférences	En poste
19	EYEBE FOUDA Jean sire	Maître de Conférences	En poste
20	FEWO Serge Ibraïd	Maître de Conférences	En poste
21	HONA Jacques	Maître de Conférences	En poste
22	MBANE BIOUELE César	Maître de Conférences	En poste
23	**MBINACK Clément**	Maître de Conférences	En poste
24	NDOP Joseph	Maître de Conférences	En poste
25	SAIDOU	Maître de Conférences	MINRESI
26	SIEWE SIEWE Martin	Maître de Conférences	En poste
27	SIMO Elie	Maître de Conférences	En poste
28	VONDOU Derbetini Appolinaire	Maître de Conférences	En poste
29	WAKATA née BEYA Annie	Maître de Conférences	*Directeur/ENS/UYI*

30	ABDOURAHIMI	Chargé de Cours	En poste
31	**CHAMANI Roméo**	Chargé de Cours	En poste
32	EDONGUE HERVAIS	Chargé de Cours	En poste
33	FOUEDJIO David	Chargé de Cours	Chef Cell. MINADER
34	MBONO SAMBA Yves Christian U.	Chargé de Cours	En poste
35	MELI'I Joelle Larissa	Chargée de Cours	En poste
36	MVOGO ALAIN	Chargé de Cours	En poste
37	OBOUNOU Marcel	Chargé de Cours	DA/Univ Inter Etat/Sangmalima
38	WOULACHE Rosalie Laure	Chargée de Cours	En poste

39	AYISSI EYEBE Guy François Valérie	Assistant	En poste
40	TEYOU NGOUPOU Ariel	Assistant	En poste

10- DÉPARTEMENT DE SCIENCES DE LA TERRE (ST) (43)

1	BITOM Dieudonné	Professeur	*Doyen / FASA / UDs*
2	FOUATEU Rose épse YONGUE	Professeur	En poste
3	NDAM NGOUPAYOU Jules-Remy	Professeur	En poste
4	NDJIGUI Paul Désiré	Professeur	Chef de Département
5	NGOS III Simon	Professeur	En poste
6	NKOUMBOU Charles	Professeur	En poste
7	NZENTI Jean-Paul	Professeur	En poste

8	ABOSSOLO née ANGUE Monique	Maître de Conférences	*Vice-Doyen / DRC*
9	BISSO Dieudonné	Maître de Conférences	*Directeur/Projet Barrage Memve'ele*
10	EKOMANE Emile	Maître de Conférences	En poste
11	GANNO Sylvestre	Maître de Conférences	En poste
12	GHOGOMU Richard TANWI	Maître de Conférences	CD/Uma
13	MOUNDI Amidou	Maître de Conférences	*CT/ MINIMDT*
14	NGUEUTCHOUA Gabriel	Maître de Conférences	CEA/MINRESI

15	NJILAH Isaac KONFOR	Maître de Conférences	En poste
16	NYECK Bruno	Maître de Conférences	En poste
17	ONANA Vincent Laurent	Maître de Conférences	*Chef service Maintenance & du Matériel/UYII*
18	**TCHAKOUNTE J. épse NOUMBEM**	Maître de Conférences	*Chef.cell / MINRESI*
19	TCHOUANKOUE Jean-Pierre	Maître de Conférences	En poste
20	TEMDJIM Robert	Maître de Conférences	En poste
21	YENE ATANGANA Joseph Q.	Maître de Conférences	*Chef Div. /MINTP*
22	ZO'O ZAME Philémon	Maître de Conférences	*DG/ART*

23	ANABA ONANA Achille Basile	Chargé de Cours	En poste
24	BEKOA Etienne	Chargé de Cours	En poste
25	ELISE SABABA	Chargé de Cours	En poste
26	ESSONO Jean	Chargé de Cours	En poste
27	EYONG JOHN TAKEM	Chargé de Cours	En poste
28	FUH Calistus Gentry	Chargé de Cours	*Sec. D'Etat/MINMIDT*
29	LAMILEN BILLA Daniel	Chargé de Cours	En poste
30	MBESSE CECILE OLIVE	Chargée de Cours	En poste
31	MBIDA YEM	Chargé de Cours	En poste
32	METANG Victor	Chargé de Cours	En poste
33	MINYEM Dieudonné-Lucien	Chargé de Cours	*CD/Uma*
34	NGO BELNOUN Rose Noël	Chargée de Cours	En poste
35	NGO BIDJECK Louise Marie	Chargée de Cours	En poste
36	NOMO NEGUE Emmanuel	Chargé de Cours	En poste
37	NTSAMA ATANGANA Jacqueline	Chargé de Cours	En poste
38	TCHAPTCHET TCHATO De P.	Chargé de Cours	En poste
39	TEHNA Nathanaël	Chargé de Cours	En poste
40	TEMGA Jean Pierre	Chargé de Cours	En poste

41	FEUMBA Roger	Assistant	En poste
42	MBANGA NYOBE Jules	Assistant	En poste

Répartition chiffrée des Enseignants de la Faculté des Sciences de l'Université de Yaoundé I

NOMBRE D'ENSEIGNANTS					
DÉPARTEMENT	**Professeurs**	**Maîtres de Conférences**	**Chargés de Cours**	**Assistants**	**Total**
BCH	8 (01)	14 (10)	13 (05)	3 (02)	**38 (18)**
BPA	15 (01)	8 (06)	18 (05)	05 (02)	**46 (14)**
BPV	07 (01)	10(01)	9 (06)	07 (01)	**33 (9)**
CI	10(01)	10(02)	10 (02)	03 (0)	**33 (5)**
CO	6 (0)	21 (05)	05 (02)	02 (0)	**34(7)**
IN	2 (0)	1 (0)	14 (01)	08 (01)	**25 (2)**
MAT	2 (0)	8 (0)	15 (01)	05 (02)	**30 (3)**
MIB	3 (0)	4 (02)	05 (01)	06 (02)	**18 (5)**
PHY	15 (0)	14 (02)	09 (03)	02 (0)	**40 (5)**
ST	7 (1)	15 (01)	18 (05)	02 (0)	**42(7)**
Total	**75 (5)**	**105 (29)**	**116 (31)**	**43 (10)**	**339 (75)**

Soit un total de **339 (**75**)** dont :

- Professeurs **75 (5)**
- Maîtres de Conférences **105(**29**)**
- Chargés de Cours **116 (31)**
- Assistants **43**(10**)**

( ) = Nombre de Femmes **75**

DEDICATION

TO:

GOD ALMIGHTY

ACKNOWLEDGEMENTS

I wish to sincerely appreciate some people without whom this work would not have been accomplished.

Firstly, my Supervisors, Professor BIGOGA Jude, Molecular Parasitology and Disease Vector Research Laboratory, who has taught me the virtue of perfection and for the rigorous training he passed me through. You brought out the best in me and made sure I always meet up with tight deadlines. You stopped at nothing to make sure that this work was properly and meticulously realized**.** Professor GOUADO Inocent, Laboratory of Food Science and Nutrition, University of Douala, who took time out of his very busy schedule, each time I needed his attention. Your finishing touches added meaning and value to this work. Professor Ngondi Judith-Laure, Laboratory of Nutrition and Nutritional Biochemistry, I specially acknowledge your guidance and support right from the conception of this work. I am immensely grateful to Professor OBEN Julius, Head of the Laboratory of Nutrition and Nutritional Biochemistry who encouraged me to enrol for this Ph.D. programme.

Professor Jean Claude MBANYA, Director of the Biotechnology Centre, Nkolbisson and all the staff of the institution, for granting me access into the center.

I also express my sincere thanks to the Head of Department of Biochemistry, Professor MOUNDIPA FEWOU Paul and all the Lecturers of the Department of Biochemistry of the University of Yaounde I, for all I learnt through them.

I am equally grateful to Drs. Djélé SALI, KOUAMBENG Célestin, all the staff/team of the National Malaria Control Programme of the North Region of Cameroon as well as all the study participants in the study area for the kind assistance you provided during the field work.

Thank you to my fellow laboratory mates of the Laboratory of Nutrition and Nutritional Biochemistry; M'BOPDA Momdjo Christelle, NKOUGNI Tchinda Jacob, CHAKOKAM Raoul, ESSOLA Nadine, ESSOUMAN Florine, BEYEGUE Eric, NGUIMKENG Boris, NGO-NLEND Marguerite as well as Molecular Parasitology and Disease Vector Research Laboratory; TABUE Raymond, FODJO Barrière, SEUMEN Clovis, ESEMU Livo, ZEUKENG Francis, NANFACK Ferdinand, TEDJOU Armel, NJEAMBOSAY Boris, for all your assistance and contributions in the realization of this work; I enjoyed working with you all.

The research team also express their appreciation to Professor GHOGOMO S.M of the University of Buea for the 3D7 antigen used in the ELISA assays.

I specially acknowledge my parents especially my father, Professor P. N. SAKWE, Pioneer Dean of the Faculty of Agriculture and Veterinary Medicine, University of Buea. Thank you, Daddy, for moulding me into the Nobelle that I am today. I'll remain forever grateful to you. Your shoes are quite big but I'll do my best to follow your footsteps and carry on with the baton which you have handed down to me.

My heartfelt thanks go to my siblings in the U.S.A, Chantal SAKWE, Louis SAKWE and Joy SAKWE. Honestly, family is gold. You have all been so supportive. You all never stopped praying for me and encouraging me, every step of the way. I couldn't wish for more.

Dr. Palmer MASUMBE, I am grateful to you for being a wonderful big brother to me, always holding my hand and pulling me along. To Marceline MASUMBE, I can't thank you enough for always being there for me throughout this PhD programme.

All those who helped me in one way or the other but whose names haven't been listed, please accept my sincere gratitude. I truly appreciate all you did for me. Thank you and God bless you all.

FUNDING SOURCE

This study received financial support from: *WHO/Bill and Melinda Gates Foundation.*

Grant award number: AFCMR 1207011-36.1-54497518

Through Prosper Ndong of the National Malaria Control Programme

To the Molecular Parasitology and Disease Vector Research Laboratory

Through Professor Bigoga Jude Daiga.

TABLE OF CONTENTS

ABSTRACT

Malaria, malnutrition and anaemia are key public-health challenges in paediatric populations in sub - Saharan Africa, with a high burden in the North Region of Cameroon. Nonetheless studies of interaction between malaria and malnutrition have remained complex and controversial. Besides, no study so far has explored the interaction between child malnutrition and specific anti-*P. falciparum* immune responses in the North Region. Thus, this study investigates the relationship between nutritional status, anaemia and anti-malarial IgG antibody response in malaria-infected under-ten children living in six localities within Pitoa and Mayo-oulo Health Districts, in the North Region of Cameroon. Accordingly, a cross-sectional survey was conducted during the peak season in 2014, involving 362 children aged 6months - 10 years. A structured questionnaire was used to assess Socio-economic status (SES). Anthropometric measurements were taken using standard methods and nutritional status assessed by calculating Height for Age (HA), Weight for Age (WA) and Weight for Height (WH) z-scores based on WHO reference values, to determine stunting, underweight and wasting respectively. Blood samples were analysed for PCV, malariometric indices (prevalence and density) by microscopy, *Plasmodium* genotyping and anti-malarial IgG1 and IgG3 antibody response by ELISA. The prevalence of malaria, malnutrition and anaemia was 32.9%, 54.1% and 20.6%, respectively. Stunting, underweight and wasting were prevalent in 56.9%, 63.5% and 34.8%, respectively of the participants. The prevalence of mild, moderate and severe anaemia was 8.1%, 9.2% and 3.3%, respectively. Malaria and malnutrition associated significantly [OR=1.89, (95% CI: 1.12–3.19); (p=0.017)] and vice versa [OR=2.07, (95% CI: 1.22 – 3.53) ;(p=0.007)]. No significant (p>0.05) associations between mean parasite density and stunting (r = -0.010; p=0.851), underweight (r = -0.005; p=0.922) or wasting (r = -0.002; p=0.973) were observed. While parasiataemia was significantly higher in the mildly wasted (p=0.031) compared to the moderately and severely wasted counterparts, haemoglobin levels did not vary (p>0.05) in stunted, underweight or wasted children when compared to well-nourished. Anti-malarial IgG1 and 3 responses were similar in malaria infected and non-infected as well as in malnourished versus well-nourished children (p>0.05). This study highlights a synergistic relationship between malaria and malnutrition warranting effective collaboration between nutritional intervention and malaria control programmes for better case management and reduced socio-economic burden.

Keywords: Malaria, malnutrition, anaemia, IgG responses, Cameroon.

RESUMÉ

Le paludisme, la malnutrition et l'anémie sont des problèmes de santé publique majeurs au sein des populations infantiles en Afrique sub-saharienne, avec une lourde charge dans la région du Nord-Cameroun. Les études sur les interactions entre le paludisme et la malnutrition sont restées complexes et controversées. En outre, aucune étude n'a jusqu'à présent exploré interaction entre la malnutrition de l'enfant et les réponses immunitaires spécifiques anti- *P. falciparum* au Nord-Cameroun. Ainsi, cette étude recherche la relation entre le statut nutritionnelle, l'anémie et la réponse des anti-corps anti-palustres IgG chez les enfants de moins de 10 ans infectés par le paludisme dans six localités des districts de santé de Pitoa et Mayo-Oulo, région du Nord-Cameroon. Ainsi, une étude transversale a été menée pendant la haute saison de forte incidence palustre de 2014, auprès de 362 enfants âgés de 6 mois à 10 ans. Un questionnaire structuré a été utilisé pour évaluer le statut socio-économique (SSE). Les mesures anthropométriques ont été prises en utilisant les méthodes standard et l'état nutritionnel a été évalué en calculant les z-scores de la Taille pour l'Age (TA), du Poids pour l'Age (PA) et du Poids pour la Taille (PT) pour déterminer respectivement le retard de croissance, l'insuffisance pondérale et l'émaciation. Les échantillons de sang ont été analysés pour le PCV, les indices malariométriques (prévalence et densité) par microscopie, ainsi que pour l'extraction de l'ADN plasmodial à partir des papiers filtre pour la PCR. La réponse en anticorps antipaludiques IgG (IgG1 et IgG3) par la technique ELISA. La prévalence du paludisme, de la malnutrition et de l'anémie était respectivement de 32,9%, 54,1% et 20,6%. Le retard de croissance, l'insuffisance pondérale et l'émaciation étaient prévalents chez 56,9%, 63,5% et 34,8% respectivement. La prévalence de l'anémie légère, modérée et sévère était respectivement de 8,1%, 9,2% et 3,3%. Le paludisme et la malnutrition étaient significativement associés [OR=1.89, (95% CI : 1.12-3.19) ; (p=0.017)] et vice-versa [OR=2,07, (IC 95 % : 1,22 - 3,53) ; (p=0,007)]. Aucune association significative (p>0.05) n'a été observée entre la densité parasitaire moyenne et le retard de croissance (r = -0,010 ; p=0,851), l'insuffisance pondérale (r = -0,005 ; p=0,922) ou l'émaciation (r = -0,002 ; p=0,973). Alors que, la parasitémie était significativement plus élevée chez les enfants souffrant d'émaciation légère(p=0,031) comparé à leurs homologues souffrant d'émaciation modérée et sévère, les taux d'hémoglobine ne variaient pas (p>0.05) chez les enfants souffrant de retard de croissance, d'insuffisance pondérale ou d'émaciation par rapport aux enfants normale. Les réponses aux anticorps antipaludiques IgG1 et 3 étaient similaires chez les enfants infectés et non infectés, ainsi que chez les enfants malnutris et bien nourris (p>0.05). Cette étude met en évidence une relation synergique entre les programmes d'intervention nutritionnelle et de contrôle du paludisme pour une meilleure prise en charge des cas et une réduction du fardeau socio-économique

Mots clés : Paludisme, malnutrition, anémie, réponses aux IgG, Cameroun**.**

LIST OF ABBREVIATIONS

Abbreviation	Full Name
ACT	Artemisinin- based Combination Therapy
ANC	Ante- Natal Care
Bp	Base pairs
DNA	Deoxyribonucleic acid
dNTPs Mix	Deoxynucleoside triphosphate mixture (dATP, dTTP, dCTP and dGTP)
ELISA	Enzyme-Linked Immunosorbent Assay
EtBr	Ethidium Bromide
GNT	Global Nutrition Targets
H/A	Height- for – Age
Hb	Haemoglobin
IPT_p	Intermittent Preventive Treatment for Malaria in pregnancy
LLINs	Long- Lasting Insecticidal Nets
NMCP	National Malaria Control Programme
OR	Odd Ratios
PBS	Phosphate Buffered Saline
PBS-T	Phosphate Buffered Saline –Tween 20
PCR	Polymerase Chain Reaction

PCV	Packed Cell Volume
P-npp	Para- Nitro- Phenyl- Phosphate
RBC	Red Blood Cell
RDT	Rapid Diagnostic Test
RPM	Revolutions per minute
SDG	Sustainable Development Goal
SES	Socio-Economic Status
SMART	Standardised Monitoring and Assessment of Relief and Transitions
SMC	Seasonal Malaria Chemoprevention
SPSS	Statistical Package for Social Science
TBE	Tris Borate EDTA
TE	Tris EDTA
UV	Ultra Violet
W/A	Weight – for – Age
W/H	Weight –for – Height
WBC	White Blood Cell
WHA	World Health Assembly
WHO	World Health Organization

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

INTRODUCTION

BACKGROUND INFORMATION

Malaria, malnutrition and anaemia are key public-health challenges in paediatric populations in sub-Saharan Africa (Deribew *et al.,* 2010) which may compromise immune responses, with malaria and under nutrition being the two major causes of childhood mortality. However, the relationship between malaria and malnutrition is controversial and complex (Das *et al.*, 2018), while the impact of malnutrition on these responses is poorly understood.

These three diseases are tightly linked to poverty (Bigoga *et al.,* 2012). Poor feeding in children greatly contributes to the development of infectious diseases most especially malaria as well as weakened immune systems that are unable to fight off infection (Thorarinsdottir *et al.*, 2005). However, infection with malaria generally affects the red blood cells, leading to the evolution of anaemia which can be worsened in children that are malnourished, (Deribew *et al.*, 2010) thereby down-regulating immune responses against malaria.

Malaria is the most serious vector borne disease known to mankind and is tightly intertwined with poverty, (Bigoga *et al*., 2012) often afflicting populations that are impoverished and malnourished (Shankar*,* 2000). Despite considerable national and international control efforts, it still remains a major threat to human health (Mfonkeu *et al.,* 2008).

The World Malaria Report of 2021 indicates that there were 241 million cases of infection globally leading to 627.000 cases of death, which were both higher than what was reported in 2019. Ninety-six (96) % of this burden was reported in Africa, amongst which 80% were in children less than 5 years old with a burden of 2.9% of malaria cases reported in Cameroon (WMR, 2021; WHO, 2019), showing that the burden continues to rise. In Cameroon, malaria is a major public health challenge with a national prevalence of 23.6%, and it is responsible for 24% of consultations, 45% hospitalizations, and 13% hospital related deaths of which 10%

deaths are observed in children less than 5 years old (NMCP, 2018). Prevalence rates also vary from one location to another (Eyong *et al.,* 2016) with the Cameroon Demographic and Health Survey of 2018 reporting a prevalence of 26% for malaria in under -five children in the North region (DHS, 2018). Earlier studies in the Garoua, Pitoa and Mayo-oulo health districts observed an overall prevalence of 31.15% for malaria infection in children (Tabue *et al.,* 2019), which is estimated to have declined from the 36% reported in 2012 and 2013 by the National Malaria Control Programme (NMCP, 2018). Implying that it is still a serious health problem in the area. Because its roots lie deep within human communities, malaria is a unique disease which causes chronic anaemia, impaired growth and delayed development in young children (Holding and Kitsao, 2004) who are often the most affected due to poor and weak immune systems that are unable to fight off the infection (Shankar, 2000).

On the other hand, globally, child malnutrition is attributable to about half of mortality among children aged under five years (UNICEF *et al.,* 2020) and more than 80% of countries face different forms of malnutrition (Ireri *et al.,* 2021). Globally the prevalence of stunting, overweight and wasting in children under-five is 21.9%, 5.9% and 23% respectively (UNICEF *et al.,* 2019). Under nutrition, most especially under-five malnutrition is considered the most common form of malnutrition in low and middle-income countries and it continues to remain high (WHO, 2019). However, the World Health Assembly has as ultimate goal for all children to be free of malnutrition in all its forms by 2030. In as much as stunting rates are dropping, 149 million children around the world are still affected. There are 40 million overweight children in the world; about 10 million more than there were 2 decades ago while wasting still threatens the lives of 49 million children across the globe (UNICEF *et al.,* 2019). Africa experiences high levels (36%) and it is postulated that poverty and ignorance are primary casual factors of malnutrition (Kimani-Murage *et al.,* 2011). Although Cameroon has made some progress towards achieving the global nutrition target of stunting, 28.9% of

children under 5 of age are still affected. In the same line, Cameroon is on course for the target for wasting, with 4.3% of children under 5 years of age affected (Global Nutrition Report 2020). The most recent 2021 Standardized Monitoring and Assessment of Relief and Transitions (SMART) nutritional survey report revealed a prevalence of 40.2% for stunting and 4.8% for wasting in children in the North Region (SMART, 2021) which aligns with the Cameroon Demographic and Health Survey report of 2018 (DHS, 2018). Also, a previous study in the area reported that prevalence rates of stunting, underweight and wasting stood at 37.7%, 26.6% and 6.6% respectively in under five children (Ngwa-Akonwi *et al.,* 2015), showing that malnutrition rates continue to remain high. The health of children is affected by lack of adequate micronutrients and it is common knowledge that nutrition plays a major role in maintaining health, but contrarily speaking, malnutrition appears to generate vulnerability to a wide variety of diseases and general ill health.

The relationship between malnutrition and malaria is controversial, complex and poorly understood (Nyakeriga *et al*., 2004; Das *et al.,* 2018). On one hand, malaria may cause malnutrition, whereas on the other hand, malnutrition itself may enhance susceptibility to the disease (Sumbele *et al.,* 2015). Some studies even report no effect at all of malnutrition on malaria and vice-versa (Deribew *et al.,* 2010; Kateera *et al.,* 2015; Nyaaba *et al.,* 2017; O'brien *et al.,* 2018). These contradictions pose the specific need for this study, to provide a better understanding of this complex relationship in these co-morbidities.

Childhood anaemia is considered a severe public health problem in sub-Saharan Africa (62.5%) and in Cameroon in particular where prevalence of 57% was reported in 2018 (WHO, 2018). This figure does not significantly differ from the 60% reported in 2011, implying that there is no change, it is stagnant. Also, the Cameroon Demographic Health Survey report of 2018 revealed a prevalence of 56.5% for anaemia in children living in the North Region. Malaria causes a substantial proportion of anaemia observed in malaria endemic settings

(Njunda *et al.,* 2016, Sumbele *et al.,* 2016). Moreso, anaemia impairs normal development in children and it constitutes a major public health problem in young children in the developing world with wide social and economic implications (Leilei *et al*., 2014), Cameroon inclusive. Furthermore, Asoba *et al.,* (2019) in the Mount Cameroon area, reported a prevalence as high as 77.3% in children while Jourdan *et al.,* even reported prevalences as high as 82% in children in Adamawa (Jourdan *et al.,* 2008). Therefore, this study will be relevant to guide in the prevention and timely management of anaemia, in a bid to achieve sustainable development goal 3 of 2030, in promoting well-being and ensuring healthy lives for all at all ages.

Children living in malaria-endemic regions are born with some level of immunity (partial immunity) due to the trans-placental transfer of Total IgG from mother to child. Between the ages of 4 to 6 months, this partial immunity wanes drastically, exposing the children to more risk and making way for each child to develop his own acquired immunity (Aribot, 1996). Acquired immunity depends on how much exposure each child gets. This level of acquired immunity may slow down in case of malnutrition and the situation may worsen upon infection with malaria, leading to the destruction of red blood cells, giving rise to anaemia. The Gamma immunoglobulin (IgG) antibodies are known to play a crucial role in protection against malaria by decreasing parasite load and clinical malaria (Kwenti *et al.,* 2019). The cytophilic antibodies IgG1 and IgG3 are considered to be the most important and have been shown to be associated with lower risk of infection and symptoms, unlike IgG4 which has been shown to be associated with severity. It is widely recognized that malnutrition compromises the immune function. There is also some evidence now that child malnutrition and particularly stunting, may down-regulate the anti-*Plasmodium falciparum* antibody response resulting in higher risk of infection (Field *et al.,* 2002; Cunningham- Rundles *et al.,* 2005; Fillol *et al.,* 2009, Juster *et al.,* 2017).

Whether under nutrition or over nutrition, both affect the immune system by decreasing immunity and this renders the children more vulnerable to infections. When a child is malnourished, he/she may be unable to mount an appropriate immune response to the malaria parasite due to; reduction in T-lymphocytes, impairment of antibody formation, decreased complement formation, and atrophy of the thymus /lymphoid tissues. (Caulfield, 2004)

PROBLEM STATEMENT AND JUSTIFICATION

Numerous studies by Genton *et al.,* (1998) in New Papua Guinea; Deen *et al.,* (2002) in rural Gambia; Caulfield *et al.,* (2004) ; Nyakeriga *et al.,* (2004) in the Coast of Kenya **;** Friedman *et al.,* (2005) in Western Kenya; Kamugisha *et al.,* (2006) in North - Eastern Tanzania; Ehrhardt *et al.,* (2006) in Ghana**;** Deribew *et al.,* (2010) in South-Western Ethiopia; Sumbele *et al.,* (2015) in South-Western Cameroon; Das *et al.,* (2018); have all investigated the problem of malaria related to nutritional status and anaemia in children, in different regions of the world, focusing on ≤ 5year olds; a few on adults and adolescents and very little in the age group between 5 and 10 years old. Most of whose findings have been inconsistent (Deribew *et al.,* 2010) and the studies so far have been very controversial, complex and poorly understood (Das *et al.,* 2018). While some studies report that malaria may cause malnutrition, others believe that malnutrition may enhance susceptibility to malaria. In contrast, very few studies have explored the interaction between child malnutrition and specific anti -*P. falciparum* immune responses. Moreover, the results of those studies are conflicting (Carswell *et al.,* 1981; Dominguez- Vazquez *et al,* 1990; Blair *et al.,* 2003; Plebanski *et al.,* 2008; Fillol *et al.,* 2009; Juster *et al.,* 2017; Tepa *et al.,* 2020). While some studies report that nutritional status could modulate immune responses directed to malaria antigens, other studies believe that malnutrition has no impact on antibody response to *Plasmodium falciparum*. Understanding the complex relationship of the immune response of individuals infected with malaria and

suffering from malnutrition is crucial to guide specific anti-malarial therapeutic approaches in the vulnerable sub-populations.

In Cameroon, however, where malaria, malnutrition and anaemia are major public health problems and individual studies have been conducted on malaria, malnutrition, anaemia and immunity, no study so far, to the best of our knowledge, has been reported on the epidemiological interaction between these interconnected health determinants with regards to child malnutrition and specific anti -*P. falciparum* immune responses, especially in the North Region of Cameroon, a typical area of high malaria transmission which bears a high burden of malnutrition. Therefore, the present study sought to investigate the relationship between malnutrition, anaemia and IgG responses amongst malaria-infected under-ten children in 2 Health districts (Pitoa and Mayo- Oulo) in the north region of Cameroon. In addition, it will generate baseline data which will serve as a guide for future studies, policy makers, programme planners and implementers, to design timely and appropriate nutrition intervention programs as well as provide improved strategies for better malaria control especially in the vulnerable populations.

RESEARCH QUESTIONS

1. How common is malaria, malnutrition and anaemia in under-ten children within 3 localities in each health district and what are the associated risk factors?

2. How does nutritional status influence malaria parasitaemia and the presence of anaemia; malaria parasitaemia influence anaemia severity in the study population?

3. What is the effect of malaria, nutritional status and its varying severity levels on the prevalence of anti-malarial IgG1 and IgG3 antibody responses to the crude 3D7 *P. falciparum* antigen amongst these children?

HYPOTHESIS

We hypothesized that;

There is a linear relationship between malnutrition, anaemia and immunity in malaria-infected under-ten children in the North Region of Cameroon.

OVERALL OBJECTIVE

To assess the relationship between malnutrition, anaemia and anti-malarial IgG antibody responses in malaria- infected under-ten children in two health districts (Pitoa and Mayo-oulo), in the North Region of Cameroon, for improved case management of malaria and malnutrition and reduced socio-economic burden.

SPECIFIC OBJECTIVES

1. To determine the prevalence of malaria, malnutrition and anaemia as well as their associated risk factors in under-ten children within 3 localities in each health district, during the peak malaria transmission period.

2. To evaluate the influence of nutritional status on malaria parasitaemia, nutritional status on the presence of anaemia as well as malaria parasitaemia on anaemia severity in the study population.

3. To evaluate the effect of malaria, nutritional status and its varying severity levels on anti-malarial IgG1 and IgG3 antibody responses to crude *Plasmodium falciparum* 3D7 antigen amongst the enrolled children.

SCIENTIFIC RELEVANCE

This study generates preliminary baseline information needed for future studies by policy makers, programme planners / implementers and NGO's for strategic planning and implementation of rapid scale-up of already proven nutrition-specific and nutrition-sensitive

interventions and improved malaria control measures for the vulnerable children in the country.

It addresses Global Nutrition Targets (GNTs) 1 and 6 of 2025 (WHO, 2014) as well as Sustainable Development Goals (SDG) 3, 4, 10 and 17 of 2030.

GNT 1: reducing by 40%, the number of children <5 who are stunted.

GNT 6: reducing and maintaining childhood wasting to less than 5%.

SDG 3: the prevention and timely management of anaemia is essential to attain SDG 3 on ensuring healthy lives and promoting well-being for all at all ages.

SDG 4: quality education

SDG 10: reduced inequalities

SDG 17: partnerships for the goals

It also elucidates the interplay between these interconnected health determinants (malaria, immunity, anaemia and malnutrition) in the Pitoa and Mayo-Oulo health districts of the North Region of Cameroon.

Understanding the complex relationship of the immune response of individuals infected with malaria and suffering from malnutrition is of public health importance and crucial to guide specific antimalarial therapeutic approaches in the vulnerable sub-populations.

CHAPTER ONE: LITERATURE REVIEW

CHAPTER ONE: LITERATURE REVIEW

1.1. MALARIA

1.1.1. Definition of Malaria

Malaria is a vector-borne disease transmitted through the bites of *Anopheles* mosquitoes; a tiny fraction of infections is transmitted via other routes (e.g. transfusion or congenital transmission) (WHO, 2015). The rising toll of deaths over the years caused by the malaria parasite world-wide and particularly in Cameroon, is alarming. Humans get this disease when bitten by mosquitoes, which transfer the parasite in saliva as she seeks human blood to feed her young eggs. Malaria is the most significant parasitic disease of human beings and remains a major cause of morbidity, anaemia and mortality worldwide. (Hassen and Ali, 2016)

1.1.2. Malaria Parasite: *Plasmodium*

Malaria has been an endemic disease in most countries of the world in the past, but today the disease is endemic in countries of the southern hemisphere. Malaria is caused by protozoan of the *plasmodiae* family called *Plasmodium.* There exist over 140 species of *Plasmodium* which infect diverse animal species, but only 5 of these species are found in human beings (NMCP, 2014-2018). Several species of the *Plasmodium* protozoan parasite can infect humans under natural circumstances – *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi.* Humans are the only known reservoirs of these parasite species, with the exception of *P. knowlesi* for which the natural hosts are the long-tailed and pig-tailed macaques (WHO, 2015). *Plasmodium falciparum* is the most dangerous and the worst (most serious) of the clinical forms responsible for malaria in the world (NMCP, 2014-2018).

All four human *Plasmodium* species have been documented in Cameroon, including *P. falcipaum, P. ovale, P. malariae and P. vivax.* (PNLP, 2012 ; Fru-Cho *et al.,* 2014 ; Russo *et*

al., 2017 ; Antonio-Nkondjio *et al.*, 2019). *Plasmodium falciparum* is by far the predominant species recorded in up to 95% of all infection cases (Kwenti *et al.*, 2017; Sandeu *et al.*, 2017). *Plasmodium malariae* and *Plasmodium ovale* represent each 1 and 3% of infection cases, respectively (PNLP, 2012).

1.1.3. Transmission: *Anopheles* vector

The *Anopheles gambiae* is the most widespread vector. Members of the *Anopheles gambiae* species complex found in Cameroon include *An. gambiae* (*s.s*), *An. arabiensis*, *An. coluzzii* and An. *melas* (Simard *et al.*, 2009). While *Anopheles arabiensis* is restricted to the northern arid and semi-arid zone, *An. gambiae (s.s)* and *An. coluzzii* are widely distributed across the country**.** Others found in Cameroon include, *Anopheles funestus, Anopheles nili,* and *Anopheles moucheti*, which are the five major malaria vectors found in Africa (Bigoga *et al.*, 2012).

Main vector species in the North Region are *An. arabiensis, An. gambiae* and *An. funestus.* Other species playing a role in malaria parasite transmission are *An. pharoensis, An. coluzzii, An. rufipes and An. ziemanni* (Antonio- Nkondjio *et al.*, 2008; Tabue *et al.*, 2017).

1.1.4. Life Cycle of *Plasmodium*

- **Asexual Phase in Man or Schizogony**

Malaria infection is established in humans following the injection of the sporozoite form of the parasite by female anopheline mosquitoes (CDC, 2013). She may inject sporozoites along with saliva into small blood vessels (Crutcher and Hoffman, 1996). Subsequent development usually takes place over 5–8 days, with multiplication of the parasite in liver cells, followed by release of parasites into the blood-stream and invasion of erythrocytes. Replication within these cells and their subsequent rupture leads to the clinical manifestations of malaria (Fig. 1).

The usual range of the incubation period, from the time of *P. falciparum* infection to initial symptoms, is 8–14 days in non-immune persons. In those with some degree of immunity, the incubation period may be much longer.

Man, and other vertebrate hosts e.g. rodents, birds and reptiles are infected with malaria on being bitten by an infected mosquito. Viable sporozoites are inoculated and invade liver cells in man, where they multiply asexually to produce merozoites.

These merozoites are liberated and invade the red cells, where they form characteristic 'rings' which grow to become mature trophozoites and undergo another asexual multiplication to produce many new merozoites. The red cells erupt, releasing the merozoites which soon invade other red cells and begin a new cycle. The cycle of growth of the human malaria parasites within the red cells is 48 or 72 hours depending on the malaria species involved, and tends to be synchronous, producing periodic fevers with successive releases of the merozoites.

- Sexual Phase in the mosquitoes or Sporogony

Some parasites develop in the red cells to become male and female gametocytes which are the forms infective to anopheline mosquitoes. The gametocytes are taken up with the blood meal,

develop into gametes and combine sexually to form the zygote in the mosquito midgut. The zygote matures into a motile ookinete, which forces its way to the external wall of the midgut,

where it forms an oocyst. The oocyst bursts after maturation, releasing thousands of spindle-shaped sporozoites into the haemocoel of the mosquito. These migrate to the salivary glands, from which they are inoculated into a new host (Fig. 1)

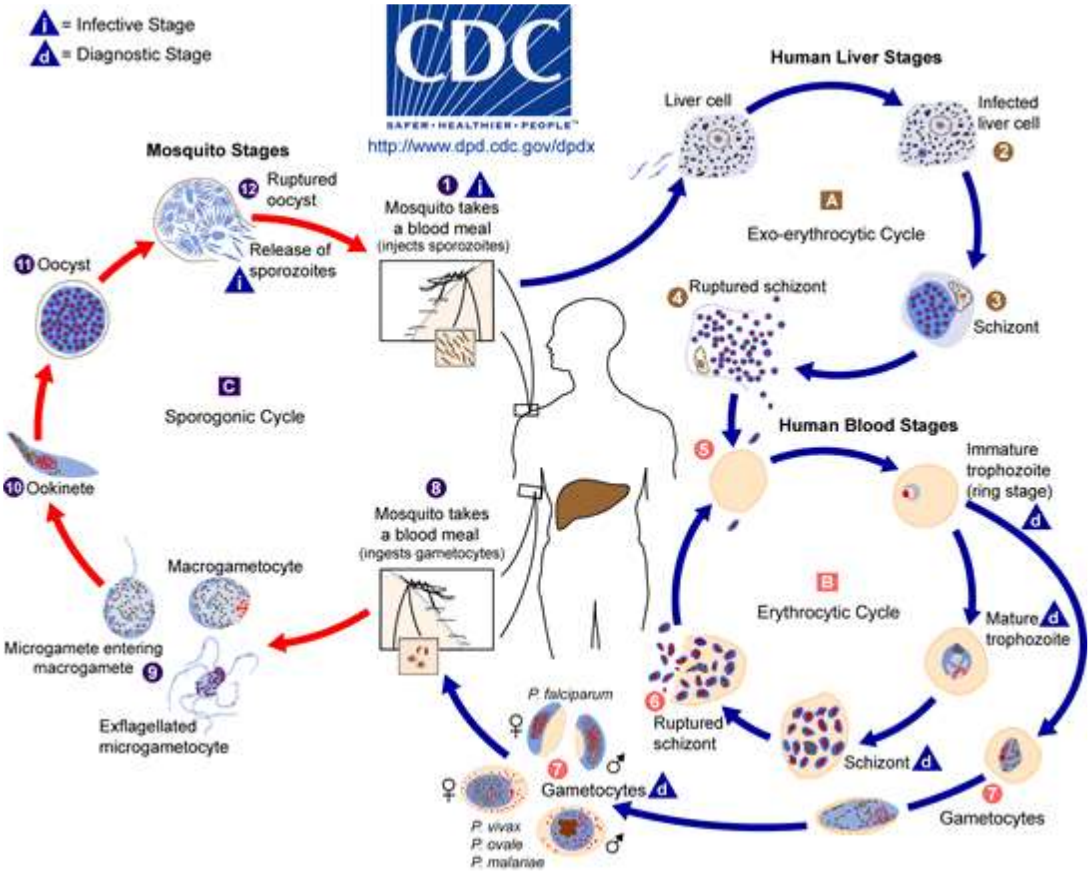


Figure 1 : Life Cycle of malaria parasite (CDC, 2013)

1.1.5. Clinical Manifestation of Malaria in Children

1.1.5.1. Malaria Symptoms and Pathology

Symptoms of malaria usually appear about 10-15days after the bite of an infected mosquito. Prodromal symptoms may include malaise, fatigue, headache, anorexia, and low- grade fever (WHO, 2006). These may last 2-3 days before an acute paroxysm occurs. An acute attack begins with a chill or rigors and then within 30 to 60 minutes, fever develops in association with profuse sweating, headache and malaise. Patients may also have nausea, vomiting, mild diarrhea, abdominal pain, flushed skin and dry skin. High fevers last for several hours and then are followed by a period of intense sweating. As untreated infections progress, the spleen gradually enlarges and anaemia worsens (WHO, 2006). Morbidity due to infection with *P. falciparum* can range from mild febrile illness which is difficult to distinguish clinically from

other similar illnesses, to life-threatening disease with coma, respiratory distress, severe anaemia or circulatory shock (Ojurongbe *et al.*, 2013).

Pathogenesis of malaria

The fever and chills of malaria are associated with the rupture of erythrocytic- stage schizonts. In severe *falciparum* malaria, parasitized red cells may obstruct capillaries and postcapillary venules, leading to local hypoxia and the release of toxic cellular products. Obstruction of the microcirculation in the brain (cerebral malaria) and in other vital organs is thought to be responsible for severe complications. Cytokines (e.g., tumour necrosis factor) are also felt to be involved, but at present their role is unclear (Crutcher and Hoffman, 1996). Anaemia in acute *falciparum* malaria is caused by increased destruction of both infected and non-infected erythrocytes and decreased erythropoiesis. Upon infection with malaria, it further compounds the problem and as a consequence RBCs are massively destroyed leading to anaemia.

1.1.6. Epidemiology of Malaria

According to WHO data, there were 241 million cases of infection globally, and an estimated 627.000 people died of malaria in 2020, with over 96% of these deaths occurring in sub-Saharan Africa, and nearly all of the others occurring in South-East Asia and South America, (WHO, 2021) with the World Malaria Report of 2021 indicating a rate of 2.9% for malaria cases in Cameroon. Africa is the continent which is most affected by this epidemic, with 80% of which represent children less than 5years old (WHO, 2021), which are all higher than the figures reported in 2019, showing that the burden continues to rise. There are 5 principal eco-epidemiological zones of malaria in Central Africa (NMCP, 2011). Equatorial**,** Tropical**,** Sahelian, Sub- desert and Sudan. In Cameroon, malaria is responsible for 24% of consultations and 45% hospitalization, 13% hospital- related deaths of which 10% are in children less than 5years old (NMCP, 2018). There are also 3 principal eco-epidemiological

zones of malaria in Cameroon (NMCP, 2011; NMCP, 2014-2018). Equatorial forest in the South**,** Tropical Sudan in the Adamawa and NorTropical Sahel in the Extreme North (Fig. 2)**.**

Figure 2: Map of Cameroon showing the different eco-epidemiological zones (Djeutchouang, 2010)

In Epidemiologic zones, favourable conditions of rainfall, temperature, and humid climate leads to multiplication of the parasite breeding. Endemicity in one zone varies from another zone, therefore control measures in one zone may not apply to another zone. Cameroon is endemic for malaria; however, the level of endemicity varies between the various eco-epidemiological zones (Bigoga *et al.*, 2012; Songue *et al.*, 2013).

1.1.6.1. Malaria Incidence

Malaria is distributed in varying degrees throughout the tropical world and in some more temperate areas, wherever ecological and sociological conditions favour sufficient interactions between humans, mosquitoes and parasites, to maintain transmission. The groups at highest risk for the adverse effects of malaria are children and pregnant women. (Shankar, 2000).

In Cameroon, malaria burden and transmission intensity is heterogenous with spatial and temporal variations between altitudes and geographical areas, with prevalence rates varying from one area to another (Eyong *et al.,* 2016). The frequency of episodes of malaria and the characteristics of malaria disease vary, depending on the infected individual's age, genetics and immune response from previous malaria infections, and the intensity and seasonality of malaria transmission.

Depending on the level of transmission and the degree of exposure and susceptibility, individuals exposed to malaria may suffer one or multiple episodes each year; repeated episodes of *P. falciparum* malaria in the same individuals are generally due to reinfections or, in some cases, to incomplete clearance of the primary infection due to inadequate antimalarial treatment. In areas of moderate or high transmission, children commonly experience 4–6 febrile illnesses per year attributable to malaria (WHO, 2015) and infants, or children younger than 12 months of age, are at greatest risk of suffering from the worst symptoms and disease outcomes from malaria infection.

In the North Region of Cameroon, the Entomological Innoculation Rate (EIR) was found to vary between 2.7-36.5 infective bites/ person /month (Antonio-Nkondjio *et al.,* 2008). After the LLINs scale-up, average malaria parasite prevalence levels of 30.4% [varying significantly from 28.6% (798/2795) for net users and 35% (243/694) for non-net users] was recorded in the health districts of Garoua, Pitoa and Mayo-Oulo in children of 6 months to 5years–old (Kleinschmidt *et al.,* 2018). Intense transmission was found to occur during the

rainy season with estimates varying from 24.5 to 60 infective bites /person /month in the health districts of Lagdo, Garoua, Pitoa, Mayo-Mbocki and Mayo-Oulo (Antonio-Nkondjio *et al.*, 2008; Tabue *et al.*, 2017; Awono-Ambene *et al.*, 2018).

1.1.6.2. Malaria Prevalence.

Prevalence in Cameroon

According to the demographic and health survey conducted in 2018, the data shows a slight decrease in malaria prevalence in children (6-59 months) from 30% in 2011 to 24% in 2018 (DHS, 2018). By region, malaria prevalence in children was as follows; Far-North, 22%; North, 26%; Adamawa, 32%; Centre, 47%; East, 35%; North-West, 10%; West, 16%; South-West, 10%; Littoral, 21% and South, 33%.

1.1.7. Malaria Co-Infection

There is no cross-protection between different *Plasmodium* species: an infection with *P. vivax* does not give any protection to a subsequent infection with *P. falciparum*. However, the co-infection with more than one *Plasmodium* species may also modulate the host's response. In situations where *P. vivax* and *P. falciparum* are co-endemic, the severity of *falciparum* malaria may be much reduced (the classical example is the malaria situation in Vanuatu, South West Pacific), (Maitland *et al.*, 1996). The same is true for *P. malariae*, where individuals infected with *P. malariae* and *P. falciparum* suffer less from their infection than individuals infected with *P. falciparum* alone. Mixed malaria infections (here *P. falciparum* and *P. vivax*) are not uncommon in certain parts of the world.

1.1.8. Malaria Case Management

Early diagnosis and treatment of malaria reduces disease, prevents death and contributes to reducing transmission. WHO recommends that all suspected cases of malaria be confirmed using parasite-based diagnostic testing (through either microscopy or a rapid diagnostic test),

to enable health providers to swiftly distinguish between malarial and non-malarial fevers, fascilitating appropriate treatment (WHO, 2021).

1.1.8.1. Malaria Diagnosis

Since the creation of the national malaria control program (NMCP) in 1998 and the reorganization of the National Roll Back Malaria Committee in 2002, the Cameroon malaria treatment and diagnostic guidelines have evolved enormously (Moyeh *et al.,* 2019). While the level of endemicity determined the diagnostic method applied, the drug efficacy and resistance profile determined the national treatment recommended (Cameroon Academy of Science, 2014).

1.1.8.1.1. Confirmatory diagnosis

The WHO recommends that a positive microscopy laboratory result confirms the presence of malaria parasite, since fever is usually not specific and may result from other infections.

1.1.8.1.2. Clinical Diagnosis

Clinical diagnosis also referred to as presumptive diagnosis is the least expensive, imprecise and most commonly used method and the basis for therapeutic care for the majority of febrile patients in malaria endemic areas, where laboratory support is often out of reach (Ojurongbe *et al.,* 2013). Fever is the most prominent symptom of malaria which is often accompanied by chills, perspiration, anorexia, headache, vomiting, temperature> 37.5°C and malaise, which are not specific. Residents of endemic areas are often familiar with these combinations of symptoms, and they usually self- diagnose malaria based on symptoms alone. Accuracy of clinical diagnosis varies with the level of endemicity, malaria season, and age group such that no single clinical algorithm can be regarded as a universal predictor (Wongsrichanalai *et al.,* 2007). Clinical findings should always be confirmed by a laboratory test for malaria.

1.1.8.1.3. Laboratory Diagnosis

Malaria can also be diagnosed microscopically by demonstrating the presence of parasites in Giemsa stained thick and thin films using the light microscope (Warhurst and Williams, 1996) or fluorescent stained blood samples observed using a fluorescent microscope (Srinivasan *et al.*, 2000). In most situations, the **"gold standard"** for individual diagnosis is the microscopical examination of thick and thin films. There are, however, situations where this may not apply. Light microscopy can detect the presence of the parasite, the infecting species, and the parasite load but requires a trained microscope technician and a constant supply of power to run the equipment, limiting the use of this method in resource poor settings. In areas of high endemicity, clinical diagnosis alone is usually the only feasible and cost-effective method for recommending the first line of treatment (Moyeh *et al.*, 2019).

1.1.8.1.4. Alternative Methods of Laboratory Diagnosis

Various methods have been designed to reduce the time spent reading slides or to enable less-trained personnel to achieve equally reliable results. The main problem with microscopy is that it is time-consuming and needs to be performed by skilled microscopists. The rapid diagnostic test (RDT) is an alternative diagnostic method to the conventional microscopy, used in malaria diagnosis. The basis for the malaria rapid immune- chromatographic test is that, the infected parasite can also produce soluble antigens such as *Plasmodium falciparum* histidine rich protein 2 and 3 (PfHRP2/3), parasite lactate dehydrogenase *(pLDH)*, and aldolase antigens that can be captured by monoclonal antibodies raised against these antigens (Moody, 2002; Abanyie *et al.*, 2011). (Fig. 3) ATFirst™ One-Step Malaria rapid test Pf/Pv Antigen (MAL Pf/Pv) Kit is a rapid and convenient immunochromato-graphic assay for the qualitative detection of *Plasmodium falciparum (P.f)* and *Plasmodium vivax (P.v)* in whole blood samples (fig 3). It is intended to aid in the rapid diagnosis of human malaria infections and to aid in the differential diagnosis of *Plasmodium falciparum* infections from *Plasmodium*

vivax infections. This assay provides only a preliminary result and all positive specimens should be confirmed with other qualified assays. A positive antigen detection assay would be expected to detect a current infection. (WHO, 2012)

Antigen detection

Figure 3: ATFirst™ Malaria Rapid Tests

Only methods based on the detection of histidine rich protein-2 (HRP-2) and the parasite lactate dehydrogenase (pLDH) have so far been commercialized. In both cases use is made of a lateral flow, immunochromatographic test format, often called "dipstick". This new generation of easy-to-perform tests has been developed to diagnose *falciparum* malaria rapidly and reliably without the need of a microscope. The most recently developed tests can also detect other malaria species. The immune-chromatographic methods have the advantage that, they are easy to perform and do not require the services of a trained technician and electricity. Notwithstanding, the test method cannot be used to determine parasite load, the infecting species, and monitor treatment follow-up (Moyeh *et al.*, 2019).

1.1.8.1.5. Molecular diagnostic methods

Other methods of diagnosis include the use of Nested Polymerase Chain Reaction (PCR), Loop mediated isothermal amplification (LAMP), and nucleic acid sequence-based amplification (NASBA) techniques (Snounou *et al.,* 1993; Mens *et al.,* 2006; Sema *et al.,* 2015; Lau *et al.,* 2016). It was the discovery of amplification methods, such as the Polymerase chain reaction (PCR), which really made molecular diagnostic feasible. Its principle is based on the use of DNA polymerase which is an in vitro replication of specific DNA sequences. This method can generate tens of billions of copies of a particular DNA fragment (the sequence of interest) from a DNA extract (template).

To amplify a segment of DNA using PCR, the sample is first heated so the DNA denatures, or separates into two pieces of single-stranded DNA. Next, an enzyme called Taq polymerase synthesizes two new strands of DNA, using the original strands as templates.

- **Types of PCR**

There are three types of PCR;

-Conventional PCR (C-PCR): used to detect specific target genes in various micro-organisms.

-Nested PCR (N-PCR): developed to improve sensitivity of the conventional PCR.

-Real- time PCR (Q-PCR): detect / quantify the concentration of the DNA species in the reaction in real time.

- **Nested PCR**

The Nested PCR method is a simple 2 – round PCR that amplifies the 18s ribosomal RNA gene (using a thermocycler) which determines the presence of parasites as well as the infecting species (Snounou *et al.,* 1993) but cannot quantitate the parasite load (Moyeh *et al.,* 2019). Nested PCR is a modification of PCR that was designed to improve sensitivity and

specificity. It involves the use of two sets of primers (outer pair and inner pair) for a single locus and two successive PCR reactions- Nest 1 and Nest 2 (Singh *et al.*, 1999). The nested PCR has been shown to detect the presence of parasites below the detection limit of light microscopy and RDTs (Snounou *et al.*, 1993). The LAMP and the NASBA techniques equally amplify, isothermally, the 18S ribosomal RNA gene and mRNA, respectively, but differ in that the NASBA can quantitate the parasite load using an in-vitro synthesized RNA competitor (Mens *et al.*, 2006).

1.1.8.2. Treatment

Following expansion of drug resistance, drug policy for malaria treatment in Cameroon gradually changed over the years from monotherapies with chloroquine and amodiaquine used as a first-line treatment for uncomplicated malaria to combination therapy (Basco *et al.*, 2006). Chloroquine was largely used from the 1970's through to 2002 (PNLP, 2012). From 1999 to 2004, following the adoption of an interim drug policy, amodiaquine was incorporated alongside chloroquine as an alternative first-line drug for uncomplicated malaria while sulphadoxine-pyrimethamine was used as second line drug (PNLP, 2012; Basco *et al.*, 2006). In 2004, following recurrent treatment failure to amodiaquine and sulfadoxine-pyrimethamine, the Ministry of Health of Cameroon reconsidered its policy and shifted to artemisinin-based combination therapy (ACT) used as a first-line treatment for uncomplicated malaria.

1.1.8.2.1. Artemisinin-based Combination Therapies (ACTs)

ACT's are the recommended first-line antimalarial treatment. To prevent malaria in pregnant women and new born infants, WHO recommends intermittent preventive treatment of malaria in pregnancy (IPTp) with a treatment dose of sulfadoxine-pyrimethamine to be offered at each scheduled antenatal care (ANC) visit (maximum monthly) after the first trimester (WHO, 2012; WHO, 2015), this policy has been adopted in most African countries. In 2014, among

reporting countries, 17% of all pregnant women received 3 doses of IPTp, as recommended by WHO whereas injectable artemether or quinine are used in case of treatment failure or for severe malaria cases (PNLP, 2012). Common ACT used in the country include artesunate-lumefanthrine, artesunate-atovaquone-proguanyl, artesunate –amodiaquine, artesunate-mefloquine (PNLP, 2012).

1.1.8.2.2. Seasonal Malaria Chemoprevention (SMC)

SMC is defined as the intermittent administration of full treatment courses of an antimalarial medicine to children during the malaria season. In the northern part of the country exposed to recurrent malaria outbreaks during the rainy season, the government introduced in 2016 seasonal malaria chemoprevention for children below 5years old (Malaria, Operational Plan Cameroon, 2017; Minsante, 2018). The combination of artesunate –amodiaquine (AQAS) which was used before for the treatment of uncomplicated malaria cases for children under 5 years- old was replaced by artemether- lumefantrine (AL) provided free of charge to all families for malaria prevention. This strategy permitted to take in charge over 80% of children in the target settings in the North and Far-North regions (Minsante, 2018). SMC is given monthly during the transmission season, for 3 or 4 consecutive months, with the objective of maintaining therapeutic antimalarial drug concentrations in the blood throughout the period of greatest risk in order to prevent malarial illness. SMC has been shown to reduce the incidence of both uncomplicated and severe malaria by 75% in children aged <5 years (Wilson, 2011; Meremikwu *et al.*, 2012). Since 2012, WHO has recommended SMC in areas of highly seasonal malaria transmission across sub-Saharan Africa, where an estimated 25 million children aged 3–59 months could benefit from this intervention every year **(WHO, 2012).** In 2015, SMC was implemented in 9 countries.

Case management in Cameroon includes: diagnosis of suspected cases; treatment of confirmed cases at health facilities and community level; scale-up of integrated community

case management; pharmacovigilance and supply chain strengthening **(Antonio-Nkondjio *et al.*, 2019).**

1.1.9. Prevention and Control of Malaria

The number of deaths from malaria has fallen by 60% globally since 2000. In most African countries, substantial malaria control activities have been implemented, including the widespread deployment of long-lasting insecticidal nets (LLINs), the use of indoor residual spraying (IRS) of insecticides in some settings, prompt diagnosis using quality-assured rapid diagnostic tests (RDTs) and treatment with highly effective artemisinin-combination therapies (ACTs) (WHO, 2015). Also, the environmental measures taken such as improved sanitation, less bushy surroundings and better drainage facilities coupled to the use of the bed nets have gone a long way to prevent the spread of the disease and reduced infective bites. These interventions are highly cost-effective. In many settings, they have been associated with reduction of incidence rates of malaria and malaria deaths by at least 50% since 2000 (Lengeler, 2004; O'Meara et *al.*, 2010).

In some geographic areas which had very high malaria prevalence there have been substantial reductions. While many factors, including economic development, may have contributed, most of the decrease is likely attributable to large-scale deployment of LLINs, IRS, RDTs and ACTs. However, many individuals and communities still do not have access to these interventions, and WHO has called for urgent scale-up of the existing control measures (WHO, 2015). Since October 2021, WHO also recommends broad use of the RTS, S/AS01 malaria vaccine among children living in regions with moderate to high *P. falciparum* malaria transmission. The vaccine has been shown to significantly reduce malaria, and deadly severe malaria, among young children (WMR, 2021).

1.1.10. Malaria and Immunity

1.1.10.1 Definition of Immunity

The human body has the ability to resist almost all types of organisms or toxins that tend to damage the tissues or organs. This capacity is called Immunity.

1.1.10.2. Naturally- acquired immunity

Natural immunity to malaria is acquired gradually with repeated exposure to malaria infection, and is acquired more rapidly for the more severe forms of the disease, so that, with increasing age, there is progressive protection first against severe malaria and ensuing mortality, then against illness with malaria, and, much more slowly, against microscopy-detectable parasitaemia (WHO, 2015).

In areas of very high transmission, malaria mortality rates begin to fall by around 2 years of age, with the incidence of acute febrile malaria falling later in childhood or adolescence with the acquisition of partial immunity (UNICEF, 2013)

The mechanisms underlying naturally-acquired immunity is not fully understood. Immunity acquired during childhood is partially lost in pregnancy which explains the increased frequency of low birth weight and of maternal anaemia during the first and sometimes second pregnancies (WHO, 2010). Naturally-acquired immunity is believed to wane substantially if an individual migrates out of a malaria- infected area for a number of years. The use of available preventive interventions affects the time course of naturally-acquired immunity: effective prevention is likely to delay the development of naturally-acquired immunity (WHO, 2015). They advocate that naturally acquired immunity should be appreciated as being virtually 100% effective against severe disease and death among heavily exposed adults. Even the immunity that occurs in exposed infants may exceed 90% effectiveness (WHO, 2015).

1.1.10.3. Acquired immunity

People residing in malaria-endemic regions acquire immunity to malaria through natural exposure to malaria parasites. Immunity can also be acquired from the passive transfer of immune sera or immunoglobulins indicating that part of the immunity is derived from antibody. Children living in areas of stable malaria transmission become infected early in life, and experience more severe disease symptoms during the first five years of life. But as immunity develops the disease becomes less severe and the number of parasites circulating in the blood declines (WHO, 2010).

The acquired immune response to malaria is strain specific and is lost if a person moves away from a malaria endemic area (WHO, 2015). Following infection with *Plasmodium* parasites, the immune system responds in a number of ways as it attempts to clear the parasite.

Antibodies against schizont and merozoite antigens bind to infected red blood cells and to merozoites, and make them easier for macrophages and other immune cells to ingest. Antibodies also help other immune mediators, called complement proteins, to destroy parasites and they prevent merozoites infecting new red blood cells (Thurnham, 2015).

Macrophages which have taken up *Plasmodium* express parasite antigens on their surface, and other immune cells called T cells recognize these antigens and bind to them. The T cells become activated and release molecules called cytokines that promote further cell activation, parasite killing and antibody production.

Our innate immune system is designed to detect foreign material entering the body, but it will also respond to damaged cellular and tissue components generated endogenously. Oxygen is essential for life but oxidation will generate free radicals, potentially causing tissue damage.

1.1.10.4. IgG responses and Immunity

1.1.10.4.1. IgG1 and IgG3 antibody responses to malaria

IgG antibodies pivotal role in anti-malarial protection was demonstrated by seminal studies involving the passive transfer of IgG, purified from sera of semi-immune adults, to non-immune patients resulting in clearance of parasitaemia (Bouharoun –Tayoun *et al.,* 1990; Sabchareon *et al.,* 1991; Courtin *et al.,* 2009). This protection reflects antibody responses directed to blood stage antigens of *Plasmodium falciparum*. Specific IgG are proposed to have either a direct (Shi *et al.,* 1999) and/or indirect effect (Bouharoun –Tayoun *et al.,* 1995) on parasite growth inhibition. Among the IgG subclasses, IgG1 and IgG3 are thought to play a key role in the protection (Aribot *et al.,* 1996; Tongren *et al.,* 2006). It is believed that these sub-classes can neutralize parasites directly, by inhibiting parasite invasion or growth in erythrocytes or indirectly by a mechanism involving cooperation between parasite-opsonizing antibodies and monocytes by binding to the Fcγ receptor IIA, leading to secretion of soluble parasite growth- inhibitory factors such as nitric oxide or tumor necrosis factor-alpha (Tebo *et al.,* 2001). In the latter case, the cytophilic IgG subclasses IgG1 and IgG3 are thought to be of paramount importance (Jafarshad *et al.,* 2007).

Numerous studies have demonstrated the role of specific Igg isotype responses in anti-malarial protective immunity (Taylor *et al.,* 1995; Aribot *et al.,* 1996; Aucan *et al.,* 2000). It is generally agreed that cytophilic IgG1 and IgG3 isotypes participate in specific protective immunity whereas IgG2 /IgG4 isotypes, block its effect (Beeson *et al.,* 2008).

1.2. MALNUTRITION

1.2.1. Definition of Nutritional Status

Nutritional Status literally means the nutritional condition of an individual relative to that of others. It is the best indicator of the general well-being of children (WHO, 1995).

1.2.2. Definition of Malnutrition

Malnutrition is a general term that has been used to refer to deficiencies, excesses and imbalance in a person's intake of nutrints and or energy (WHO, 2020). **(Fig 4)** It continues to be a primary c[Type a quote from the document or the summary of an interesting point. You can position the text box anywhere in the document. Use the Drawing Tools tab to change the formatting of the pull quote text box.]

ause of ill health and mortality among children in developing countries. It is also a major public health problem and accounts for about half of all child deaths worldwide.

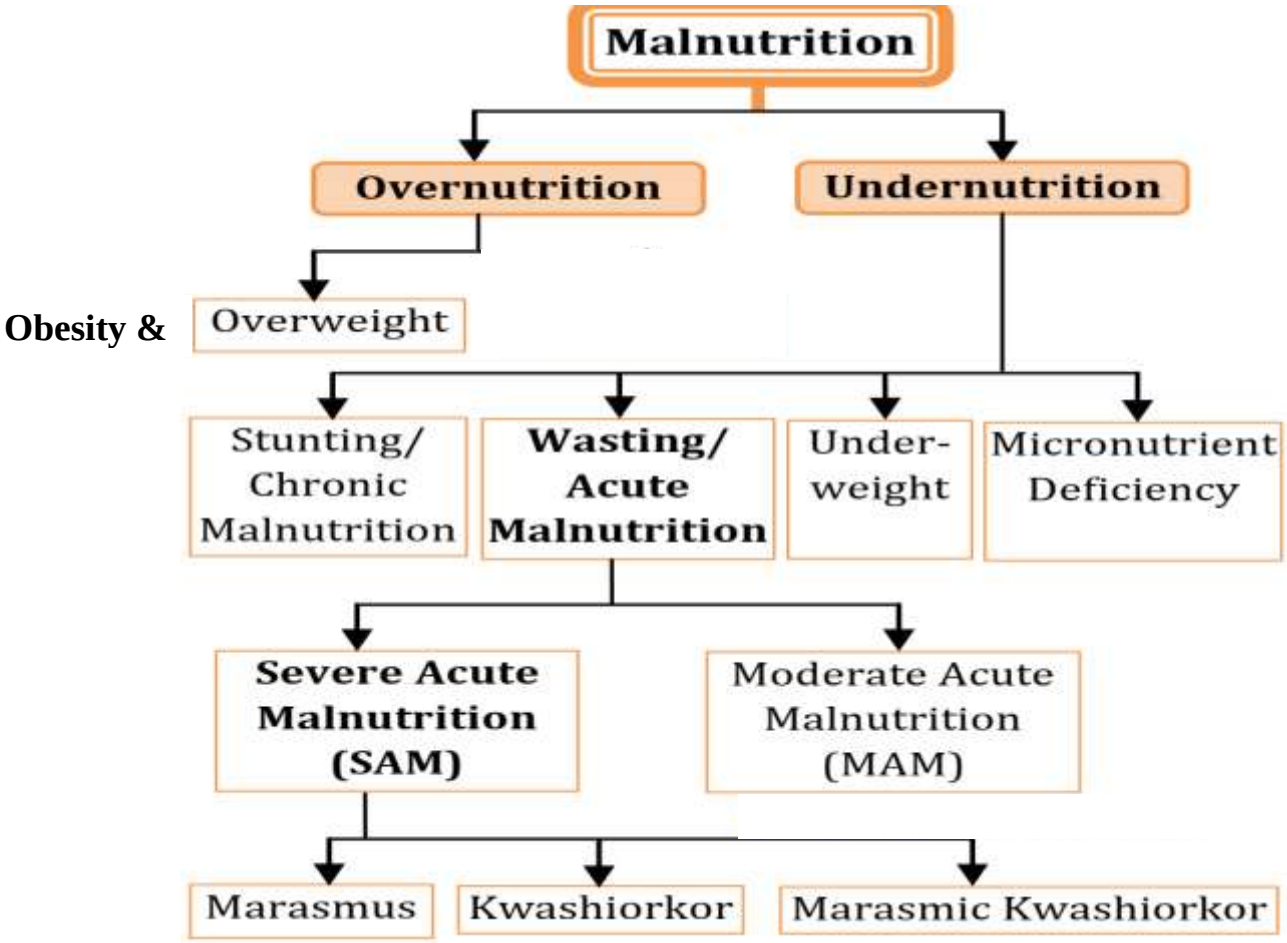


Figure 4: Definition of malnutrition (Shisir Kumar Adhikari, 2021)

1.2.3. Causes of malnutrition

This conceptual framework on the causes of malnutrition was developed in 1990 as part of the Unicef nutrition strategy. The framework shows that causes of malnutrition are multisectoral, embracing food, health and caring practices. They are also classified as immediate, underlying, and basic, whereby factors at one level influence other levels. (Fig 5) The framework is used at national, district and local levels, to help plan effective actions to improve nutrition. It serves as a guide in assessing and analysing the causes of the nutrition problem and helps in identifying the most appropriate mixture of actions. **(UNICEF, 1990)**

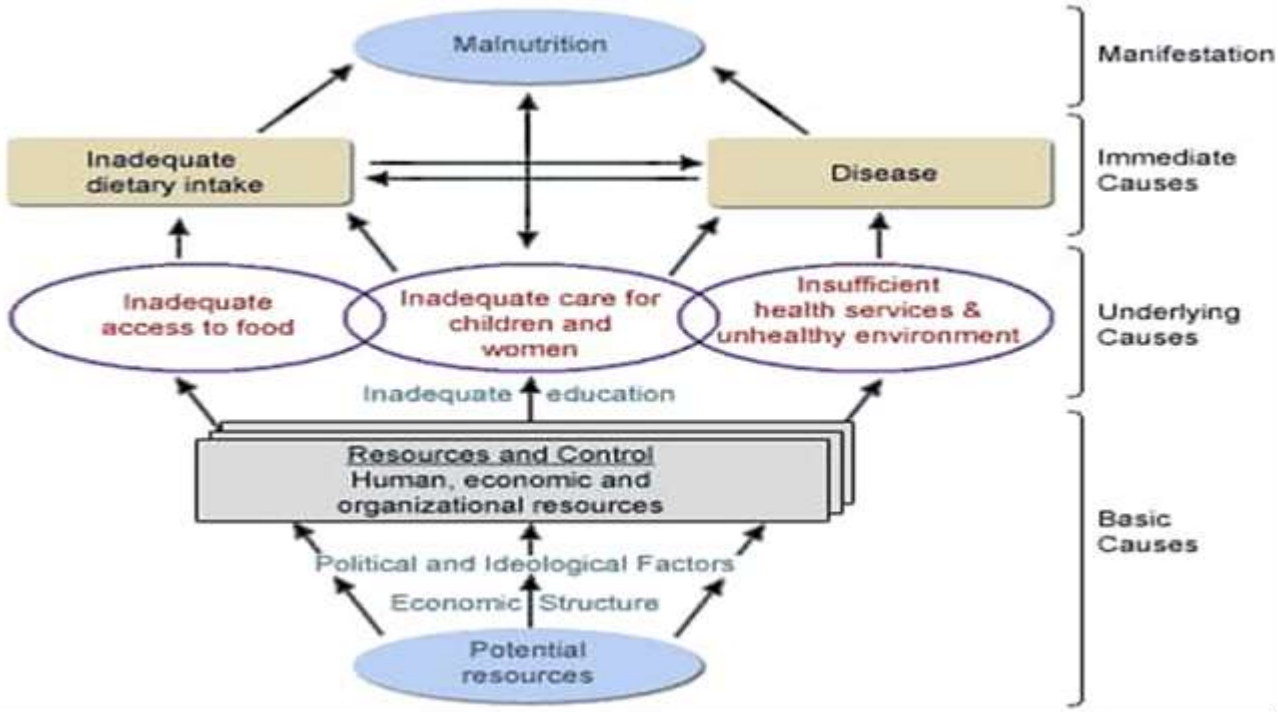


Figure 5: The UNICEF Conceptual Framework of Malnutrition (Source: UNICEF, 1990)

1.2.4. Prevalence of Undernutrition

1.2.4.1. Prevalence of Undernutrition Worldwide

The most recent data from the global nutrition report of 2021 indicates that there were 149.2 million children under 5 who were stunted (22%), 45.5 million wasted (6.7%), and 38.9 million overweight (5.7%) globally in 2020 (UNICEF *et al.*, 2021); which were similar to the

149 million children under 5 who were stunted (21.9%), 49 million were wasted (7.3%) and 40 million were overweight (5.9%) globally in 2018 (WHO, 2019).

1.2.4.3 Prevalence of Undernutrition in Cameroon

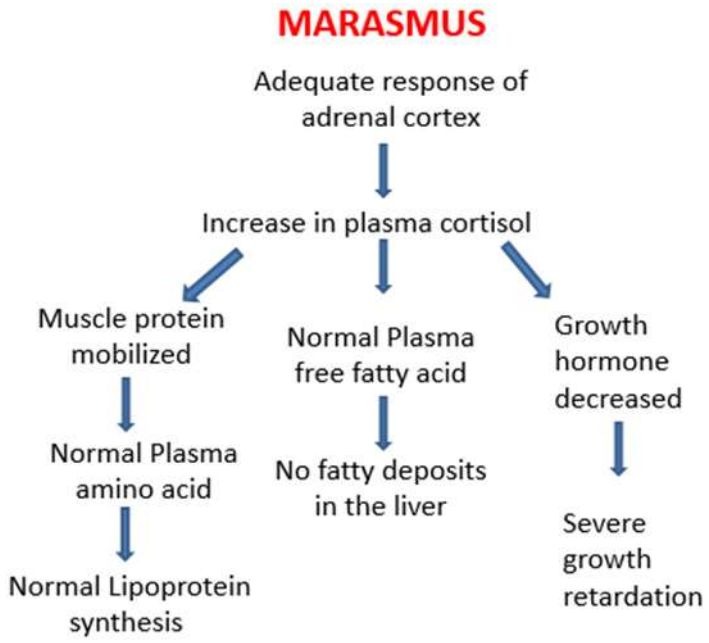
In Cameroon, 52.000 are reported to die each year of malnutrition. Four regions out of the Country's 10 have high malnutrition rates; North, Far North, Adamawa and East. According to the National DHS report of 2011, the data revealed Stunting 30.4%; Underweight 19.3%; Wasting 6.1% (Demographic Health Survey, 2011).

Also, findings in the 2011 Nutrition Survey carried out in Northern Cameroon showed a prevalence of stunting to be 43.1% (44.9% in the Far North and 40.2% in the North regions). A similar study conducted in Northern Cameroon reported the prevalence of stunting observed among school children at 42.0% (46.3% in the Far North and 37.7% in the North regions). This same study revealed that 42.0%, 26.0% and 6.6% of the school children were respectively stunted, underweight and wasted (Ngwa-Akonwi *et al.,* 2015). However, the most recent 2021 SMART Nutritional Survey report, revealed a prevalence of 40.2% for stunting and 4.8% for wasting in children living in the north region of the country.

Nevertheless, reports from the Demographic Health Survey of 2018, showed prevalence rates similar to those of 2011 for stunting, underweight and wasting, respectively (DHS, 2018). Indicating that it is stagnant. There is no change.

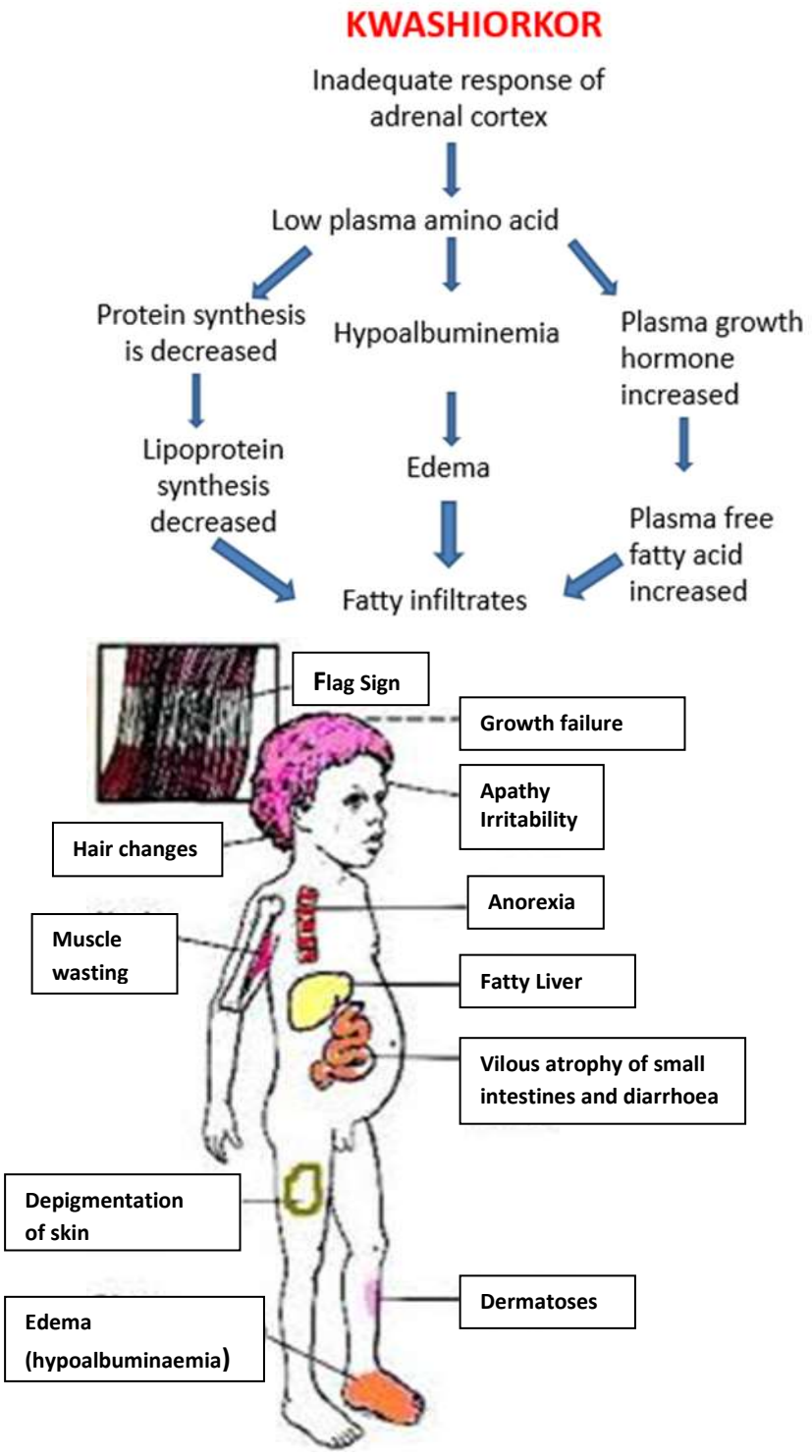
1.2.5. Pathophysiology of Malnutrition

A. Severe acute malnutrition: Marasmus (<-3SD Weight – for - Height)



No obvious pathology

B. Severe acute malnutrition: Kwashiokor (<-3SD Weight –for - Height)



- **Oedema** may mask weight loss
- **Dermatosis –** lighter hair colour
- **Hypoalbuminaemia –**Fatty liver (up to 50% wet weight)
- **Abnormal plasma amino acids (**reduced essential amino acids)
- **K^+ deficiency** (Apathy and anorexia)
- **Infection**

Figure 6: Marasmus versus Kwashiokor in children

Figure 7: Child suffering from Malnutrition (Marasmus)

Source: Global Nutrition Report, 2019

1.2.6. Indicators of Nutritional Status

The main indicators include; stunting, underweight, wasting, overweight, thinness and Mid-Upper arm circumference. However, other biochemical markers of malnutrition are; Fe, Zn and Vit A deficiency, children mortality rates as well as poverty incidence.

Because nutritional inputs are necessary for children's growth, undernutrition is generally characterized by comparing the weights or heights (or lengths) of children at a specific age

and sex with the distribution of observed weights or heights in a reference population of presumed healthy children of the same age and sex and then calculating *z*-scores, that is, the difference between a child's weight or height and the median value at that age and sex in the reference population, divided by the standard deviation (SD) of the reference population. (WHO, 2006).

A child whose height-for-age is less than −2 SD is considered stunted, because the chances of the child's height being normal are less than 3 percent. A child whose weight-for-age is less than −2 SD is considered underweight, and one whose weight-for-height is less than −2 SD is deemed wasted. Stunting results from chronic undernutrition, which retards linear growth, whereas wasting results from inadequate nutrition over a shorter period, and underweight encompasses both stunting and wasting.

Typically, growth faltering begins at about six months of age, as children transition to foods that are often inadequate in quantity and quality, and increased exposure to the environment increases their likelihood of illness.

1.2.7. The Global Nutrition Targets of 2025

In 2012, the World Health Assembly Resolution 65.6 endorsed a Comprehensive implementation plan on maternal, infant and young child nutrition (WHO, 2012), which specified six global nutrition targets for 2025 (WHO, 2014). The targets are vital for identifying priority areas for action and catalyzing global change. The World Health Assembly has as ultimate goal for all children to be free of malnutrition in all its forms- Stunting, Overweight, Wasting (UNICEF *et al.*, 2015). Fig. 8

Figure 8: Global Nutrition Targets 2025. (Source: WHO, 2014)

1.2.7.1. Stunting

The Global Nutrition Target No. 1 is 40% reduction in the number of children under-5 who are stunted.

1.2.7.1.1. Definition of Stunting

Stunting refers to a child who is too short for his/her age. Stunting is the failure to grow both physically and cognitively and is the result of chronic or recurrent malnutrition. Its effects often last a life time (UNICEF *et al.*, 2015).

Stunting, or being too short for one's age, is defined as a height that is more than two standard deviations below the World Health Organization (WHO) child growth standards median (WHO, 2009).

1.2.7.1.2. Prevalence of Stunting

Prevalence of Stunting Worldwide

Childhood stunting is one of the most significant impediments to human development, globally affecting approximately 149 million children under the age of 5 years, representing 21.9% (UNICEF *et al.*, 2019).

The global trend in stunting prevalence and numbers of children affected is decreasing but not fast enough. Between 1990 and 2018, stunting prevalence declined from 39.6% (255 million) to 21.8% (149 million) (WHO, 2019).

Prevalence of Stunting in Cameroon

Cameroon has made some progress towards achieving the global nutrition target of stunting, 28.9% of children under 5 of age are still affected (Global Nutrition Report 2020). In Cameroon, the percentage of stunted children less than 5years rose from 28% in 2011 to 28.9% in 2018, and even most recently, the SMART nutritional survey report revealed a prevalence of 36.4% in the Far North, 32.8% in the East and 34.6% in Adamawa regions. These percentages remain problematic. The SDG's target is to end all forms of malnutrition by 2030, including by achieving internationally agreed targets (reducing the number of stunted children under five by 40%) (Demographic Health Survey, 2011; 2018; WHO, 2019, SMART 2021).

1.2.7.1.3. Causes of Stunting

Factors that contribute to stunted growth and development include poor maternal health and nutrition, inadequate infant and young child feeding practices, and infection. Specifically,

these include maternal nutritional and health status before, during and after pregnancy, which influences a child's early growth and development, beginning in the womb (Ozaltin *et al.*, 2010). For example, intrauterine growth restriction due to maternal undernutrition (estimated by rates of low birth weight) accounts for 20% of childhood stunting (Black *et al.*, 2013).

1.2.7.1.4. Consequences of Stunting

Stunting has long-term effects on individuals and societies, including: diminished cognitive and physical development reduced productive capacity and poor health, and an increased risk of degenerative diseases such as diabetes (UNICEF, 2013).

Stunting is a well-established risk marker of poor child development. Stunting before the age of 2 years predicts poorer cognitive and educational outcomes in later childhood and adolescence (Walker *et al.*, 2007; Black *et al.*, 2013) and has significant educational and economic consequences at the individual, household and community levels.

Recent longitudinal studies of children from Brazil, Guatemala, India, the Philippines and South Africa associated stunting with a reduction in schooling, where adults who were stunted at the age of 2 years completed nearly one year less schooling than non-stunted individuals (Martorell *et al.*, 2010; Adair *et al.*, 2013). Similarly, a study of Guatemalan adults found that those who were stunted as children had less total schooling, lower test performances, lower household per capita expenditure and a greater likelihood of living in poverty (Hoddinott *et al.*, 2013). For women, stunting in early life was associated with a lower age at first birth and a higher number of pregnancies and children (Hoddinott *et al.*, 2008).

Stunting is an enormous drain on economic productivity and growth. Economists estimate that stunting can reduce a country's gross domestic product by up to 3% (The World Bank, 2006).

According to World Bank estimates, a 1% loss in adult height due to childhood stunting is associated with a 1.4% loss in economic productivity (The World Bank, 2006). It is estimated

that stunted children earn 20% less as adults compared to non-stunted individuals (Grantham-Mc Gregor *et al.*, 2007).

1.2.7.2. Wasting

The World Health Assembly wasting target (Global Nutrition Target 6) (WHO, 2014) has two aspects – reducing and then maintaining levels of childhood wasting to below 5% – both of which are major challenges (UNICEF *et al.*, 2015).

1.2.7.2.1. Definition of Wasting

Wasting refers to a child who is too thin for his/her height. Wasting is the result of sudden or acute malnutrition, where the child is not getting enough calories from food and faces an immediate risk of death. (UNICEF *et al.*,2015). The World Health Organization (WHO) classifies wasting in children as severe or moderate, according to the WHO growth reference for weight-for-height (WHO, 2006).

Severely wasted children are, on average, 11 times more likely to die than their healthy counterparts (Mc Donald *et al.*, 2013). Furthermore, even higher mortality has been reported when children are both wasted and stunted (with low height-for-age) (Mc Donald *et al.*, 2013).

1.2.7.2.2. Prevalence of Wasting

Prevalence of Wasting in the World

The recent data from the global nutrition report of 2019 indicates that 7.3% of children under 5 were wasted globally (UNICEF *et al.*, 2019). In 2014, the global wasting rate was 7.5%. Approximately 1 out of every 13 children in the world was wasted in 2014 (WHO, 2015). Globally, wasting accounts for 4.7% of all deaths of children aged under 5 years (Mc Donald *et al.*, 2013). It is estimated that, at any point in time in the world, 52 million children aged under 5 years are wasted, with 17 million of those estimated

to be severely wasted, based on national-level prevalence data. The majority of all moderately (69%) and severely (71%) wasted children live in Asia (WHO, 2012). Just over one quarter of all moderately (28%) and severely (28%) wasted children live in Africa (UNICEF *et al.*, 2015).

Prevalence of Wasting in Cameroon

In the same line, Cameroon is on course for the target for wasting, with 4.3% of children under 5 years of age affected. The percentage of wasted children in Cameroon decreased from 5.6% in 2011 to 4% in 2018, with the SMART nutritional survey reporting prevalence rates of 4.8% in the North, 5.9% in the Far North, 4.5% in the East and 3.8% in Adamawa region. The SDG's target is to end all forms of malnutrition by 2030; including by 2025 reaching, the internationally agreed targets (reduce and maintain under 5% wasting). (Demographic Health Survey, 1, 2018; WHO, 2019, SMART, 2021).

1.2.7.2.3. Causes of Wasting

Children become wasted when they lose weight rapidly, usually as a direct result of a combination of infection and diets that do not cover nutritional needs (WHO, 2012). The main underlying causes of wasting are:

a/ Poor access to appropriate, timely and affordable health care;

b/ Inadequate caring and feeding practices (e.g. exclusive breastfeeding or low quantity and qualit of complementary food).

c/ Poor food security – not only in humanitarian situations, but also an ongoing lack of food quantity and diversity, characterized in many resource-poor settings by a monotonous diet with low nutrient density, together with inadequate knowledge of patterns of food storage, preparation and consumption; and

d/ Lack of a sanitary environment, including access to safe water, sanitation and hygiene services (UNICEF *et al., 2015*).

1.2.7.2.4. Consequences of Wasting

Suboptimum growth indicative of wasting has been shown to increase the risk of death in childhood from infectious diseases such as diarrhoea, pneumonia and measles (Pelletier *et al.,* 2013). It is not yet well understood how much wasting contributes to conditions such as stunting, low birth weight and anaemia. Evidence does suggest, how-ever, that episodes of wasting negatively affect linear growth and, therefore, undermine child growth and development (Khara and Dolan, 2014).

1.2.7.3. Overweight

Global Nutrition Target 4 is no increase in childhood overweight. There has been a dramatic rise in the numbers of children under 5 years of age who are overweight.

1.2.7.3.1. Definition of Overweight

Overweight refers to a child who is too heavy for his/her height. This form of malnutrition results from expending too few calories for the amount consumed, and increases the risk of non-communicable disease later in life (UNICEF *et al.,* 2015).

1.2.7.3.2. Prevalence of Overweight

According to the new 2019 United Nations Children's Fund (UNICEF), World Health Organization (WHO) and World Bank estimates between 2000 and 2019, the number of overweight children worldwide increased from 32 million to 40 million (5.9%).

The prevalence of childhood overweight is increasing in all regions of the world, particularly in Africa and Asia. Between 2000 and 2019, the prevalence of overweight in children under 5

years of age increased from 1% to 19% in southern Africa, and from 3% to 7% in south-east Asia. (UNICEF *et al.*, 2019).

In terms of regional breakdowns in numbers of overweight children in 2013, there were an estimated 18 million overweight children under 5 years of age in Asia, 11 million in Africa and 4 million in Latin America and the Caribbean. Low levels of overweight in children under 5 years of age were observed in the regions of Latin America and the Caribbean, with little change over the last 13 years. Nevertheless, countries with large populations, such as Argentina, Brazil, Chile, Peru and the Plurinational State of Bolivia, observed levels of 7% and higher (WHO, 2019).

In Cameroon, the prevalence of overweight children under five years of age increased from 6.2% to 11% between 2011 and 2018. The SDG's target is to end all forms of malnutrition by 2030, including by 2025 reaching the internationally agreed goals on overweight children (no increase on overweight children) (WHO, 2019).

1.2.7.3.3. Consequences of Overweight

Children who are overweight or obese are at a higher risk of developing serious health problems, including type 2 diabetes, high blood pressure, asthma and other respiratory problems, sleep disorders and liver disease. They may also suffer from psychological effects, such as low self-esteem, depression \and social isolation. Childhood overweight and obesity also increase the risk of obesity, non-communicable diseases (NCDs), premature death and disability in adulthood. Finally, the economic costs of the escalating problem of childhood overweight and obesity are considerable, both in terms of the enormous financial strains it places on health-care systems and in terms of lost economic productivity. It is possible for a child to show combinations of malnutrition, such as being stunted and overweight or stunted and wasted (UNICEF *et al.*, 2015).

1.2.8. Malnutrition and Immunity

Relating immunity and malnutrition, there is now clear evidence that malnutrition down-regulates immunity functioning, resulting in higher risk of infection. (Fillol *et al.,* 2009)

The cycle of malnutrition and infection

Malnutrition can make a person more susceptible to infection, and infection also contributes to malnutrition, which causes a vicious cycle **(Figure 9).** An inadequate dietary intake leads to weight loss, lowered immunity, mucosal damage, invasion by pathogens, and impaired growth and development in children. **(Katona and Katona, 2008)** Undernutrition and infectious diseases exist in a baleful synergy: undernutrition reduces immunological capacity to defend against diseases, and diseases deplete and deprive the body of essential nutrients. Undernutrition and micronutrient deficiencies contribute substantially to the global burden of disease **(Ezzati *et al.*, 2002).**

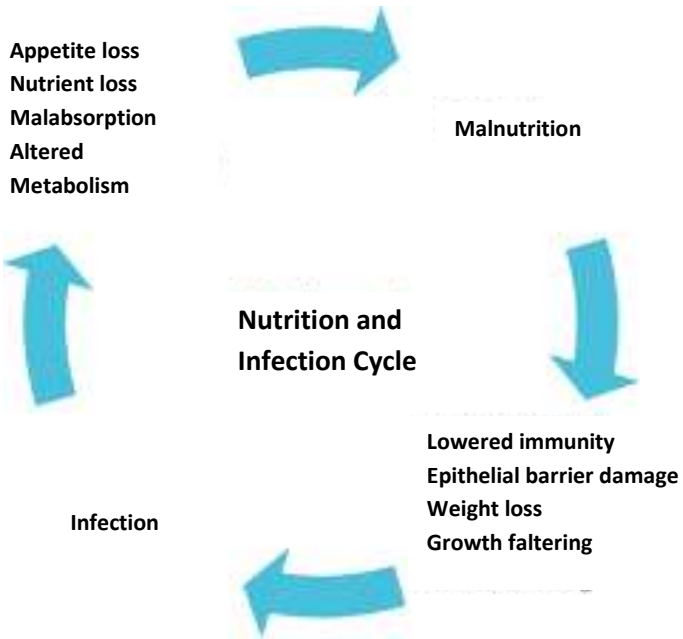


Figure 9: The "vicious cycle" of malnutrition and infection. (Katona and Katona, 2008)

A sick person's nutrition is further aggravated by diarrhoea, malabsorption, loss of appetite, diversion of nutrients for the immune response, and urinary nitrogen loss, all of which lead to

nutrient losses and further damage to defense mechanisms. These, in turn, cause reduced dietary intake. In addition, fever increases both energy and micronutrient requirements. Malaria and influenza, for example, have mortality rates proportionate to the degree of malnutrition (Muller *et al.*, 2003). The causes of malnutrition and disease operate at different levels.

Underlying the problem of malnutrition and disease is inadequate house-hold food security, which the US Department of Agriculture defines as "access by all members at all times to enough food for an active, healthy life," not merely as adequate food for survival (Nord *et al.*, 2006). Access to health services and environmental health conditions relate to essential drugs and immunizations,

Malnutrition is the primary cause of immunodeficiency worldwide, with infants, children, adolescents, and the elderly most affected. There is a strong relationship between malnutrition and infection and infant mortality, because poor nutrition leaves children underweight, weakened, and vulnerable to infections, primarily because of epithelial integrity and inflammation (Katona and Katona, 2008). Impoverished communities experience high rates of undernutrition and increased exposure to infectious diseases caused by crowding and inadequate sanitation. Women of reproductive age and children experience devastating health consequences as a result of limited resources, cultural influences, and biological vulnerabilities. Undernutrition and infectious diseases further exacerbate poverty through lost wages, increased health care costs, and—most insidiously—impaired intellectual development that can significantly reduce earning potential. Health experts have recently recognized the long-term effects of early undernutrition and inadequate infant feeding for obesity and chronic diseases, including diabetes and cardiovascular diseases (Katona and Katona, 2008).

1.2.8.1. The macronutrient era

Human studies and better animal models led to the recognition that malnutrition is not unique to children.

1.2.8.2. Micronutrients and immunity

Good nutrition is fundamental to improve immunity. The immune system is the body's defense against disease and infection and it has long been established that several factors influence the function of the immune system including stress, sleep and nutrition (Patel *et al.,* 2012; Song *et al.,* 2019; Gombart *et al,* 2020). WHO guidance on diet states that "good nutrition is crucial for health, particularly in times when the immune system might need to fight back" (WHO, 2020).

Providing a diet high in nutritious foods rich in vitamins and minerals supports optimal function of the immune system by providing antioxidants to slow damage of cells caused by free radicals (Lobo, 2010) or assisting in T-cell production (Cohen, 2017).

It is now recognized that the complex integrated immune system requires several micronutrients that have essential, often synergistic roles at every phase of the immune response (Gombart *et al.,* 2020). In fact, even marginal deficiencies in certain nutrients have been shown to impair the immune system (Gombart *et al.,* 2020). Micronutrients are believed to work collectively to support an optimum immune system. Micro-nutrients have a relationship to antibody formation and the development of the immune system. Based on a variety of systematic and clinical data, vitamins A, B6, B12, C, D, E, Folate, zinc, Iron, Copper, and Selenium are particularly important to boosting immune response (Calder *et al.,* 2020).

Worldwide, ~2 billion people are affected by micronutrient deficiencies, including vitamins A, C, and E and minerals zinc, iron, and iodine. The effects are poor growth, impaired

intellect, and increased mortality and susceptibility to infection. These ill effects are preventable by supplements, fortification, and diet change. The Copenhagen Consensus of 2008, project on hunger and malnutrition even suggested that efforts to provide vitamin A, iron, iodine, and zinc generates higher returns than do trade liberalization or malaria, water, and sanitation programs (WHO, 2020).

1.2.8.2.1. Vitamin A and Immunity

Vitamin A is often referred to as the "anti-inflammatory vitamin" because of its important role in enhancing immune function. It has a central role in the development of the immune system and plays regulatory roles in cellular immune responses and humoral substances found in the humors, or bodily fluids) immune processes (Huang *et al.,* 2018). Vitamin A Deficiency results from inadequate intakes of vitamin A or provitamin A because of low intakes of animal foods; inadequate intakes of non-animal sources of carotenoids that are converted to vitamin A; and inadequate intakes of fat, which facilitates the absorption of carotenoids. Dietary sources of preformed vitamin A include liver, milk, and egg yolks. Dark green leafy vegetables such as spinach, as well as yellow and orange non-citrus fruits (mangoes, apricots, papayas) and (red palm oil, pumpkins, squash, carrots), are common sources of carotenoids (vitamin A precursors), which are generally less bioavailable than preformed vitamin A but tend to be more affordable (Rice *et al.,* 2004).

1.2.8.2.2. Vitamin E and Immunity

Vitamin E acts as a powerful antioxidant that scavenges/protecting cells against oxidative damage from free radicals. Vitamin E supplementation has been shown to have an important role in modulating immune functions in the elderly, with delayed hypersensitivity skin response and antibody production after vaccination. Even a slight Vitamin E deficiency has been shown to weaken the immune response and its role in promoting a healthy immune system is particularly evident in older people (Moriguchi and Muraga, 2000; Meydani and

Han, 2005). A study by Lee and Hang also demonstrated a particularly protective role against several infectious diseases (Lee and Hang, 2018). Vitamin E increases both cell-dividing and interleukin-producing capacities of naive T cells but not of memory T cells. This enhancement of immune function is associated with significant improvement in resistance to influenza virus infection in aged mice and a reduced risk of acquiring upper respiratory infections in nursing home residents (Meydani *et al.,* 2005). Vitamin E is stored in the liver and is safe even at high intakes.

1.2.8.2.3. Vitamin D and Immunity

Although more clinical trials are required, promising results have been shown in studies to date. It appears that supplementation with Vitamin D lowers the probability of developing acute respiratory tract infections to varying degrees (Charan *et al.,* 2012 and Gysin *et al.,* 2016). Among the infected, flu symptoms were less experienced with recovery sooner, if higher doses of Vitamin D greater than 1.000 IU were administered (Zhou *et al.,* 2018). The benefits appear greater in those with Vitamin D deficiency than in those who had sufficient levels. Vitamin D supplements may offer a cheap and effective immune system boost against tuberculosis (Martineau *et al.,* 2007).

Vitamin D was used to treat tuberculosis in the pre-antibiotic era, when special sanatoria were built in sunny locations, such as the Swiss Alps. Investigators reported that a single 2.5-mg dose was sufficient to enhance the immune system's ability to withstand infection. These findings came from a study that identified an extraordinarily high incidence of vitamin D deficiency among tuberculosis-susceptible women in Muslim communities in London (Diamond *et al.,* 2002).

1.2.8.2.4. Vitamin C and Immunity

Vitamin C has an important role in wound healing and as an antioxidant, potentially protecting cells from oxidative damage caused by free radicals. Vitamin C helps in the protection against infections and inflammation by supporting various cellular functions of both the innate and adaptive immune systems. Regarding respiratory tract infections, a Cochrane review demonstrates a significant reduction in the incidence of infections when participants were supplemented with Vitamin C (Hemilä and Louhiala, 2013).

1.2.8.2.5. Vitamin B_{12} and Immunity

Vitamin B_{12} is required for proper red blood cell formation, nerve system function and DNA synthesis. It works together with folate and Vitamin B_6, to help maintain normal blood homocysteine levels. Research points to a significant role for B_{12} as an immunomodulator for cellular immunity (Tamure *et al.,* 1999).

In Mikkelssen and Apostolopoulos chapter on the role of B_{12} on immunity, they conclude that B_{12} together with folic acid plays a key role in the healthy functioning of the immune system. Indeed, they found that insufficient levels changed immune responses by impacting development of nucleic acid, protein production, inhibiting the action of immune cells and inhibiting metabolic processes (Mikkelsen and Apostolopoulos, 2019).

1.2.8.2.6. Vitamin B_6 and Immunity

B_6 is required for maintenance of normal blood homocysteine levels (Raised homocysteine is a risk factor for cardiovascular disease). Vitamin B_6 comprises 3 forms: pyridoxine, pyridoxal and pyridoxamine. All three forms of B_6 can be converted to the co-enzyme PLP. Vitamin B_6 in its coenzyme form is involved in more than 100 enzyme reactions and it is fair to say that Vitamin B_6 is required for the majority of biological reactions in our body.

While more research is necessary to understand B_6's role in immunity, studies suggest Vitamin B6 deficiency impacts both humoral and cell-mediated immune responses (Rall and Meydani, 1993) and thus impairs immune responses.

1.2.8.2.7. Zinc and Immunity

Zinc is a trace mineral that is essential for all species and is required for the activity's of 1300 enzymes, carbohydrate and energy metabolism, protein synthesis and degradation, nucleic acid production, heme biosynthesis, and carbon dioxide transport. It is a cofactor in the formation of enzymes and nucleic acids and plays a critical role in the structure of cell membranes and in the function of immune cells. Zinc deficiency reduces nonspecific immunity, including neutrophil and natural killer cell function and complements activity; reduces numbers of T and B lymphocytes; and suppresses delayed hypersensitivity, cytotoxic activity, and antibody production (Fawzi and Msamanga, 2004).

Inadequate zinc supply prevents normal release of vitamin A from the liver; clinically, it is associated with growth retardation, malabsorption syndromes, fetal loss, neonatal death, and congenital abnormalities. Low blood zinc concentrations have also been found in patients with tuberculosis, Crohn disease, diarrhoeal disease, and pneumonia. Zinc deficiency is associated with abnormal pregnancy outcomes (Fawzi and Msamanga, 2004) and conditions of relative immunocompromise, including alcoholism, kidney disease, burns, inflammatory bowel disease, and HIV infection. Many drugs, including corticosteroids, also cause excessive excretion.

Zinc supplementation reduces the duration and intensity of diarrhoeal illness and pneumonia among children living in developing nations. It limits growth stunting in children affected by acute diarrhoeal illness and reduces clinical disease caused by *P. falciparum* (Caulfield *et al..*, 2004; Cuevas and Koyanagi, 2005). Zinc deficiency results from inadequate intakes and, to some extent, increased losses. Only animal flesh, particularly oysters and shellfish, is a good

source of zinc, and fiber and phytates inhibit absorption. Thus, as with iron deficiency, populations consuming a primarily plant-based diet are susceptible. Deficiency can also result from losses during diarrhoeal illness (WHO, 2020).

1.2.8.2.8. Iron and Immunity

Iron deficiency is the most common trace element deficiency worldwide, affecting 20%–50% of the world's population; mainly infants, children, and women of childbearing age (Patterson *et al.,* 2001). It is associated with impairments in cell-mediated immunity and reductions in neutrophil action, with decreased bacterial and myeloperoxidase activity. It lowers the body's defenses against disease and diminishes body and brain functions. Despite this, iron deficiency has unclear effects on infectious disease risk.

In the treatment of malaria, correcting iron deficiency is important, because malaria causes hemolysis and anaemia. Supplementation in some cases, however, may actually aggravate infection, because the malaria parasite requires iron for its multiplication in blood and thus may be less infective in the iron-deficient person. The mechanism for this may also be related to the inhibition of zinc absorption (Oppenheimer, 2001). Many microorganisms require trace elements, such as iron and zinc, for survival and replication in the host and may increase in pathogenicity with supplementation (Shankar, 2000); thus, there is a concern about iron supplementation in malaria chemoprophylaxis programs. In general, iron (preferably with folate) should be administered to all pregnant women undergoing malaria chemoprophylaxis (Garner and Brabin, 1994) much like the need for pyridoxine (B6) supplementation during isoniazid treatment.

Iron is found in all plant foods but is more plentiful and bioavailable in meat. Deficiency results from insufficient absorption of iron or excess loss. Absorption is tightly regulated in the intestines, depending on the iron status of the individual, the type of iron, and other

nutritional factors. Once iron is absorbed, it is well conserved. Iron is depleted primarily through blood loss, including from parasitic infections such as schistosomiasis and hookworm (WHO, 2020).

1.2.9. IgG1 and IgG3 responses in relation to malnutrition

Although some studies earlier reported no impact of malnutrition on antibody responses to *Plasmodium falciparum* (Tepa *et al.,* 2020), others believe that nutritional status could modulate immune responses directed to malaria antigens. Nevertheless, there is now clear evidence that malnutrition down-regulates immune responses directed to malaria antigens (Plebanski *et al.,* 2008, Juster *et al.,* 2017). It is also believed that malnutrition (most precisely stunting) seems to be the most likely cause of down- regulation of anti-*Plasmodium falciparum* IgG antibody response (Fillol *et al.,* 2009). Indeed, childhood nutritional status could regulate development of the acquired protective immunity response to malaria antigens. It is now widely admitted that undernutrition during critical periods of childhood growth impairs normal development of the immune system (Cunningham-Rundles *et al.,* 2005). In addition, nutritional status could modulate the immune response directed to malaria antigens, in particular, to major vaccine candidates (Plebanski *et al.,* 2008). Indeed, a previous study revealed that IgG Ab levels against RESA and Spf66 antigens were lower in wasted children compared to well-nourished children (Genton *et al.,* 1998). Therefore, future phase 2/3 vaccine trials including major candidates should take into account child nutritional status when evaluating the specific immune response acquired after immunization. (Fillol *et al.,* 2009).

Malnutrition causes atrophy of the thymus and other lymphoid tissues, reduced B-cell activation and complement formation. Consequently, both acquired immunity and innate host defence mechanisms are affected in malnourished children (Schaible and Kaufmann, 2007). Moreover, many studies highlighted the role of micronutrients in host resistance to infection

(Field *et al.*, 2002; Cunningham-Rundles *et al.*, 2005). Deficiencies in some micronutrients such as VitA, Zinc and Iron are thought to play an important role in modulation of malaria morbidity.

Experimental evidence in the last decades shows that iron is a fundamental element for normal development of the immune system. Its deficiency affects the capacity to have an adequate immune response. The role of iron for immunity is necessary for immune cell proliferation, particularly lymphocytes, associated with the generation of a specific response to infection. Humoral immunity appears to be less affected by iron deficiency than is cellular immunity (Beard *et al.*, 2001). Iron is required for monocyte/macrophage differentiation, while macrophages require iron as a co-factor for the execution of important antimicrobial infector mechanisms, including the nicotinamide adenine dinucleotide phosphate hydrogen-dependent oxidative burst (Collins *et al.*, 2003)**.**

1.3. ANAEMIA

1.3.1. Definition of Anaemia

Anaemia is a physiological or haematological condition in which the number and size of red blood cells, or the `haemoglobin concentration, falls below an established cut-off value, for sex, age, environmental and physiological status, consequently impairing the capacity of the blood to transport oxygen around the body. Anaemia is an indicator of both poor nutrition and poor health (WHO, 2008; Stevens *et al.*, 2013; WHO 2014).

1.3.1.1. Hamoglobin threshold

Normal Haemoglobin distributions vary with age, sex, and physiological status e.g during pregnancy (Koller, 1982). WHO thresholds were used to classify individuals as follows;

Table 1: Haemoglobin thresholds used to define anaemia

Age or gender group	Haemoglobin threshold (g/L)
Children (0.50 – 4.99 years)	110
Children (5.00 – 11.99 years)	115
Children (12.00 – 14.99 years)	120
Non-pregnant women (≥15.00 years)	120
Pregnant women	110
Men (≥15.00 years)	130

Source: Adapted from WHO, (2001). Iron deficiency anamia

1.3.2. Causes of Anaemia

Anaemia is the result of a wide variety of causes that can be isolated, but more often coexist. Globally, Iron deficiency is indicated as the most common cause of anaemia in under-five children with a smaller proportion due to other micronutrient deficiency such as folate, Vitamin A and B12 (Villalpando *et al.*, 2003). The main risk factors for iron deficiency anaemia (IDA) include a low intake of iron, poor absorption of iron from diets high in phytates or phenolic compounds and periods of life when iron requirements are especially high (growth and pregnancy), (WHO, 2011).

Among the other causes of anaemia, heavy blood loss as a result of menstruation, or parasite infection such as *plasmodium*, hookworms, ascaris, and schistosomiasis can lower blood haemoglobin (Hb) concentrations (WHO, 2011) as well as other infections which cause blood loss also contribute to iron deficiency and anaemia, often severe anaemia (Caulfield *et al.,* 2006). Acute and chronic infections, including malaria, cancer, tuberculosis and HIV can also lower blood Hb concentration. Additional risk factors of anaemia include glucose-6-phosphate dehydrogenase deficiency, haemoglobinopathies, and infectious diseases endemic in African countries (Koremromp *et al.*, 2004).

The presence of other micro- nutrient deficiencies, including Vit A and B12, folate, riboflavin and copper can increase the risk of anaemia. However, other factors, such as early weaning, poor health of safe drinking-water, inadequate hygiene and sanitary conditions, and poverty which increases the likelihood of all the above-mentioned factors, may contribute to the development of the disease (WHO, 2001).

1.3.3. Prevalence of Anaemia

1.3.3.1. Prevalence of Anaemia Worldwide

Globally, approximately 1.62 billion people are affected by anaemia (Kebede *et al.,* 2021; Gaston *et al.,* 2021).

1.3.3.2. Prevalence of Anaemia in Developing countries, Africa and North America

In developing countries, having low- and middle-income, the prevalence of anaemia among 6-59 month age children was >20% based on latest demographic and health survey (DHS) report rounds between 2005-2018, and it is classified as severe public health problem (hassan *et al.,* 2021). .. In the same age-group, the prevalence was 70% in Togo (Nambiema *et al.,* 2019), 41.1% in North East Ethiopia (Gebreweld *et al.,* 2019) and 37.9% in Tanzania at Rombo district (Mboya *et al.,* 2020). A prevalence of 27.3% was reported by Huang *et al.,* (2018) in Huaihua, Hunan Province, China.

1.3.3.3. Prevalence of Anaemia in sub- Saharan Africa

Sheltering approximately 84.5 million 6-59 months aged children suffering from anaemia, Sub- Saharan Africa remains the most affected region with a prevalence reaching 62.3% (Nambiema *et al.,* 2019). Approximately 36.4 to 61.9% of under-five children in sub-Saharan Africa are affected (Roberts *et al.,* 2020). Recently, in sub-Saharan Africa, the prevalence of anaemia among children has been reported to be 64.1% (Tesema *et al.,* 2021).

1.3.3.4. Prevalence of Anaemia in Cameroon

Anaemia is a major public health problem in Cameroon with most recent estimates from the World Bank showing a prevalence of 62.5% in children (< 5 years) and 49.3% in pregnant women (World Bank, 2020, Sama *et al.,* 2021). The national prevalence of anaemia among children under five years old was almost 57%, with rates as high as 67% in certain regions (Helen Keller International, 2013; DHS, 2018; WHO, 2019). A prevalence which decreased by 3% from 60% in 2011. Meanwhile, Jourdan *et al.,* 2008 also reported a prevalence as high as 82% in children attending a clinic in Northern Cameroon, as well as 84% by Sumbele *et al.*, (2020) in the conflict- hit Mount Cameroon area and 77.3% by Asoba *et al.*, (2019) in similar localities.

1.3.4. Indicators of Anaemia

At the population level and in clinical practice, haemoglobin concentration is the most common indicator and the most common haematological assessment method used (CDC, 2013). Cut offs in children aged under 5 years with haemoglobin lower than 110 g/L; in children >5, Hb lower than 115 g/L (WHO, 2014).

1.3.5. Consequences of Anaemia

Anaemia is a global public health problem affectiong both developing and developed countries with major consequences for human health as well as social and economic development.

Indeed, the consequences of anaemia among pre-school children are serious (McCann *et al.,* 2007; Lozoff *et al.*, 2010; Stoltzfus, 2011) and include: impairment of cognitive function, impaired motor development and growth, declining academic performance, decreased immune function which exposes children to infections, decreased responsiveness and activity, and fatigue. These can irreversibly compromise the future development of a child. This

situation has awoken a particular interest both nationally and internationally, leading to the implementation of prevention programs through food fortification and intermittent iron supplementation (Sylvetsky *et al.,* 2011; OMS, 2012).

1.3.6. Clinical symptoms of anaemia

The critical role of Hb to carry oxygen to the tissues explains the clinical symptoms of anaemia, which include fatigue, shortness of breath, bounding pulses or palpitations, and conjunctival and palmar pallor (WHO, 2005)

1.3.7. Diagnosis of anaemia

Haemoglobin concentration is the most reliable indicator of anaemia at the population level, as opposed to clinical measures which are subjective and therefore have more room for error. Though most commonly diagnosed by a low haemoglobin concentration or a low haematocrit (Schreir, 2018), anaemia can also be diagnosed using RBC count, Mean Corpuscular Volume, Blood reticulocyte count, blood film analysis or Haemoglobin electrophoresis (Balarajan *et al.*, 2011).

1.3.8. Classification of anaemia by degree of Public Health significance

The level of the public health problem of anaemia has been illustrated by WHO, across age groups in several countries, most especially for pre-school children, pregnant and non-pregnant women, based on Hb concentrations. The prevalence of anaemia as a public health problem is categorized as follows;

Table 2: Classification of anaemia by Public Health significance

PublicHealthProblem	Prevalence (%)
None	**<5**
Mild	**5 – 19.9**
Moderate	**20 – 39.9**
Severe	**≥40**

Source: WHO, (2001)

1.3.9. Control of anaemia

1.3.9.1. Correcting anaemia: Prevention and Management

Given the multifactorial nature of this disease, correcting anaemia often requires an integrated approach. In order to effectively combat it, the contributing factors must be identified and addressed. In settings where iron deficiency is the most frequent cause, additional iron intake is usually provided through iron supplementation at low doses to vulnerable goups; young children and pregnant women in particular.

Also, food-based approaches to increase iron intake through food fortification and dietary diversification are important, sustainable strategies for preventing iron deficiency anaemia (IDA) in the general population. In settings where iron deficiency is not the only cause of anaemia, approaches that combine iron interventions with other measures are needed. Strategies should include addressing other causes of anaemia (Crompton *et al.*, 2003; WHO, 2006).

1.4. RELATIONSHIP BETWEEN MALNUTRITION, ANAEMIA, IMMUNITY AND MALARIA.

Children's immune systems are not well developed, so they rely on maternal immunity transferred from the mother to the child at birth. Their immunity develops gradually therefore

making them very prone to infection. Naturally acquired immunity to *falciparum* malaria protects millions of people routinely exposed to *Plasmodium falciparum* infection from severe disease and death. However, there is no clear concept about how this protection works (Doolan *et al.,* 2009). Thus, this study is necessary to provide a better understanding.

Nutritional status is closely tied to immune responses to infection (Caulfield *et al.,* 2004). Poor feeding in children greatly contributes to the development of infectious diseases, most especially malaria as well as weakened immune responses that are unable to fight off infection (Thorarinsdottir *et al.,* 2005). However, the impact of malnutrition on antibody responses is poorly understood. It is thought that malnutrition impairs the immune system, rendering undernourished children more susceptible to infections and death (Rytter *et al.,* 2014). Malnutrition causes atrophy of the thymus and other lymphoid tissues, reduced β-cell activation and complement formation. The simultaneous presence of both malnutrition and infection results in an interaction that has more serious consequences for the host than the additive effect would be if the two worked independently. Infections make malnutrition worse and poor nutrition increases the severity of infectious diseases (Nyakeriga *et al*., 2004). Based on previous reports on malnutrition-induced immunodeficiency, an undernourished child may be unable to mount an appropriate immune response to the malaria parasite leading to poor outcome (Bourke *et al.,* 2016). Malnutrition, particularly chronic malnutrition (stunting) has been reported to down-regulate anti- *P. falciparum* antibody responses in pre-school children in rural Senegal (Fillol *et al.,* 2009). Indeed, the childhood nutritional status could regulate development of the protective immune responses to malaria antigen.

Owing to the fact that few studies have explored the interaction between child malnutrition and anti-*P. falciparum* antibody responses (Fillol *et al.,* 2009), data on this aspect is scarce. Although some studies (Plebanski *et al.,* 2008; Fillol *et al.,* 2009; Juster *et al.,* 2017) report that nutritional status could modulate immune responses directed to malaria antigens, others

observed that there is no impact of malnutrition on antibody response to *P. falciparum* (Tepa *et al.,* 2020) in Eastern Cameroon**.** Nutrition is a critical determinant of immune responses and malnutrition the most common cause of immunodeficiency worldwide (Katona and Katona, 2008). When a child is undernourished, he/she may be unable to mount an appropriate immune response to the malaria parasite due to reduction in T-lymphocytes, impairment of antibody formation, decreased complement formation and atrophy of thymus and other lymphoid tissues. Consequently, both acquired immunity and innate host defense mechanisms are affected in malnourished children (Cunningham-Rundle *et al.,* 2005).

These conflicting results show the pertinent need for this study on the impact of child malnutrition on anti- malarial immunity, hence an advancement of knowledge.

However, to further complement the role of nutritional status, many studies have highlighted the role of micronutrients in host resistance to infection (Field *et al.,* 2002; Cunningham-Rundles *et al*., 2005). Undernutrition and infectious diseases exist in a baleful synergy: undernutrition reduces immunological capacity to defend against diseases, and diseases deplete and deprive the body of essential nutrients (Katona and Katona, 2008). Majority of the children in Africa live in poor and food insecure households and thus are more likely to consume inadequate and low-quality diets and experience nutrient deficiencies (Lewnard *et al.,* 2014). As a result of poor nutrition, these children may have compromised antibody immune systems, increasing their risk for malaria and death (Waithaka *et al.,* 2017). Many studies highlighted the role of micronutrients in host resistance to infection (Gombart *et al.,* 2020). Several micronutrients play a role in the immune system and have been associated with malaria incidence; Fe, Zn, Vit A, Folate, Long chain PUFA, Anti-oxidants like Vit E and C, Riboflavin, and Thiamine (Barua *et al.*, 2018).

Micronutrient deficiencies are of particular interest since their mode of action is more often on host response to infection and immunity than direct effect on parasite development. Both zinc and vitamin A are, for instance, essential for effective immune responses (Thurnham, 2015).

The relation between malnutrition and malaria is controversial, complex and poorly understood (Das *et al.,* 2018). On one hand, malaria may cause malnutrition, whereas on the other hand, malnutrition itself may modulate susceptibility to malaria (Nyakeriga *et al*., 2004). Some studies even report that there is no effect of malnutrition on malaria (Deribew *et al.,* 2010; O'brien *et al.,* 2018) and vice versa**.** These discrepancies in results, brings about the need for further studies, to contribute to a better understanding of the relationship between malaria and malnutrition.

Undernutrition or over nutrition both affect the immune system by decreasing immunity and this renders the children more vulnerable to infections. Upon infection with malaria, it further compounds the problem and as a consequence, RBCs are massively destroyed leading to anaemia which can be worsened in children that are malnourirshed, thereby down- regulating immune responses against malaria (Deribew *et al.,* 2010).

Anaemia, an indicator of both poor nutrition and poor health is a common and sometimes serious complication of *P. falciparum* infection (Benoist *et al*., 2008). Anaemia and nutritional deficiency are associated with severe malaria (Ansong *et al.,* 2013). Anaemia and malnutrition potentially could impact on the outcome of children reporting to the emergency units with severe malaria (Ansong *et al.,* 2013). The development of anaemia in children with malaria has as potential contributing factors, the degree of parasitemia with the resultant splenic destruction of the parasitized and the non-parasitized red cells and the level of malnutrition (Mulenga *et al.,* 2005), hence reducing haemoglobin levels leading to anaemia (Sumbele *et al.,* 2016). The cyclical relationship between poor nutrition, increased susceptibility to

infectious diseases, leads to immunologic dysfunction and metabolic responses that further alter nutritional status (Katona and Katona, 2008).

Individuals living in areas where malaria is transmitted, often become immune to the disease, with antibodies being an important component of immunity (Bouharoun- Tayoum *et al.*, 1990).

Fillol *et al*., (2009); Juster *et al.,* (2017); Tepa *et al.,* (2020) explored some relationships among immunity, nutrition, and malaria in groups of children from populations with a high endemic incidence of malaria in rural Senegal, Kenya and Eastern Cameroon respectively. Findings showed a relationship was observed between the anthropometric indicators of malnutrition (especially stunting) on antibody responses to *Plasmodium falciparum* in Senegal, both wasting and stunting reduced IgG responses in children below 5 years to *P. falciparum in* Kenya, but no association in Cameroon. These conflicting results show the need for this study, so as to provide a better understanding of the relationship between child malnutrition and anti-*P. falciparum* antibody responses. Indeed, the childhood nutritional status could regulate development of the protective immune responses to malaria antigen. (Cunningham-Rundle *et al.,* 2005). In fact, even marginal deficiencies in certain nutrients have been shown to impair the immune system (Gombart *et al.,* 2020). Providing a diet rich in nutritious foods rich in vitamins and minerals supports optimal function of the immune system by providing anti-oxidants to slow the damage of cells caused by free radicals (Lobo, 2010).

CHAPTER TWO: MATERIALS AND METHODS

CHAPTER TWO: MATERIALS AND METHODS

2.1. Description of the Study Site(s)

2.1.1. Field Sites

The study was conducted in two health districts, namely, Pitoa Health District, 9.21° N, 13.31° E – 9.23° N, 13.32° E and Mayo-Oulo Health District, 9.46° N, 13.44° E – 9.74° N, 13.37° E and both peri-urban settings, in the North region of Cameroon. **(Fig. 10)** The area is the center of trade of the surrounding agricultural region and also houses several textile processing facilities. Pitoa has a population of about 108.611 inhabitants while Mayo-Oulo is inhabited by about 91.501 individuals. The population depends almost entirely on agriculture (including gardening) for subsistence. Crops grown include; rice, maize, millet, cotton, and sorghum for Pitoa whereas beans, peanuts and maize are cultivated in Mayo-oulo.

The study area is predominantly populated by individuals of the Hausa ethnic group. Polygamy is a common practice with a number of wives and their children living in separate huts within the family compound. Both Health Districts have a sudanese type climate with an annual average rainfall of 700 – 1000mm, annual average temperature of 26.0 – 33.0°C and relative humidity of 15%. Malaria is endemic in the region, with a seasonal mode of transmission, lasting about 6months and peaking generally during the months of September – November when rainfall is highest. *Plasmodium falciparum* is the predominant (>98%) parasite species in the area (Tabue *et al.*, 2019)

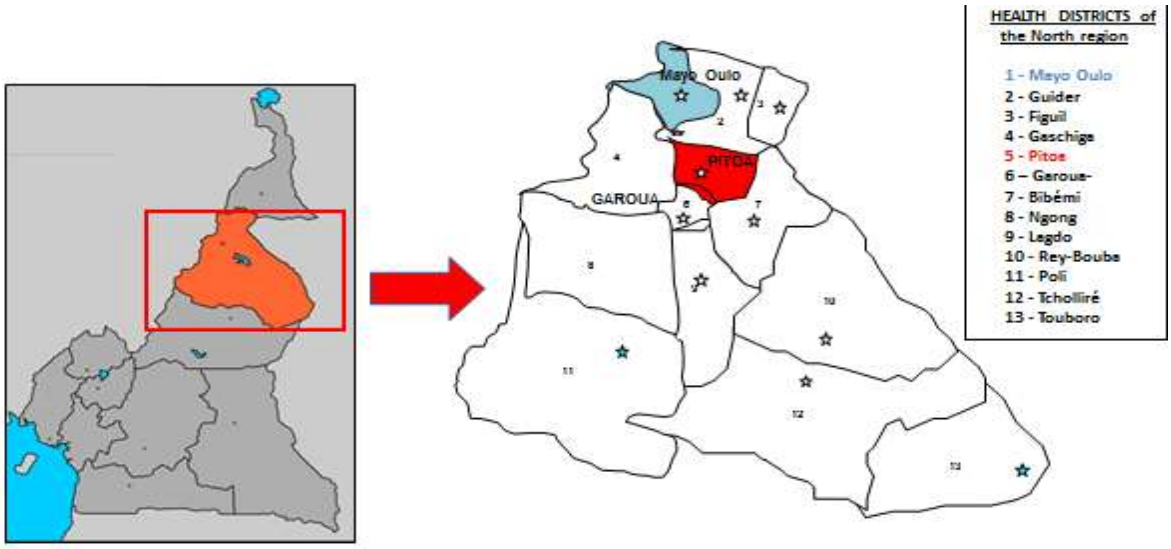


Figure 10 : Map of the North Region showing the Pitoa and Mayo-Oulo Health Districts. Source: (Tabue *et al.*, 2019)

This study site was chosen because of previous evidence on high prevalence of malaria and malnutrition morbidity data in the children of the study age group (Ngwa-Akongwi *et al.*, 2015).

2.2. Study Population

The study population consisted of children aged 6months to 10 years and of both sexes, after obtention of full informed consent of parents or legal guardians. They weighed between 4kg and 20.8 kg**.** Children with recent (during the last 48hours) or current fever, were considered symptomatic, and were tested using Rapid Diagnostic Tests and immediately treated for free following the national guidelines for managing uncomplicated malaria, if found positive. Blood smears were taken for all children irrespective of fever status for microscopy.

2.2.1. Calculation of sample size

The sample size was calculated using the formula (Manly, 1992)

$$n = \frac{Z^2 pq}{d^2}$$

where n= the sample size required

z= 1.96 : confidence level test statistic at the desired level of significance

p= previous proportion of malaria prevalence in study area (64.9%) (Garba and Mbofung, 2010)

q= (1-p) : proportion of malaria negative children

d= acceptable error willing to be committed

The minimum sample size was estimated as n = 350

This was adjusted by 10% to a maximum of at least 385 samples for each health district, taking into consideration the loss of samples and incomplete data entry.

2.3. Study design, period and sampling procedure

This was a cross- sectional study, conducted in the Pitoa and Mayo-oulo health districts, North Region of Cameroon in 2014, during the rainy season and peak malaria transmission period. The Investigation methods included the use of a structured questionnaire, clinical evaluation and laboratory investigations.

2.3.1. Sampling Procedure

Initially, a census of all households in the study localities was conducted and all houses given unique survey numbers. Following completion of the census, six (6) villages/cludters in both Health Districts were randomly selected: Kirambo, Guizigaré, Boussa (belonging to Pitoa

Health District); Mayo-oulo, Bala and Dourbeye (belonging to Mayo-oulo Health District). The study participants were recruited from 58 households through systematic random sampling. Visits to households were in the afternoon to include children attending school. The head of each household was the principal respondent and provided all necessary information for the household.

Subjects in each household were registered and information including name, sex, age, relationship to the head of the household, demographic and socio- economic data as well as risk factors like, village of residency, marital status, caregiver level of education, employment status of family head, type of housing, availability and principal source of electricity, drinking water and type of toilets at home, possession of household equipment and frequency of usage as well as participation in social groups, were recorded. Housing was described as separate rooms, apartments, buckaroo, several huts in one building or several huts in different buildings.

Three hundred and sixty-two children from 58 selected households were screened for malaria to detect malaria cases, anaemic cases as well as assess nutritional, socio-economic and immunity status. **(Fig. 10)** All diagnosed cases of malaria by Rapid Diagnostic Tests (RDT) were treated for free, following the national government guidelines.

Samples were then transported on ice packs in coolers from the field sites in the North Region to the Laboratories. This work was realized in the Laboratory of Nutrition and Nutritional Biochemistry in collaboration with the Laboratory for Molecular Parasitology and Disease Vector Research as well as the Immunology Laboratory, both of the Biotechnology Centre, Nkolbisson, University of Yaounde I, Yaounde.

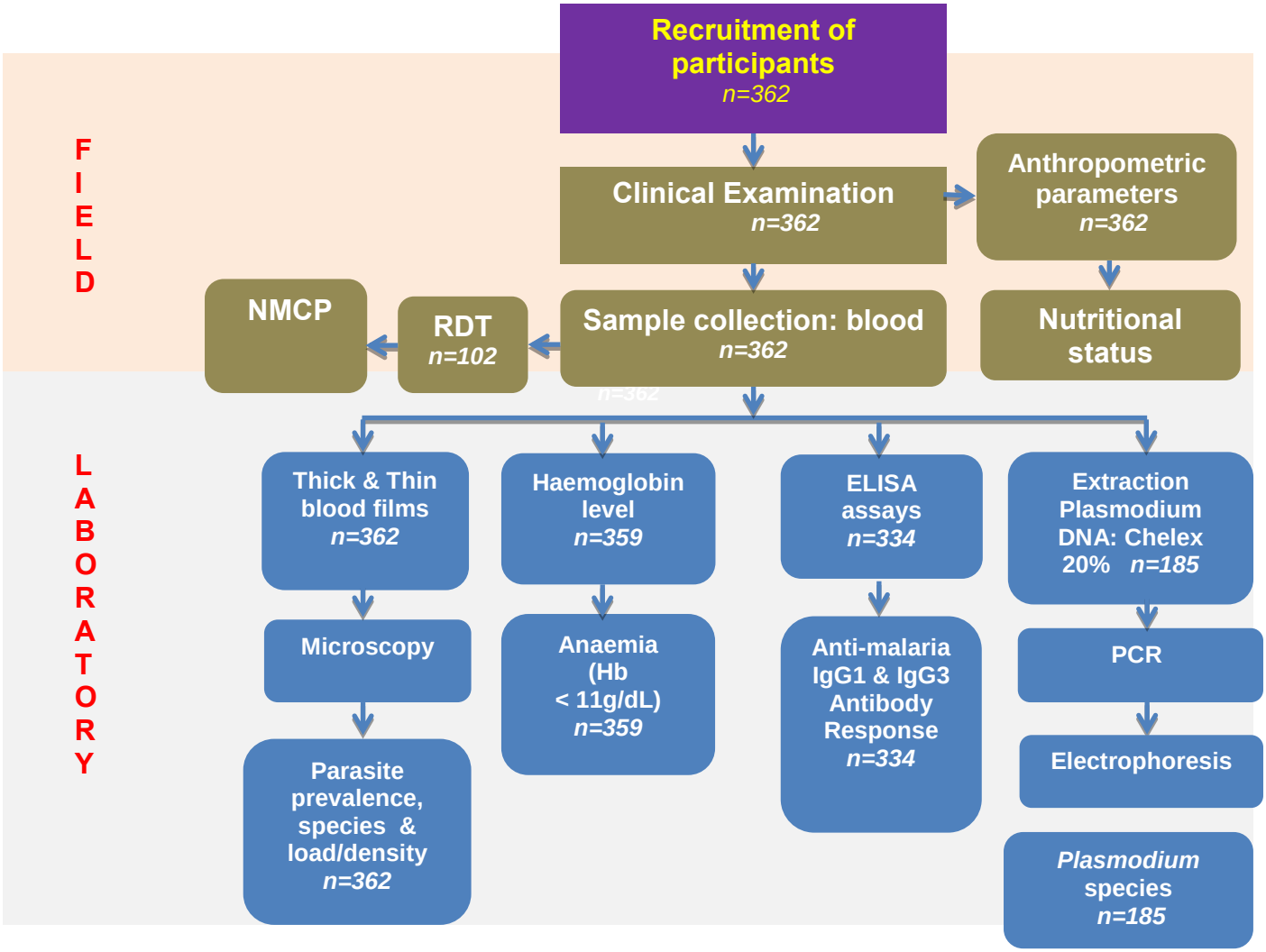


Figure 11 : Study Design **n= sample size**

2.3.2. Inclusion Criteria

- Children less than 10 years, both sexes.
- Children infected with the malaria parasite and manifesting malaria symptoms (high body temp > 37.5°C) or a history of fever, within the preceding 48 hours.
- Children with no malaria parasite.
- Children with written and verbal informed consent from parent/ legal guardian (assent).
- They must have resided in the study area for a duration of at least 4 weeks.

2.3.3. Non-inclusion Criteria

- Children who had no informed consent from parent/guardian resident in the study area were not included in the study.
- Children with incomplete data entry resulting from inadequate volume of blood for analysis.

2.4. Data Collection and Processing

2.4.1. Collection of Demographic and Socio-economic Data

Two different questionnaires were used for this study. One for Malaria data with medical history as concerns malaria and the other for Nutrition data (Appendix 2 and 3). Questionnaires were administered following an informed consent process, to record Socio-Economic and Demographic Data as well as nutrition /feeding habits for each household.

To determine the Socio-economic status (SES) of the households, the development index (a composite variable) was computed using socio-economic indicators. Indicators for socio-economic status included; the number of people living in a household, the level of education of caregiver and family head, employment status of family head, type of housing, type of toilet/waste disposal system, source of lighting and cooking energy, availability of potable water and possession of household assets. All the socio-economic indicators were recoded in ranking order of importance and the sum of scores calculated for each household. A straight forward count of the possessions was made where one point was given for each of the possessions to obtain the development index. The index was then divided into quintiles, SES1 to SES5 to determine poorest, poor, average, rich and richest SES.

2.4.2. Questionnaire for Malaria

State Registered Nurses, Medical Laboratory Technicians and Interviewers were trained for data and sample collection by the National Malaria Control Program. A Medical Doctor was

present on the field to supervise/oversee the sample and data collection procedures. The questionnaire was written in French, but had to be administered in the local dialect (Fufuldé) by the interviewers.

2.4.3. Questionnaire for Nutrition

Uniform structured questionnaires (Appendix 3), from the Cameroon Central Bureau of Census and Population Studies (BUCREP), prepared in English and then translated to French, were used for nutritional data collection. This was administered by 2 trained personnel/ data collectors/interviewers with at least University education, hailing from the region with knowledge of Fufuldé, the local language. However, they also spoke French, which is the main language spoken in the North Region of Cameroon. The questionnaires were administered to the head of households and comprised of the following parts; Demographic characteristics, nutritional status and food consumption frequency the previous 7 days, nutrition/diets and feeding habits, were all recorded through the administration of structured questionnaires to the head of each household.

2.4.4. Collection of Clinical Data

Clinical evaluations were carried out by trained medical personnel (Certified Nurses). Axillary body temperatures of the children were taken using digital thermometers. The sensitive portion of the thermometer was cleaned with alcohol using cotton, placed in the armpit of the child, taken out after a while and the temperature recorded in °C (+ 0.5°).

The conjunctivas were also examined for paleness (signs of anaemia). Conjunctival pallor was evaluated by everting the lower eyelid and examining the palpebral conjunctiva and recorded as either “coloured” or “not coloured”.

2.4.5. Collection of Anthropometric Data

Anthropometric measurements were taken by well trained personnel. Training sessions were held twice between the Ph.D. student researcher and the data collectors on the technique of interviewing and anthropometric measurements. Then the tools were tested in 5% of the sample size in the study area. The weighing scale was checked daily against the standard weight for accuracy. The weighing instrument was also calibrated before weighing each child by setting it to zero. Measurements were performed according to standard anthropometric methods (Cogill, 2003) and related to age and sex. Weight was measured to the nearest 0.1kg while height was read to the nearest 0.1cm.

Age; The age of the child was given by the parent or head of the household to whom the questionnaires were administered. Child's age was recorded in years.

Weight; Children >2kg were weighed wearing light clothes only and no shoes by 2 trained measurers using a portable electronic scale accurate to 0.1 kg positioned on a flat surface. In the case of infants (< 2 years), the weight of the mother was recorded and subtracted from the weight of the mother plus the infant, to get the weight of the infant. Weight was recorded in kg.

Height; Height (for children >2 years of age) and Length (for infants and children aged < 2 years), was measured to the nearest 0.1cm using a portable non-stretchable tape. Height was measured in a standing position while Length was measured using the same tape, with the child in a horizontal position. Both were recorded in cm.

Mid Upper Arm Circumference (MUAC); a composite measure of upper arm muscle and subcutaneous fat reserves, with no clothes covering the arm, was measured to 0.1cm at the midpoint of the left upper arm, between the acromion process(elbow) and the tip of the

olecranon(shoulder), using a portable non- stretchable tape. MUAC was also recorded in cm. (UNICEF, 2013)

- ≥ 14 cm: normal child,
- 12,5-14 cm: moderate Malnutrition,
- < 12,5 cm: severe Malnutrition.

2.4.5.1. Nutritional Status Assessment

Nutritional status was assessed from the anthropometric parameters; the Weight and Height of the boys and girls were compared to those of same aged boys and girls measured in the National Centre for Health Statistics (NCHS)/WHO growth reference curves (WHO, 2006) using the nutrition module of the Epi Info 2000 programme.

The Z-scores < -2.0 was used to classify stunted, underweight and wasted children, based on their HAZ (Height - for- Age), WAZ (Weight - for- Age) and WHZ (Weight -for –Height) values, respectively.

The WHO Classification for assessing severity of malnutrition by percentage prevalence ranges of these 3 indicators among children were followed. Children with <−2 and <−3 SD were classified as malnourished and severely malnourished, respectively.

2.4.6. Collection of blood specimens

Blood samples for each child, were collected through finger- prick, by piercing the third or ring finger.

2.4.6.1. Collection of blood samples on slides

One to two drops of blood were immediately dispensed on properly labelled slides bearing the participant's identification code, to prepare blood films (thick and thin) on the same slide.

2.4.6.2. Collection of blood samples in Micro-capillary haematocrit tubes

Haematocrit micro-capillary tubes were filled up to the mark with blood (2 per child) for PCV determination. Micro-capillary tubes were sealed with crysta- seal and stored on ice.

2.4.6.3. Collection of blood samples on filter papers

Blood spots were done on filter papers (Whatman 3MM). The filter papers were air dried and packed in drug sachets and kept at room temperature.

2.4.7. Malaria diagnosis by Rapid Diagnostic Tests (RDTs)

Two to three drops (approx. 5µL) of blood were transferred using an inverted cup into the round sample well of the test devise (SD- Bioline, Standard Diagnostics, INC. Lot No.05FK60). After which, four drops of buffer (assay diluent) were put in the square buffer well. Then, the sample was allowed to develop for 15 minutes, based on manufacturer's instruction.

2.4.8. Preparation of blood films for malaria parasite diagnosis by Microscopy

One to two drops of blood were immediately dispensed on properly labelled slides bearing the participant's identification code, to prepare blood films (thick and thin) on the same slide. Slides were air- dried and the thin films fixed with methanol. They were then stained with 10% Giemsa for 20 minutes, washed, air- dried and packed in slide boxes.

2.5. Transportation and Storage of Samples

Slides were stained and air-dried, then stored in slide boxes. Those collected in micro-haematocrit capillary tubes were stored on ice in cool boxes. Upon return from the field, they were stored at -20°C. The samples collected on filter papers were air-dried at room temperature and stored in drug sachets. All labelled samples were then transported from the field in the North, to the Laboratory for Molecular Parasitology and Disease Vector Research (LMPDVR) at the Biotechnology Centre, Nkolbisson, Yaounde, (blood samples were

transported on ice in ice boxes) where they were processed and stored at -30° C for further analyses.

2.6. Laboratory Investigations

2.6.1. Malaria Diagnosis

2.6.1.1. Malaria Diagnosis using Rapid Diagnostic Tests (RDT's) - SD Bioline 05FK60

This was done using the Method recommended by WHO, (2012).

- **Principle**

This qualitative membrane based immune-chromatographic assay for the detection of *P. falciparum* antigen in whole blood is based on the principle that, the membrane is pre-coated with *P. falciparum* antibody. During testing, the whole blood specimen reacts with the dye conjugate in the test strip. The mixture then migrates upward on the membrane chromatographically by capillary action and reacts with *P. falciparum* antibody on the membrane of the test line. (Fig 12)

If the specimen contains *P.f.* antigens, a coloured line will appear in the test region.

As a procedural control, a coloured line will always appear in the control region.

- **Procedure**

Gloves were put on and the test pouch opened. The test device was set and patient's name or code written on test. Alcohol swab was opened and finger cleaned. The protective cap was removed and lancet used to prick the finger (lancet was immediately discarded after pricking finger into sharps box). Approx. 5µL of blood was collected using an inverted cup and the blood put into the round sample well of test device (the inverted cup was immediately discarded into the sharps box).

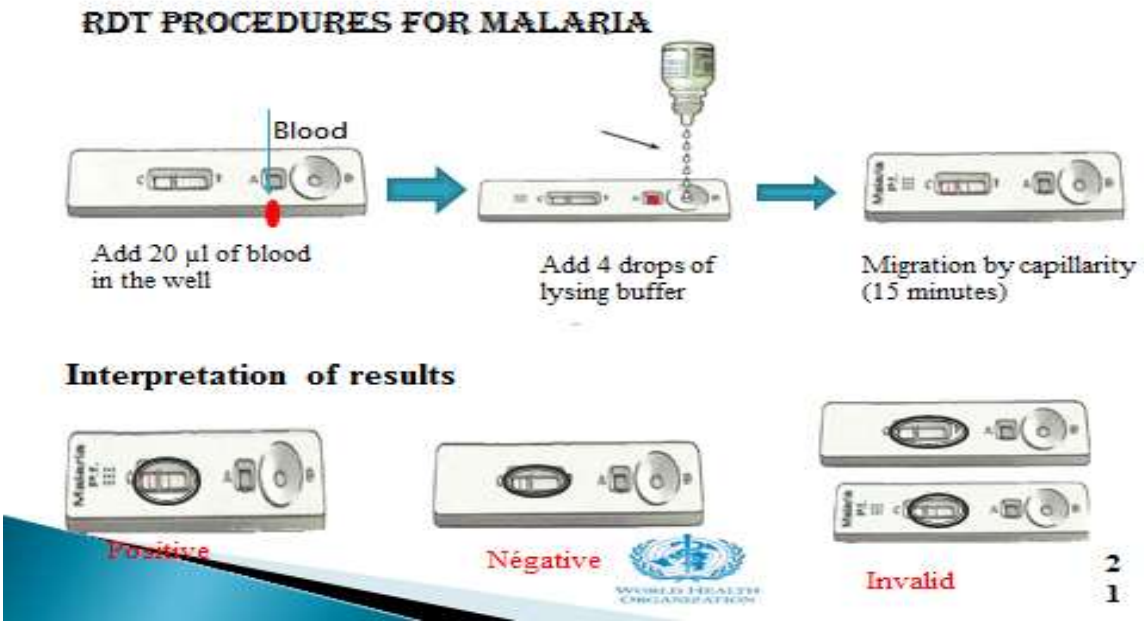


Figure 12: RDT Procedure for Malaria. (WHO, 2012)

Then, the results were interpreted as follows;

Reading and Interpretation of Results

NEGATIVE - One line "C" on result window

POSITIVE *P. falciparum* - Two colour bands ("P. f." test line / "C" control line)

POSITIVE Pan - Two colour bands ("Pan" test line / "C" control line)

MIXED INFECTION *P. f.* and Pan - Three colour bands "P. f." / "Pan" test line / "C" control line.

INVALID RESULT - No "C" line in result window

2.6.1.2. Malaria Diagnosis using Microscopy

Parasitaemia assessment

A blood sample for parasite density (thick and thin films) was collected from a finger prick. Thin and thick films combination slides were prepared immediately on the same slide on the field according to CDC, (2007) method. Upon arrival at the laboratory, the thick and thin blood films were examined following standard protocols (Cheesbrough, 1998). The films were then screened before a slide was considered negative. A thick blood smear was declared negative after observing more than 100 fields at a X100 high power magnification and no malaria parasite seen. Parasite identification was done using the standard morphological characteristics. Positive slides were quantified by counting the number of parasites against 200 white blood cells, and the parasites/μL of blood was calculated by assuming a leucocyte count of 8000 per microliter as reported by Nankabirwa *et al.* (2011).

The thin smears were examined to identify the *Plasmodium species* with the aid of identification charts of Cheesbrough (Cheesbrough, 2005).

As a quality control measure, each slide was read or examined by two readers. Where there was a discrepancy, a third person was assigned to examine the slide or the two microscopists re-read the slide.

However, species identification was further confirmed by PCR, using *Plasmodium* DNA isolated by the Chelex Method from a portion of each blood sample collected on filter paper (Plowe *et al.*, 1995).

2.6.2. Diagnosis of Anaemia

2.6.2.1. Determination of Packed Cell Volume (PCV)

- **Method** Micro-centrifugation
- **Procedure**

Finger prick blood samples were collected in micro-haematocrit tubes by pricking the finger. The tubes were sealed with crysto – seal and placed in the micro-centrifuge and the security

lead sealed tightly. Heparinized micro-capillary sample tubes were centrifuged using a Micro-centrifuge at 10.000 x g for 5mins. The samples were then removed. The centrifuged Micro-Haematocrit tube was placed vertically on the chart, with the bottom edge of the CRITOCAP just touching the red line below the 0 percent line. The bottom of the column of blood was at the 0 percent line. The tube was then slid along the chart until the meniscus of the plasma intersected with the 100 percent line. The height of the packed red cell column was then read directly as percent cell volume.

Expression of Results

-A Micro- haematocrit capillary tube reader (CRITOCAPS) was then used to directly read off the PCV value in %. PCV values (%) were converted to Haemoglobin levels (g/dL) by dividing by 3 (WHO, 1968).

2.6.3. Blood sample processing and storage

2.6.3.1. Separation of the plasma and the red cells

After centrifuging, and PCV values read, the capillary tubes were cut open by using a fountain pen. A micropipette was then used to aliquot the plasma and the pellets into separate eppendorf tubes which were stored in the freezer at -30° C until needed for ELISA assays.

2.6.4. Identification of *Plasmodium species by* Polymerase Chain Reaction - PCR

The molecular identification by PCR was done according to the nested PCR method described by Snounou *et al.* (1993). It involved amplification of a target DNA fragment in vitro. Two sets of primers instead of one were used to amplify the target fragment in two successive PCRs, Nest 1 and Nest 2. The primers rPLU5: (5'-CCT GTT GTT GCC TTA AAC TTC-3') and rPLU6:(5'-TTA AAA TTG TTG CAT TTA AAA ACG -3') were the *Plasmodium* Genus specific primers (ssrRNA primers) used to amplify the target fragment in Nest 1 while rFAL1 and rFAL2, rMAL1 and rMAL 2, rOVA1 and rOVA2, rVIV1 and rVIV2, were specific to the

species *falciparum, malariae, ovale* and *vivax* respectively for Nest 2. They served as forward and reverse primers, respectively.

The Molecular identification by PCR took place in 3 stages as follows; *Plasmodium* DNA Extraction, Amplification of the target DNA fragment and Revelation of the amplified products by agarose gel electrophoresis.

2.6.4.1. *Plasmodium* DNA Extraction

The extraction of *Plasmodium* DNA was done using the Chelex 20% Method described by Plowe *et al.* (1995) with minor modifications.

- **Principle**

Chelex is a chelating agent and saponin complexes with cholesterol in the RBC membrane leading to haemolysis, such that chelex, by means of Mg^{2+} ions, binds to the DNA on the sample, thereby preventing DNA degradation from degradative enzymes (DNAses), and through a displacement reaction, causes the DNA to be released into solution.

- **Procedure**

Portions of dried blood spots on filter paper (Whatman 3 mm) were excised and incubated overnight at 4°C in 1mL of 0.5% saponin in phosphate buffered saline- 1X PBS (sterile). After which the brown solution was removed and discarded. Next, it was replaced with 1 mL of PBS and further incubated at 4°C for 30 minutes. Then, the filter papers were placed in hot chelex, 50uL of 20% Chelex solution (mixed well before use) was added to 150 µl of DNase – free water in a 1.5ml microfuge tube containing the filter paper and heated to 100 °C in a water bath for 15 minutes to elute the DNA. Vigorously, the tube was vortexed and centrifuged at 10,000 x g for two minutes and then replaced in the water bath at 100°C for a further 10minutes, vortexing again once during the incubation and once afterwards (the forceps was cleaned after each sample by dipping in acid, base and rinsing with distilled water

as before). The tubes were then spun at 10.000 X g in a centrifuge for 2 minutes and 200µl of the supernatant removed into a fresh tube (labelled ‘’), and further spun for 2 minutes at 10.000 X g, and 150µl of the supernatant transferred again to another fresh tube (labelled ‘”), care was taken so no chelex matrix is transferred during the final transfer. The DNA solution was stored at 4°C (short term) or at -70°C (long term).

- **Expression of Results**

The total number of samples extracted = 185 samples.

2.6.4.2. Amplification of the DNA target fragment (18s rRNA gene)

This was done using the method described by Snounou *et al.* (1993) with minor modifications.

- **Principle**

This method is based on the in-vitro amplification of regions of the DNA by successive reactions of denaturation, annealing and elongation of DNA strands with respect to temperature due to a thermostable DNA polymerase which possesses enzymatic synthetic properties. With the aid of specific primers, large quantities of DNA can be obtained from very little samples.

- **Procedure**

The reaction mixture (master mix, **Table 3**) was prepared in a 1.5mL Eppendorf tube. After homogenization, 17 µL of this solution was dispensed into each PCR tube, after which 3 µL of *Plasmodium* DNA was added. Next, the tubes were capped, homogenized and placed in a thermocycler (Gene Amp. PCR systems 9700) for DNA amplification (it was ensured that, the final reaction volume was adjusted when programing)

Table 3: Reaction Mixture for NEST 1, for Identification of *Plasmodium* genus by PCR

REAGENT	VOLUME (µl) X1tube	FINAL CONCENTRATION
PCR H_20	8.3	
PCR Buffer (10X)	2.5	1.25X
$MgCl_2$ (25mM)	2	2.5 mM
dNTPs mix (2.5mM)	2	0.25 mM
Forward Primer 1 (10µM)	1	0.5 µM
Reverse Primer 2 (10µM)	1	0.5 µM
Taq DNA Polymerase (5U/µl)	0.2	0.05U/µl
Total Volume / reaction	**17**	
DNA Matrix	**3**	
Total volume per tube	**20**	

*Use **3µl** of DNA template.
* PCR buffer 1X: 10mM Tris – HCL, 50mM KCL, 2mM $MgCl_2$ (pH 8.3) 0.1mg/ml.
* dNTPs mix: mixture of deoxyribonucleotides: dATP, dTTP, dCTP, dGTP.
(Appendix 1.4).

Stages of PCR

Denaturation: In this study, it was achieved at 95°C.The double helical strands of DNA were denatured giving rise to 2 single strands.

Annealing: was characterized by the appearance of primers with bases complementary to the DNA.

Elongation: was characterized by the synthesis of complementary strands by addition of dNTPs. **(Fig. 13)**

NEST 1 AMPLIFICATION CONDITIONS

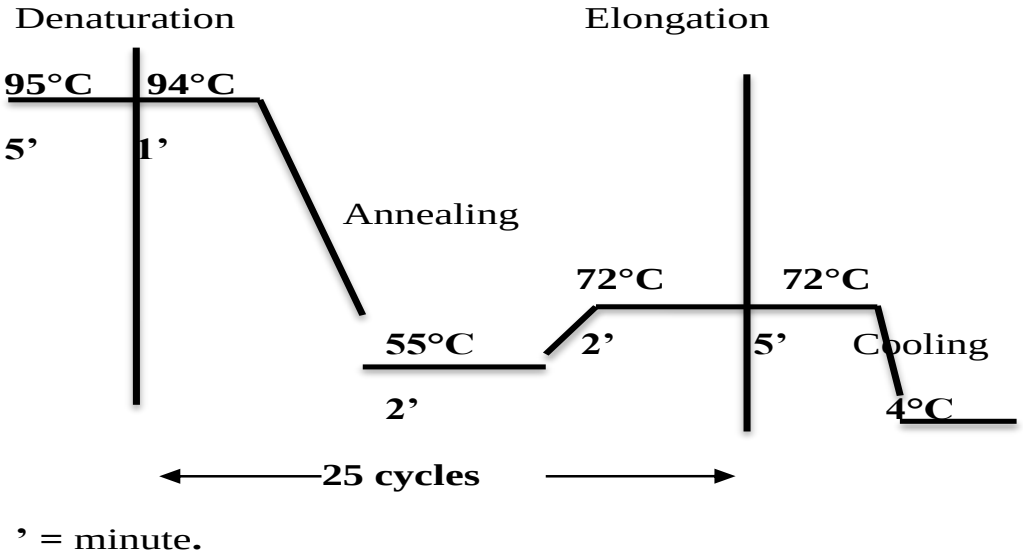


Figure 13: PCR Cycle conditions for Nest 1 Reaction.

95°C/5min ……...Denaturation step

(94°C/1min, 55°C/2min, 72°C/2min) X 25cycles…….. ….. Annealation step

72°C/5min…….....Elongation step

4°C hold…….....Cooling step

In the first PCR (Nest 1) amplification, *Plasmodium*- specific primers were used.

Nest 1 amplification products then served as precursors (DNA template) for four separate second PCR (Nest 2) amplifications with primers specific for each of the 4 human malaria species (*P. falciparum, P. malariae, P. ovale and P. vivax)* labeled as F, M, O, and V. (Table 4)

It was vortexed vigorously. After which, **18µl** of the reaction mixture was dispensed into PCR tubes and **2µl** of Nest 1 product added. (Care was taken to ensure that, the positive and negative controls labeled as PCF, PCM, PCO, PCV and NCF, NCM, NCO, NCV were included). Next, the tubes were capped, homogenized and placed in the thermocycler for DNA amplification (it was ensured that programming was done as Nest 2).

Table 4: Reaction Mixture for NEST 2, for Identification of *Plasmodium* species by PCR

REAGENT	VOLUME (µl) X1tube	FINAL CONCENTRATION
PCR H_20	9.3	
PCR Buffer (10X)	2.5	1.25X
$MgCl_2$ (25mM)	2	2.5 mM
dNTPs mix (2.5mM)	2	0.25 mM
Forward Primer 1 (10µM)	1	0.5 µM
Reverse Primer 2 (10µM)	1	0.5µM
Taq DNA Polymerase (5U/µl)	0.2	0.05U/µl
Total Volume / reaction	**18**	
DNA Matrix	**2**	
Total volume per tube	**20**	

*Use **2µl** of Nest 1 product.

* Primers: rFAL1 and rFAL2 : Forward and Reverse respectively, specific for *P. falciparum*
: rMAL1 and rMAL2 : Forward and Reverse respectively, specific for *P. malariae*
: rOVA1 and rOVA2 : Forward and Reverse respectively, specific for *P. ovale*
: rVIV1 and rVIV2 : Forward and Reverse respectively, specific for *P. vivax*
(Appendix 1.4)

NEST 2 AMPLIFICATION CONDITIONS

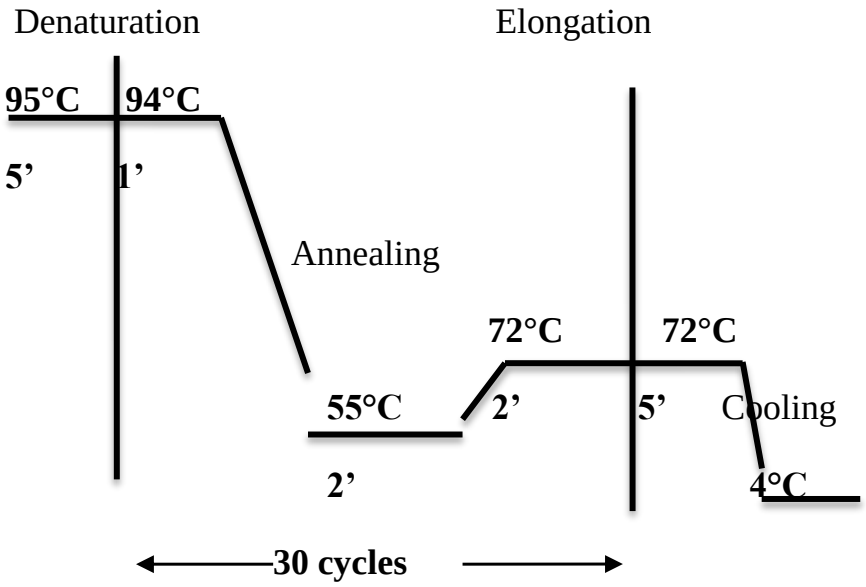


Figure 14: PCR Cycle conditions for Nest 2 Reaction.

Expression of Results

Expected size of amplified products (along a 100 base pair DNA ladder)

P. falciparum 205 bp

P. malariae 144 bp

P. ovale 800 bp

P. vivax 120 bp

2.6.4.3. Revelation by Agarose Gel Electrophoresis

- **Principle**

This technique is based on the separation of charged molecules according to their molecular weight across a gel upon introduction of an electric field into the medium. The molecules (DNA) migrate towards oppositely charged electrodes at a speed which is inversely

proportional to their molecular weight. Particles migrate based on their charge, molecular weight and porosity of the gel. (Snounou *et al.,* 1993)

- **Procedure**

A 2% agarose gel was prepared by weighing 6g of Agarose ultra-pure (Gibco. BLG, Molecular Grade), dissolved in 300ml TBE 1x buffer in a 500ml erlenmeyer conical flask. The cloudy white solution was brought to the boil in a microwave until complete dissolution. After which, it was allowed to cool sufficiently (40°C>) but not solidifying. Next, 9 µL of Ethidium Bromide (Sigma Chemical co., 10mg/ml) was added and the mixture was poured into a gel mould (Biorad ^{R}, sub-cell Model 96). Then, combs were fitted in and the gel allowed to solidify for approximately 30 minutes. After solidification, the combs were carefully removed and the gel transferred from the mould into the electrophoretic tank containing TE buffer, ensuring that the gel was submerged in the buffer. TE buffer served as the migration buffer. 10 µL of amplified product was mixed with 2 µL of loading buffer and dispensed into the wells at the same time as the positive and negative controls, as well as the Molecular weight marker. The electrodes of the electrophoretic tank were connected to a generator for current (Biorad^{R}, Power Pac, Basic) and migration was set at 100V for 1 hour. The visualization of the migrated products was seen by a UV transilluminator (Biorad^{R} Gel Doc XR Systems) with the help of fluorescence emitted by Ethidium bromide which binds to the DNA of the sample. The expected sizes of the amplified products were; *P. falciparum* – 205bp, *P. malariae* -144bp, *P ovale* – 800bp, *P. vivax* – 120bp (Snounou *et al.,* 1993).

Expression of Results

Samples with Nest 2 PCR amplicons showing expected band sizes of 205, 144, 800 and 120 base pairs on the agarose gel indicated positive results for *Plasmodium falciparum, malariae, ovale* and *vivax,* respectively.

2.6.5. Determination of anti-malarial IgG1 and IgG3 antibody responses against crude *Plasmodium falciparum* 3D7 antigen by Enzyme-Linked Immunosorbent Assay (ELISA).

This was done using the Indirect ELISA method described by **Courtin *et al.*, (2009)** with little modifications.

- **Principle**

The indirect ELISA method is based on the principle that a secondary antibody which is complementary to a primary antibody, is conjugated to the detection enzyme which upon addition of a substrate, leads to the formation of a coloured product whose intensity is directly proportional to the level of specific antibody being measured.

- **Procedure**

Ninety-six (96) well Polystyrene ELISA microplates (COSTAR, Seracluster "U" vinyl plate) were coated overnight with 100µL per well of crude *P. falciparum* 3D7 antigen diluted with coating buffer to a concentration of 5µg/ml, containing 0.1M Sodium Carbonate buffer (pH 9.5; SIGMA-Aldrich) at 4°C. The following morning, the coating buffer was removed, the plate washed 3X by filling the wells with 200µL washing buffer (PBS-0.5%-Tween 20), using a multi-channel pipette. After which the remaining protein-binding sites were blocked by adding 200µL/well of Blocking buffer (10% milk in 0.5% PBS-T) and incubated for 2hours at room temperature. Next, the Blocking buffer was removed and the plate washed again 3X with 300µL washing buffer (PBS-T). Dilutions (1:500) of primary antibody (serum samples) and controls (positive and negative) were prepared in dilution buffer (1% Milk in PBS 0.02%-Tween 20). Diluted Sera were then added to antigen-coated wells (100µL/well) and incubated for 1 hour at Room temperature. After five washings with washing buffer (PBS 0.5%-Tween 20), 100µL of the enzyme- conjugated secondary antibody, Alkaline phosphatase linked Goat anti-Human Whole IgG, diluted (1:4000), IgG1 (1:6000) and IgG3 (1:6500) were added to the

wells and incubated for 1 hour at room temperature. The plates were washed 5X with 300µL washing buffer (PBS-T) and wells were added with 10mg para-nitro phenyl phosphate in substrate buffer (SIGMA- Aldrich) and incubated for a further 1 hour in the dark at room temperature. The reaction was stopped after 30 minutes by adding 25µL stopping buffer (2N H_2SO_4). After sufficient colour development, the absorbance/ Optical Density (OD) was read at 30 and 60**minutes at 405/ 630nm (405 as the primary wavelength and 630 as the reference) using a Micro plate reader (iMARK^{TM}- BIORAD). Negative Controls (NC) were used for each assay. The threshold for positivity was set at the mean of the optical densities of the Negative Controls plus twice their Standard Deviation (ODNC + (2SD). The superior values to this cut-off were considered positive while those inferiors were negative.

2.7. Definitions and end points

- Fever was defined as axillary temperature ≥ 37.5˚C.

- Confirmed malaria was defined as the presence of any species of *Plasmodium*, with an axillary temperature of ≥ 37.5˚C or reported fever in previous 48 hours.

- Malaria Parasitaemia was defined as the presence of *Plasmodium* by microscopy and with an axillary temperature of ≥37.5°C and no record of fever within the past two weeks.

- Malnutrition was defined as Z-score <-2SD from the WHO reference median and severe malnutrition as a Z-score <-3SD.

- Nutritional status was categorized as stunting, underweight or wasting if the HAZ, WAZ and WHZ was < -2 SD of the NCHS/WHO reference median, respectively. They were classified as having severe stunting, wasting or underweight, if the HAZ, WHZ, or WAZ was <-3, respectively.

- Anaemia was defined as Hb<11.0g/dL (<5year age group), and Hb<11.5g/dL (>5year age group) and further categorized as severe (Hb<7.0g/dL), moderate (Hb between 7.0 and 10.0g/dL), and mild (Hb between 10.1 and < 11g/dL or 11.5g/dL).

- The threshold for positivity was set at the mean of the Optical Densities (OD) of the Negative Controls plus twice their Standard Deviation (ODNC +(2SD). The values ≥ cut -off were considered positive, while all those below considered negative.

2.8. Statistical Analyses

All data collected were entered in Microsoft Excel 2010, cleaned- up and statistically analyzed using the IBM-Statistical Package for Social Sciences (IBM-SPSS) Standard version, Release 20.0 (SPSS Inc. 2012) and Epi- Info version 6.0. Continuous variables were summarized into means and standard deviations (SD) and categorical variables which were reported as frequencies and percentages, were used to evaluate the descriptive statistics. The differences in proportions were compared using Cramer's V test. Differences between group means were compared using non- parametric tests, the Mann–Whitney U test and Kruskal Wallis test. Parasite density was log transformed before analysis. Associations between predictor variables and primary outcomes were assesessed using bivariate logistic regression analysis and degree of associations between dependent and independent variables evaluated using Spearman's correlation test. Odd ratios (ORs) and 95% confidence intervals (CI's) were computed. Significant levels were measured at 95 % confidence interval (CI) with level of significance set at $P<0.05$.

2.9. Ethical Considerations

This study was carried out within the framework of a larger project by the National Malaria Control Programme whose ethical clearance was obtained from the National Ethics Committee of Cameroon - N°019/CNE/SE/12; FWA IRB00001954) (Appendix 2). The objective of the larger project was to assess the Impact of Insecticide Resistance on the effectiveness of Long-Lasting Insecticide Nets (LLINs) and malaria burden in the North Region of Cameroon. Informed consent (written for those who could read and verbal for those who were illiterate) was obtained from the heads of households (parents/guardians).

Participation was voluntary based. Participants were informed of their rights to withdraw from the study at any time. Participants names and other identifying characteristics were not documented and records were encoded to ensure anonymity and confidentiality during data collection and reporting.

CHAPTER THREE: RESULTS AND DISCUSSION

CHAPTER THREE:RESULTS AND DISCUSSION

3.1. Baseline Characteristics of the study participants

3.1.1. Children demographic data

3.1.1.1. Gender

Out of 362 children sampled, 187 (51.7%) of them were females which greater than the 175 (48.3%) which were males. (Table 5)

3.1.1.2. Age group

One hundred and eighty-two (50.3%) of the study participants belonged to <5 years age group while 180 (49.7%) of them belonged to the ≥ 5 age group. (Table 5)

In this study, malaria parasite, GMPD, confirmed malaria, malnutrition and anaemia all showed higher proportions in the <5 age group when compared to the >5 age group. Meaning that they were all inveresely related to age. Such increased susceptibility of the younger children to infections has been attributed to their poorly developed immune systems (Thorarinsdottir *et al.*, 2005). However, Deribew *et al.*, (2010) in South -Western Ethiopia, reported the contrary in the <5age group.

Table 5: Baseline Characteristics of the study participants (N=362)

	Parameter			All
Age group		≤5	>5	Total
Sex	Females n(%)	85(45.5%)	102(54.5%)	187(51.7%)
	Males n(%)	97(55.4%)	78(44.6%)	175(48.3%)
	Mean WAZ	-1.462±1.986	-1.503±1.258	-1.489±1.622
	Mean HAZ	-1.621±2.898	-1.346±2.315	-1.484±2.607
	Mean WHZ	-0.269±2.547	-0.520±2.610	-0.395±2.578
	Children by age group n(%)	182(50.3%)	180(49.7%)	362(100%)
	Malaria parasite prevalence n(%)	86(23.8%)	33(9.1%)	119(32.9%)
	GMPD (range)	(40-129600)	(80- 69200)	(40-129600)
	Confirmed malaria RDT n(%)	52(89.7%)	39(88.6%)	91(89.2%)
	Malnutrition n(%)	101(55.5%)	95(52.8%)	196(54.1%)
	Stunting n(%)	104(57.1%)	102(56.6%)	206(56.9%)
	Underweightn(%)	106(58.2%)	124(68.9%)	230(63.5%)
	Wasting n(%)	57(31.3%)	69(38.3%)	126(34.8%)
	Anaemia n(%)	40(22.2%)	34(19.0%)	74(20.6%)

Legend: Means are presented with Standard Deviations (SD)
Malnutrition was defined as Z-score <-2SD; Severe Malnutrition as Z-score <-3SD

GMPD – Geometric Mean Parasite Density
≤5 - Children less than or equal to 5 years old
>5 - Children greater than 5 years old
Mean HAZ – Mean Height –for- age Z-Score
Mean WAZ - Mean Weight –for- age Z-Score
Mean WHZ – Mean Weight –for- height Z-Score

3.1.1.3. Weight, Height and MUAC

The mean age was 4.719±2.5 years, mean weight was 14.914±5.5 Kg, mean height 98.53±22.1 cm while the mid upper arm circumference was in average 15.21±1.8 cm. (Table 6)

3.1.1.4. Study sites

Amongst 38 villages in the study area, 6 villages from 2 different health districts were sampled for the study. In the Health district of Mayo-Oulo, the villages were Bala 21.8% (79), Mayo-Oulo 18.5% (67) and Dourbeye 7.7% (28). In the Health district of Pitoa, the villages were Guizigaré 22.4% (81), Kirambo 18.5% (67) and Boussa 11.0% (40). (Table 6)

3.1.1.5. Distribution of people living in the households

In average 15 people lived in a household. The number of children aged 0-11months varied from 0 to 4 with the median at 0, implying that half or more of households had no child aged 0-11. As for the children aged 12-59months, in average they were 5 per household. Children aged 5 – 15years ranged from 0 to 14, median at 4, with an average of 5 persons per household. Women of procreation age (15-49 years) were in average 3 per household. Men aged more than 15 years were in average 2 per household while women aged more than 49 years were in average 1 per household, implying there were half or more households without a woman aged 49 years and above (Table 6).

Table 6: Characteristics of the study population by demographic variables

Demographic variables					
Parameter	**N**	**Mean±SD**	**Min-Max**	**Median**	**Range**
Age (years)	362	4.719±2.501	0.5-10	4.250	9.5
Weight (kg)	362	14.914±5.533	4.0-30.8	14.650	26.8
Height(cm)	362	98.53±22.080	27-198	100.00	171
MUAC	362	15.21±1.767	7-21	15.00	14
Distribution of People in households					
Age group		**Mean ±SD**	**Min-Max**	**Median**	**Range**
Aged]0-11]		.54±.798	0-4	.00	4
Aged 12-59 months		4.18±3.476	0-14	3.00	14
Aged 5-15 years		4.85±3.336	0-14	4.00	14
Women of procreation age (15-49years)		2.70±4.416	0-16	1.00	16
Men more than 15 years		1.75±2.848	0-12	1.00	12
Women more than 49years		0.90±1.538	0-6	.00	6
Villages sampled for the study					
Health district		**Village**	**Frequency**	**Percentage(%)**	
Mayo-oulo		Bala	79	21.8	
		Mayo-oulo	67	18.5	
		Dourbeye	28	7.7	
Pitoa		Guizigaré	81	22.4	
		Kirambo	67	18.5	
		Boussa	40	11.0	
Total			**362**	**100.00**	
Level of education of care giver					
		Frequency		**Percentage(%)**	
None		184		50.8	
Primary		171		47.2	
Secondary		07		1.9	

Legend : Min = Minimum Max = Maximum SD = Standard Deviation

] 0-11] meaning 0 exclusive

. Socio-economic characteristics of the study population

Socio-economic status has been defined as a position that an individual or family occupies with reference to prevailing standards of cultural and material possessions, income and education. Our findings revealed that majority (29.3%) of children in the study population belonged to the poor SES quintile, (Table 17) which is not surprising, and is in support of previous studies in the same area by Ngwa-Akonwi *et al.* (2015) considering the fact that the community being studied has a low standard of living.

Furthermore, as revealed by the logistic regression model on Table 16, although not statistically significant [OR= 1.08, (95% C.I: 0.94-1.24); p= 0.256], the non-employment status of the family heads in our study, may also have caused increased malnutrition rates. Out of the total number of them who responded to this question, only 6.7% of them were employed (Table 17). Indicating that, most of them were un-employed. Making it difficult for them to afford appropriate meals for the children. (Gelu *et al.,* 2018)

With regards to poor SES level and housing conditions, majority (29.3%) of the children in our study belonged to the poor SES level (Table 17). Although, poor sanitation and poor SES have been reported to directly impact on food security (Gelu *et al.,* 2018), however, our study did not find any significant association between development index and malnutrition [OR= 1.09, (95% C.I: 0.85-1.40); p=0.497]. (Table 16)

Table 17: Socio-economic and Demographic characteristics of children and their parents in the Pitoa and Mayo-oulo health districts, North Region of Cameroon 2014 (N=362)

Characteristics	Frequency(n)	Percentage (%)
- Level of education of care giver		
✓ None	**184**	**50.8**
✓ Primary	171	47.2
✓ Secondary	07	1.9
- SES level		
✓ Poorest	72	21.6
✓ Poor	**97**	**29.3**
✓ Average	87	26.3
✓ Rich	22	6.6
✓ Richest	54	16.2
-Care-giver principal activity		
✓ Skilled	04	1.1
✓ Business	06	1.7
✓ Personal land farming	19	5.2
✓ Mechanized land farming	5	14.0
✓ Rented land faming	3	0.8
✓ House wife	**280**	**77.3**
✓ Petit trader	45	12.4
-Level of education of family head		
✓ None	96	26.5
✓ Primary	**260**	**71.8**
✓ Secondary	6	1.7
-Caregiver employment status		
✓ No work since 12 months	**42**	**11.6**
✓ Working for 12 mths/5days/wk	10	2.8
✓ Seasonal Worker	14	3.9
✓ No response	296	81.8
-Type of toilet/waste disposal system		
✓ Modern	13	3.6
✓ Improved Pit Latrine	4	1.1
✓ Pit Latrine	104	28.7
✓ Open Pit	54	14.9
✓ Others (open areas/plastic bags)	**187**	**51.7**
- Source of drinking water		
✓ Pipe-borne water	31	8.6

✓ Bore-hole/wells	**187**	**51.7**
✓ Sachet water	5	1.4
✓ Rain water	139	38.4
- Housing condition		
✓ Separate rooms	**193**	**53.3**
✓ Appartments	25	6.9
✓ Several same huts	84	23.2
✓ Several different huts	26	7.2

The foremost aim of nutritional assessment studies is to determine the actual magnitude of under nutrition and thereby introduce appropriate nutritional intervention programmes to improve the existing nutrition situation (de Onis *et al.,* 1997; WHO, 1995; WHO, 2007).

According to UNICEF, the Global rates for stunting, underweight and wasting are 23%, 16% and 7% respectively (UNICEF, 2019). Our results from this study are higher than these rates. Therefore, the government should launch policy measures to reduce the burden of all types of malnutrition in Cameroon.

In order to meet up with the World health Assembly's Global Nutrition Targets 1 and 6 for 2025 on a 40% reduction in the number of children under-5 who are stunted as well as reduce and maintain childhood wasting to less than 5%, then effective strategies need to be sought (Unicef *et al.,* 2017).

Therefore, appropriate measures could be recommended in areas with high malnutrition such as improved nutritional practices like supplementation of children and infants (between the ages of after 6 months to about 36 months) with quality complementary foods. This way, the long-term negative consequences of stunting and wasting may be minimised, since they are known to begin in childhood, although poverty was the major cause of the problem in the North Region of Cameroon as well as other developing parts of the world. The way forward would be to expand the health benefits and improve control strategies from the Ministry of Public health for prompt and rapid scale-up of already proven nutrition-specific and nutrition-

sensitive interventions (Unicef *et al.,* 2015) for the vulnerable malnourished children in the country.

3.1.2. Nutritional Status Assessment of the study population

3.1.2.1. Indicators of Nutritional Status in the study population

If the HAZ, WAZ and WHZ were <-2SD of the WHO reference median, malnutrition was categorized as stunting, underweight and wasting respectively. However, if the Z-scores were <-3SD of the WHO reference median, it was categorized as severe stunting, severe underweight and severe wasting respectively (WHO, 2006).

The mean ± SD of HAZ, WAZ and WHZ scores were −1.621±2.898, −1.462±1.986, and -0.269±2.547 respectively in the <5 age group while for the ≥5 age group it was −1.346±2.315, −1.503±1.258, and -0.520±2.610, respectively (Table 7). A statistically significant (p=0.027) difference was observed between the means WHZ of the two age groups in wasted and normal children. Indicating that there were morewated children (lower WHZ) in the <5 age group when compared with the ≥ 5 age group.

Table 7: Means ± SD of Nutritional Indicators summarized by age-group

Indicators	Categories	Age group in years			Test Statistic
		<5 (n=182)	≥5 (n=180)	All	
Nutritional status (mean±SD)	HAZ	-1.621±2.898	-1.346±2.315	-1.484±2.607	U=15454.0 P=0.352
	WAZ	-1.462±1.986	-1.503±1.258	-1.489±1.622	U=16235.5 P=0.885
	WHZ	-0.269±2.547	-0.520±2.610	-0.395±2.578	U=14185.0 **P=0.027**

Legend: P= p-value (p= 0.027 - statistically significant difference between ≤5 and >5 wasted and normal children); Mann-Whitney U Test statistics
HAZ = Height-for-age Z score (Stunting);
WAZ = Weight –for–age Z score (Underweight);
WHZ = Weight-for-height Z score (Wasting)

The mean ± SD of WHZ scores was significantly different (p=0.007) between the <5 and ≥5 wasted girls, (-0.147±2.636 and -0.698±2.761) respectively, while it was (p=0.018) between the wasted boys and girls in the ≥5 age group, (-0.287±2.397 and -0.698±2.761) respectively (Table 8). Showing that, there were more wasted girls in the <5 age group (lower WHZ) when compared to the ≥5 age group, while there were more wasted boys in the ≥5 age group when compared to wasted girls.

A statistically significant (p=0.049) difference was equally observed between the mean ± SD HAZ scores (stunted boys and girls) in the <5 age group, (-1.861±2.634 and -1.348±3.166) respectively; while it was (p=0.044) between the stunted boys and girls in the ≥5 age group, (-1.495±2.559 and -1.231±2.115) respectively. Implying that, there were more stunted girls (lower HAZ) in both the <5 age group and the ≥5 age group of children, when compared to boys.

Table 8: Means ± SD of Stunting, Underweight and Wasting summarized by sex

Indicators			Nutritional status (mean±SD)					
Categories			HAZ		WAZ		WHZ	
Boys	<5	(n=97)	-1.861±2.634		-1.667±2.077		-0.375±2.475	
	≥5	(n=78)	-1.495±2.559		-1.482±1.309		-0.287±2.397	
	P-value		U=3457.000	P=0.328	U=3567.000	P=0.518	U=3699.500	P=0.802
Girls	<5	(n=85)	-1.348±3.166		-1.230±1.865		-0.147±2.636	
	≥5	(n=102)	-1.231±2.115		-1.520±1.224		-0.698±2.761	
	P-value		U=4240.000	P=0.797	U=3953.500	P=0.301	U=3339.000	**P=0.007**
Comparing between sex and within <5			U=3427.500	**P=0.049**	U=3647.000	P=0.180	U=4071.000	P=0.885
Comparing between sex and within 5+			U=3279.500	**P=0.044**	U=3832.000	P=0.673	U=3160.000	**P=0.018**

Legend: P= p-value (p= 0.007 – statistically significant difference between <5 and ≥ 5wasted girls)
(p= 0.049 – statistically significant difference between stunted boys and girls <5)
(p=0.044 – statistically significant difference between stunted boys and girls ≥5)
(p=0.018 – statistically significant difference between wasted boys and girls ≥5)
Mann-Whitney U Test statistics

HAZ = Height-for-age Z score (Stunting);
WAZ = Weight –for–age Z score (Underweight);
WHZ = Weight-for-height Z score (Wasting)

3.2. Prevalence of Malnutrition, Malaria and Anaemia

3.2.1. Prevalence of Malnutrition

Malnutrition is a general term that has been used to mean overnutrition, undernutrition, specific nutrient deficiencies, or imbalances (Chen *et al.*, 2001). It is an imbalance - a deficiency or an excess - in a person's intake of nutrients and other dietary elements needed for healthy living. It can manifest itself as hunger (or under nutrition), deficiency in vitamins or minerals, or overfeeding (WHO, 2014).

Malnutrition prevalence in this study overall was 54.1% (196/362), with a prevalence of 56.9% for stunting. This is comparable to an overall prevalence for malnutrition of 58.1% reported by Nkuo-Akenji *et al.* (2008) in the Muea village of the South West Region of Cameroon but higher than 24.4% observed by Sumbele *et al.* (2020) in Muyuka, South-Western Cameroon; higher than 16.4% reported by Nzefa *et al.* (2019) in Bandja village,

Western Cameroon, as well as 31.7% by Wilson *et al.* (2018) in rural Gambia. Our findings also align with UNICEF's State of the World's Children's report of 2019 which indicates that, one third of children under age 5 are malnourished –stunted, wasted or overweight- while two thirds are at risk of malnutrition and hidden hunger because of the poor quality of their diets (UNICEF, 2019). The high levels of malnutrition in our study may be explained by the low level of education of the care-givers as well as their non-employment status. It may also be linked to the poor socio-economic status of the study population.

In addition, the elevated levels of malnutrition observed in our study may further be linked to the high prevalence in Zn, Fe and Vit A deficiency, as earlier reported by Ngwa –Akonwi *et al.* (2015), in similar localities within the same geographical area in the North Region, suggesting a lack or insufficient dietary intake of foods rich in these micro nutrients. This suggests the inadequate intake of foods rich in Zn such as red meat, chicken, sea foods, kidney beans, pumpkin or melon seeds, nuts; foods rich in Vit A (red palm oil, carrots, mangoes, papaya) and Fe (lean meat, beetroot, potato, watermelon etc). Judging from the poor dietary habits (consumption of staple foods, mostly cereals grown in the area, which are carbohydrates in nature and which tend to be lacking in many essential nutrients, vitamins and minerals), it is evident that foods rich in Zn, Vit A and Fe are rarely consumed, which are all necessary for proper nourishment of children. Most of these nutrients are known to play vital roles in the functioning of the body; Zn is known to act as a co-factor for the synthesis of a number of enzymes, DNA, and RNA, proteins, cellular growth and cellular differentiation (Brown *et al.,* 2002). Its absence may manifest physically as very scanty and brown/rust coloured hair as was observed in the malnourished children in our study population, severe manifestations include growth retardation, impaired immune function, skin disorders, hypogonadism, anorexia and cognitive dysfunction (Caulfield *et al.,* 2006). Zinc deficiency has been associated with poor growth in childhood, reduced immune-competence, and

increased infectious disease related morbidity (Hess *et al.*, 2009). Vit A is necessary for healthy vision, immune function and reproductive health while Fe is responsible for binding and transporting oxygen throughout the body, as well as for the regulation of cell growth and differentiation (Beard, 2001). A study in Cameroon (Engle-Stone *et al.,* 2012) also reported that Iron and Zinc intakes were below the expected levels, especially in the north and among households with lower socio-economic status.

Stunting, underweight and wasting were 56.9%, 63.5% and 34.8%, respectively, showing that they were the most common forms of malnutrition prevalent in the study area. This underscores the fact that chronic malnutrition and underweight are still a heavy burden in the area, and confirms earlier findings (Ngwa-Akonwi *et al.,* 2015). Wasting is not as common as stunting and underweight in any region of the world (Sumbele *et al.,* 2015). The same trend for stunting, underweight and wasting pointed out by Sumbele *et al.* was observed in our study. However, our results revealed the prevalence of stunting to be higher than 42.3% previously observed by Gelu *et al.* (2018) in Gondar city, North Western Ethiopia as well as the 31.7% stated by Wilson *et al.* (2018) in rural Gambia.

Stunting is generally associated with long term under nutrition whereas wasting is a manifestation of recent and acute under nutrition (Caulfield *et al.,* 2004). The prevalence of malnutrition shows about half of the children in our study to be malnourished. This is in line with the WHO report which states that fully half of the human family, some 3 billion people, suffer from malnutrition of one kind or another (WHO, 2011).

3.2.1.1. Prevalence of malnutrition by Health District

Although not significantly different, the prevalence of malnutrition was higher in the Pitoa Health District (55.6%) than Mayo-Oulo (44.4%), with the highest prevalence rates recorded in Dourbeye (60.7%) and lowest in Mayo-oulo (40.3%) villages. The high prevalence of undernutrition generally in our study may on one hand be linked to the types of foods grown

in the study area as well as the unavailability or lack of certain foods and nutrients consumed in the diet especially during the study period. Interviews carried out during these studies with the parents and observations made by the research team revealed that the staple foods grown and consumed in Pitoa are cereals; such as rice, maize, millet, cotton and sorghum (mostly carbohydrates) whereas beans and peanuts are cultivated in Mayo-Oulo. Implying that nutrient- rich foods, fruits and vegetables are rare in Pitoa than Mayo-Oulo and may explain the higher malnutrition rates observed in Pitoa. The higher prevalence of malnutrition in the Pitoa health district compared to Mayo-Oulo, may also have been due to the fact that Pitoa was more highly populated than Mayo-Oulo. Large family sizes and overcrowding greatly influences malnutrition (Shah *et al.,* 2003; Asim *et al.,* 2018).

Table 9: Prevalence of malnutrition in Pitoa and Mayo-Oulo Health districts

Health district	Villages	Frequency(n)	Prevalence (%)	N
Mayo-Oulo	Bala	43	54.4	79
	Mayo-Oulo	27	40.3	67
	Dourbeye	17	60.7	28
Total Mayo-oulo		87	**44.4%**	**174**
Pitoa	Guizigaré	48	59.3	81
	Kirambo	39	58.2	67
	Boussa	22	55.0	40
Total Pitoa		109	**55.6%**	**188**
Total		**196**	**54.1**	**362**
Cramer V test comparing between health districts		**V=0.080**	**P=0.128**	

3.2.1.2. Proportion of malnourished children in the study population by age group

One hundred and ninety-six (196) children were malnourished making a proportion of 54.1% (95% CI: 48.9-59.2) among which 101 (55.5%) were aged <5 years, not significantly different (P>0.05) from the proportion of those aged 5 years and above 95 (52.8%) (Table 10). The

overall prevalence of stunting, underweight and wasting were 56.9%, 63.5% and 34.8% respectively, showing that most of the children were stunted and underweight (Table 10).

However, we observed a higher prevalence in malnutrition among the <5years (55.5%) compared to their counterparts. This was not consistent with earlier work by Deribew *et al.*, (2010) in South Western Ethiopia, whose findings showed that older children were more likely to have under-nutrition as compared to the younger ones. It may have been due to neglect of proper attention from the mothers to the older kids as a result of short birth intervals which predisposes them to malnutrition, so more attention is given to the younger ones. On the contrary, our results are higher in the younger ones presumably due to the fact that the younger ones may have been more vulnerable to diseases due to their weak immune systems that are unable to fight off infection (Shankar, 2000). Poor feeding habits may also have contributed to the higher prevalence in the <5 age group.

The overall prevalence of stunting was significantly higher in the <5 age group compared to the ≥5 age group (p= 0.012), with the same trend observed for underweight (p< 0.001). This result was much higher than the 1% observed by Maketa *et al.*, (2015) in the Democratic Republic of Congo. However, for wasting, although the difference was not significant (p=0.649), the ≥5 age group were more underweight than the <5 age group.

Table 10: Prevalence of malnutrition in the study population by age group

Age group	Frequency(n)	Prevalence (%)	N
<5	101	55.5	180
≥5	95	52.8	182
Total	**196**	**54.1**	**362**

Comparing malnutrition between ≤5 and >5 age groups; Cramer's V= 0.089 P= 0.078

3.2.1.3. Proportion of malnourished children in the study population by sex

With regards to sex, amongst the children examined, males 106(60.6%) were significantly more malnourished than females 90(48.1%). Prevalence of stunting showed a similar trend to malnutrition, males (66.9%); females (47.6%). Stunting and underweight showed the same trend in both sexes, though not statistically significant (p=0.588). For wasting, it was the reverse, though not statistically significant too (p= 0.613), more wasting was observed in females (37.9%) than in males (31.4%). Stunting was significantly higher in males than females (p=0.018). Although the difference was not significant (p>0.05), underweight was also more in males (65.7%) than in females (61.5%) while wasting, was the reverse, more wasting was observed in females (37.9%) than in males (31.4%). The observed result was consistent with the findings of Nzefa *et al.* (2019), in Bandja village of Western Cameroon as well as Sumbele *et al.* (2015) who reported the same trend in the Mount Cameroon area. It is also supportive of earlier studies by Wamani *et al.* (2007) who reported that boys are more stunted than girls. This could be due to biological predisposition of male children than females and also confirms previous studies in Tanzania (Kamugisha *et al.*, 2006) which reported that stunting and underweight were common among males than females in all age groups. Nevertheless, some studies in Pakistan stated that there was no significant difference in stunting and wasting in male and female children (Khattak and Shah, 2010). Others reported that female children were three times more likely to be stunted than male children (Ansari *et al.*, 2006).

Table 11: Proportion of malnourished children in the study population by sex

Parameter				**All**
Malnutrition	**Sex**	**Frequency (n)**	**Prevalence (%)**	**N**
	Males	106	60.6	175
	Females	90	48.1	187
Total		**196**	**54.1**	**362**
Stunting	Males		66.9	
	Females		47.6	
Total			**56.9**	
Underweight	Males		65.7	
	Females		61.5	
Total			**63.5**	
Wasting	Males		31.4	
	Females		37.9	
Total			**34.8**	

Comparing malnutrition between sexes; Cramer's V= 0.125 P= 0.018
Comparing stunting between sexes; Cramer's V= 0.789 P>0.05
Comparing underweight between sexes; Cramer's V= 0.652 P=0.588
Comparing wasting between sexes; Cramer's V= 0.741 P=0.613

3.2.1.4. Proportion of mild, moderate and severely malnourished children by age group categorized

The prevalence of severe stunting, severe underweight and severe wasting were 29.1%, 20.9% and 9.3% in the <5 age group and 18.3%,10.0% and 7.8% in the ≥5 age group respectively (Table 12). The same trend was observed for severe underweight ($p<0.001$), however, the reverse was seen for severe wasting. The difference in our study may be explained by the vulnerability of the younger children to infection (Thorarinsdottir *et al.*, 2005), though contrary observations were made by Deribew *et al.* (2010) in the <5 age group.

Table 12: Proportion of mild, moderate and severely malnourished children in the study population by age group

	Category	≤5 %(n)	>5 %(n)	N	P- value
HAZ % (n)	Normal	33.5%(61)	38.9%(70)	131	V=0.188 **P=0.012**
	Mild stunting	13.7%(25)	23.3%(42)	67	
	Moderate stunting	14.3%(26)	15.0%(27)	53	
	Severe stunting	**29.1%(53)**	**18.3%(33)**	86	
	Overall stunting	**57.1%**	**56.7%**		
WAZ % (n)	Normal	38.5%(70)	31.1%(56)	126	V=0.300 **P<0.001**
	Mild underweight	17.6%(32)	38.3%(69)	101	
	Moderate underweight	19.8%(36)	22.8%(41)	77	
	Severe underweight	**20.9%(38)**	**7.8%(14)**	52	
	Overall underweight	**58.2%**	**68.9%**		
WHZ % (n)	Normal	61.5%(112)	56.1%(101)	213	V=0.083 P=0.649
	Mild wasting	13.7%(25)	18.9%(34)	59	
	Moderate wasting	8.2%(15)	9.4%(17)	32	
	Severe wasting	**9.3%(17)**	**10.0%(18)**	35	
	Overall wasting	**31.4%**	**38.3%**		
Nutritional status % (n)	Malnourished	55.5%(101)	52.8%(95)	196	V=0.027 P=0.604
	Normal	44.5%(81)	47.2%(85)	166	

Legend : HAZ = Stunting; WAZ = Underweight; WHZ = Wasting P= p-value

Cramer's V test: Comparing between age groups for stunted children ; p=0.012
Comparing between age groups for underweight children; p<0.001
P in bold- statistically significant difference (p<0.05)

3.2.1.5. Proportion of mild, moderate and severely malnourished children by sex categorized

In females, severe stunting and severe underweight were significantly higher (p=0.028 and p=0.003) respectively) in the <5 age group compared to the ≥5 while severe wasting was higher in the ≥5 age group than the <5, but not statistically significant (p=0.185) with males being generally more severely malnourished than females (p<0.05) as well (Table 13). While in males, only underweight showed a significant difference (p=0.002) between the <5 and ≥5 age groups. However, in Pakistan, it was the contrary, severe malnutrition was observed to be higher in females than males (Laghari *et al.*, 2015).

Table 13: Proportion of mild, moderate and severely malnourished children in the study population by age within sex

	Category	Boys			P-value	Girls			P-value	P-value (compare boys and girls)
		≤5 N=97	>5 N=78	Overall boys N=175		≤5 N=85	>5 N=102	Overall girls N=187		
HAZ % (n)	Normal	25.8%(25)	24.4%(19)	25.1(44)		42.4%(36)	50.0%(51)	46.5(87)		
	Mild stunting	14.4%(14)	26.9%(21)	20.0(35)	V=0.168	12.9%(11)	20.6%(21)	17.1(32)	V=0.241	V=0.228
	Moderate stunting	18.6%(18)	17.9%(14)	18.3(32)	P=0.296	9.4%(8)	12.7%(13)	11.2(21)	**P=0.028**	**P=0.001**
	Severe stunting	**33.0%(32)**	**23.1%(18)**	**28.6(50)**		**24.7%(21)**	**14.7%(15)**	**19.3(36)**		
WAZ % (n)	Normal	33%(32)	30.8%(24)	32(56)		44.7%(38)	31.4%(32)	37.4(70)		
	Mild underweight	16.5%(16)	34.6%(27)	24.6(43)	V=0.310	18.8%(16)	41.2%(42)	31.0(58)	V=0.291	V=0.132
	Moderate underweight	23.7%(23)	28.2%(22)	25.7(45)	**P=0.002**	15.3%(13)	18.6%(19)	17.1(32)	**P=0.003**	P=0.176
	Severe underweight	**22.7%(22)**	**6.4%(5)**	**15.4(27)**		**18.8%(16)**	**8.8%(9)**	**13.4(25)**		
WHZ % (n)	Normal	60.8%(59)	64.1%(50)	61.5(112)		62.4%(53)	50.0%(51)	56.1(101)		
	Mild wasting	15.5%(15)	14.1%(11)	13.7(25)	V=0.059	11.8%(10)	22.5%(23)	18.9(34)	V=0.182	V=0.071
	Moderate wasting	9.3%(9)	6.4%(5)	8.2(15)	P=0.963	7.1%(6)	11.8%(12)	9.4(17)	P=0.185	P=0.771
	Severe wasting	**8.2%(8)**	**9.0%(7)**	**9.3(17)**		**10.6%(9)**	**10.8%(11)**	**10.0(18)**		
Nutritional status % (n)	Malnourished	62.9%(61)	57.7%(45)	60.6(105)	V=0.053	47.1%(40)	49.0%(50)	48.1(90)	V=0.020	V=0.125
	Normal	37.1%(36)	42.3%(33)	39.4(69)	P=0.485	52.9%(51)	51.0%(52)	51.9(97)	P=0.789	**P=0.018**

Legend : HAZ = Stunting; WAZ = Underweight; WHZ = Wasting P= p-value

Cramer's V test: Comparing between overall sex for stunted children ; p=0.001
Comparing between overall sex for malnourished children; p= 0.018
P in bold- statistically significant difference (p< 0.05)

3.2.1.6. Proportion of mild, moderate and severely malnourished children by age categorized

In the ≥5 age group, severe stunting was significantly higher (p=0.007) in the boys when compared to the girls, with the <5 age group being generally more stunted than the ≥5 age group (p=0.001) (Table 14). Furthermore, in the <5 age group, boys were significantly (p=0.032) more malnourished than girls, with the <5 age group being generally more malnourished than the ≥5s (0.018).

Table 14: Proportion of mild, moderate and severely malnourished children in the study population by sex within age

	Category	≤5			P-value	>5				P-value (compare ≤5 and >5)
		Boys	Girls	Overall ≤5		Boys	Girls	Overall >5	P-value	
HAZ % (n)	Normal	25.8%(25)	42.4%(36)	33.5%(61)	V=0.207 P=0.100	24.4%(19)	50.0%(51)	38.9%(70)	V=0.279 **P=0.007**	V=0.228 P=**0.001**
	Mild stunting	14.4%(14)	12.9%(11)	13.7%(25)		20.6%(21)	20.6%(21)	23.3%(42)		
	Moderate stunting	18.6%(18)	26%(9.4)	14.3%(26)		17.9%(14)	12.7%(13)	15%(27)		
	Severe stunting	**33.0%(32)**	**24.7%(21)**	**29.1%(53)**		**15%(23.1)**	**14.7(15)**	**18.3%(33)**		
WAZ % (n)	Normal	33%(32)	44.7%(38)	38.5%(70)	V=0.151 P=0.388	30.8%(24)	31.4%(32)	31.1%(56)	V=0.120 P=0.455	V=0.132 P=0.176
	Mild underweight	16.5%(16)	18.8%(16)	17.6%(32)		34.6%(27)	41.2%(42)	38.3%(69)		
	Moderate underweight	23.7%(23)	15.3%(13)	19.8%(36)		28.2%(22)	18.6%(19)	22.8%(41)		
	Severe underweight	**22.7%(22)**	**18.8%(16)**	**20.9%(38)**		**6.4%(5)**	**8.8%(9)**	**7.8%(14)**		
WHZ % (n)	Normal	60.8%(59)	62.4%(53)	61.5%(112)	V=0.084 P=0.866	64.1%(50)	50.0%(51)	56.1%(101)	V=0.165 P=0.297	V=0.071 P=0.771
	Mild wasting	15.5%(15)	11.8%(10)	13.7%(25)		14.1%(11)	22.5%(23)	18.9%(34)		
	Moderate wasting	9.3%(9)	7.1%(6)	8.2%(15)		6.4%(5)	11.8%(12)	9.4%(17)		
	Severe wasting	**8.2%(8)**	**10.6%(9)**	**9.3%(17)**		**9.0%(7)**	**10.8%(11)**	**10.0%(18)**		
Nutritional status % (n)	Malnourished	62.9%(61)	47.1%(40)	55.5%(101)	V=0.159 P=**0.032**	57.7%(45)	49.0%(50)	52.8%(95)	V=0.086 P=0.248	V=0.125 **P=0.018**
	Normal	37.1%(36)	52.9%(45)	44.5%(81)		42.3%(33)	51.0%(52)	47.2%(85)		

Legend : HAZ = Sunting; WAZ = Underweight; WHZ = Wasting P= p-value

Cramer's V test: Comparing between overall age group for stunted children ; p=0.001
Comparing between overall age group for malnourished children; p= 0.018
P in bold- statistically significant difference (p< 0.05)

3.2.1.7. Prevalence of malnutrition by Socio-economic level

Although not significantly different (p=0.348), the malnutrition prevalence was highest (64.9%) in the poor SES quintile and least (48.0%) in the very rich SES quintile. (Table 15)

Table 15: Prevalence of malnutrition in the different Socio-economic levels in the study Population

Socio-economic status	Frequency(n)	Prevalence of malnutrition (%)	N
Very low	59	(55.7)	106
Low (poor)	**37**	**(64.9)**	**57**
Average	48	(52.2)	93
High (rich)	27	(48.2)	56
Very high	**24**	**(48.0)**	**50**
Total	**196**	**(54.1)**	**362**

Comparing malnutrition by SES levels; Cramer's V= 0.879 P= 0.348

3.2.1.8. Predictors of Malnutrition

The logistic regression model demonstrated sex of child [OR=1.65, (95% CI: 1.05-2.59); (p= 0.029)], level of education of care giver [OR=2.41, (95% CI: 1.39-4.16); (p=0.002)], and malaria status [OR=1.89, (95% CI: 1.12-3.19); (p=0.017)] as significant predicting risk factors of malnutrition as shown in Table 16. Furthermore, the model also identified other risk factors for malnutrition like family head relationship with child (p=0.031). Males were 1.65 times more likely to become malnourished compared to females [OR=1.65, (95% CI: 1.05-2.59); (p=0.029)]. Malaria positive children were also 1.89 times more likely to become malnourished compared to malaria negative children [OR=1.89, (95% CI: 1.12-3.19); (p=0.017)]. The level of education of caregiver (r= 0.88; p=0.002), gender of child (r= 0.50; p=0.029) and malaria status (r=0.64; p=0.017) all showed a positive correlation to malnutrition. However, family head relationship with child was negatively correlated with malnutrition (r = -0.23; p=0.031).

Based on the logistic regression model (LRM), malnutrition observed in the study population may also be greatly explained by poor care giver education [OR=2.408, (95% C.I: 1.39-4.16); p=0.002] in the studied area (50.8% had no education at all, 47.2% had only primary level education). Children from mothers with little education were 2.4 times more likely to become malnourished compared to those who had educated mothers. This may have resulted to caregivers not being able to understand the nutritional requirements of the children, leading to poor feeding. It may also have resulted to care givers not being able to follow recommended procedures for feeding the children. The lack of proper knowledge on proper preparation methods and balanced diets may also have contributed to the high rate of malnutrition. These findings are consistent with earlier studies by Gelu *et al.* (2018) in North Western Ethiopia.

Table 16: Logistic regression model predicting risk factors of [a]malnutrition

Predictors	**B**	**P**	**OR**	**95% C.I.**	
				Lower	**Upper**
[b]Villages	-.168	0.131	.845	.680	1.051
[c]Religion	-.291	0.299	.747	.431	1.295
[d]Gender of familyhead	-.241	0.692	.786	.238	2.594
[e]Family head relationship with child	**-.229**	**0.031**	**.796**	**.647**	**.979**
[f]Care giver relationship to family head	1.072	0.085	2.921	.864	9.877
[g]Marital status of family head	.294	0.409	1.342	.667	2.702
[h]Level of education of care giver	**.879**	**0.002**	**2.408**	**1.395**	**4.156**
[i]Level of education of family head	-.478	0.098	.620	.352	1.093
[j]Type of housing	-.062	0.416	.939	.808	1.092
[k]Household size	.087	0.627	1.090	.769	1.546
[l]Gender of child	**.502**	**0.029**	**1.652**	**1.054**	**2.591**
[m]Development index	.087	0.497	1.091	.848	1.404
[n]Anaemic status	.228	0.421	1.257	.721	2.191
[o]Malaria status	**.638**	**0.017**	**1.892**	**1.120**	**3.196**
[p]Child age	-.004	0.985	.996	.632	1.568
[q]Family head employment status	.080	0.256	1.083	.944	1.243

Legend: B= Significant correlation; P=P-value; OR=Odds Ratio; C.I= Confidence Interval
P is significant at <0.05

3.2.2. Malaria Prevalence Survey in the study population

3.2.2.1. Confirmed Malaria

Out of the 362 children in the study population, RDT (SD- Bioline, Standard Diagnostics, INC. Lot No.05FK60), was done on 102 of them who showed symptoms of malaria (body temperature >37.5°C and fever). PCR was conducted on 185 samples which were randomly selected. However, microscopy was performed on all 362 samples. Based on microscopy, 119 children (119/362) were malaria positive making a proportion of 32.9% (95% CI: 28.2-37.8). Based on RDT, 91 children (91/102) were positive making a proportion of 89.2% (95% CI: 82.0-94.2). Based on PCR, 123 children (123/185) were malaria positive, making a proportion of 66.5% (95% CI: 62.4-75.9). (Fig. 15)

Malaria represents a major public health problem, mainly linked to poverty (Bigoga *et al.*, 2012). The overall prevalence of malaria parasitaemia as revealed by our study was 32.9% (based on microscopy as the standard). This result was lower than 46.7% and 44.3% earlier observed in the Mount Cameroon area by Teh *et al.* (2021) and Kimbi *et al.* (2012) resectively, an area of perennial malaria transmission. However, this was lower than the 36% reported in 2012 and 2013 in the North region (NMCP, 2018). Our results were also by far lower than the 76.3% observed by Ndiabamoh *et al.* (2020) in the Ngali II and Mfou villages of the Central Region of Cameroon.

The lowered malaria prevalence from our study and presently in Cameroon as a whole could be explained by the continuous scaled-up intervention measures put in place at the time by the Cameroonian government through the National Malaria Control Program (NMCP) in the Ministry of Public Health, such as the free treatment for malaria positive children (0-5years old) as well as the free distribution of LLIN's (Long Lasting Insecticide-treated Nets) especially to pregnant women, which may have contributed to lower the infective mosquito

bites. Improved environmental management such as better drainage, less stagnant water as well as few bushy surroundings may also have contributed a great deal to the lowered malaria prevalence.

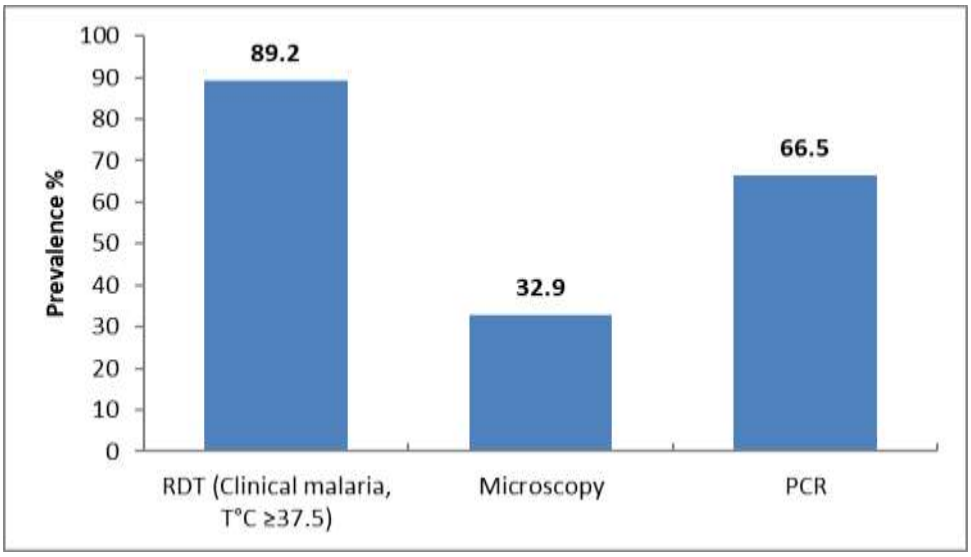


Figure 15: Individual proportions of confirmed malaria cases according to the type of parasitological test used in the study population

The overall prevalence reported in this study was obtained from microscopy. The prevalence of malaria by microscopy, RDT and PCR was 32.9%, 89.2% and 66.5%, respectively. This trend in our study was not consistent with studies by Mfuh *et al.* (2019) in selected health facilities in Maroua, Nkolbisson and Bamenda; Cameroon, who showed microscopy, RDT and PCR to be 31%, 45% and 54% respectively, reporting the highest prevalence from PCR. The difference in our study may be due to the fact that Rapid Diagnostic Test (RDT) was only done on the field on 102 children who presented with confirmed malaria (fever or body temperature > 37.5°).

Malaria prevalence by RDT (SD- Bioline) was 89.2%, very much higher than microscopy results. However, examination of blood smears using the light microscopy remains the "gold standard" for malaria diagnosis, but it is labour intensive, requires skilled microscopists and

generally there is a limited supply and maintenance of microscopes and reagents thus leading to delays in delivery of results. Although findings by Moyeh *et al.* (2019) in coastal Cameroon showed the SD Bioline ^{TM} to be as good as the light microscope in the diagnosis of malaria in remote areas of perennial transmission. New Rapid Diagnostic Techniques (RDTs) have been developed and evaluated in recent years to overcome the limitations of light microscopy. However, the rapid introduction, withdrawal and modification of commercially available RDTs, variable quality control in manufacturing, and potential decrements in test performance related to the stability of stored test kits have rendered these reviews obsolete (Murray *et al.*, 2008). However, PCR, a more sensitive technique, revealed a prevalence of 66.5%, although it was not run on all samples, it was however representative of the total population.

3.2.2.2. Prevalence of malaria in the study population by health district, sex and age group

A total of 362 samples were collected from 6 villages in both health districts in the north region with malaria prevalence relatively highest in Bala 45(57%) and lowest in Mayo- Oulo 11(16.4%) villages, ($p< 0.001$). (Table 18) Of these 119, more males 66(37.7%) were infected than females 53(28.3%), ($p= 0.058$). (Table 18) Generally, malaria prevalence was higher in the ≤5 age group compared to the >5.

Table 18: Prevalence of malaria in the study population by health district, age group and sex.

Category		Malaria		
Health district	**Villages**	**Frequency(n)**	**Percent (%)**	**N**
Mayo-Oulo.	Bala	45	57.0	79
	Mayo-Oulo	11	16.4	67
	Dourbeye	11	39.3	28
Overall Mayo-oulo		67	38.5	174
Pitoa	Guizigaré	27	33.3	81
	Kirambo	16	23.9	67
	Boussa	9	22.5	40
Overall Pitoa		52	27.7	188
Total		**119**	**32.9**	**362**
Age group	**≤5**	70	38.5	182
	>5	49	27.2	180
Total		**119**	**32.9**	**362**
Sex	Males	66	37.7	175
	Females	53	28.3	187
Total		**119**	**32.9**	**362**

Comparing between health disticts **Cramer's V= 0.115; P=0.028**
Comparing between age groups **Cramer's V= 0.120; P=0.023**
Comparing between sex **Cramer's V= 0.100; P=0.058**

The results further showed that malaria prevalence was significantly higher (p=0.028) in Mayo- oulo health district (38.5%) than Pitoa health district (27.7%), though both were peri-urban settings, (Table 18) and contradicts studies by Tabue *et al.* (2019) who reported a significantly higher prevalence of malaria in Pitoa (47.83%) than Mayo-oulo health district (17.53%). Probably due to the fact that our study sampled only 6 villages, while theirs was a larger study covering a wider range of villages.

Furthermore, malaria prevalence was higher in males than females, confiming earlier findings. This again may be as a result of the biological disposition of the boys than the girls

Also, our findings revealed that, malaria prevalence was significantly higher (p=0.023) in the <5 age group (38.5%) compared to the ≥5 (27.2%), similar to the trend observed for malnutrition, although the difference was not significant (p>0.05) (Table 10). This increased susceptibility of young children to infections has been attributed to low levels of IgA and IgG subclasses, as a result of a slow maturation of immunoglobulin, among other factors (Thorarinsdottir *et al.,* 2005).

3.2.2.3. Distribution of malaria in the study population by age group, sex, and by test used.

Parasitaemia ranged from 40parasites/µL to 129600parasites/µL of blood, with a geometric mean parasite density (GMPD) of 1783. The GMPD showed no significant difference (P=0.096) between the two age groups**.** (Table 19)

Table 19: Distribution of confirmed malaria in the study population by age group and according to the type of parasitological test that was used

	RDT (Confirmed malaria, T°C ≥37.5)		**Microscopy**		**PCR**		**Parasitaemia (GM ± SD ; Min-Max)**	
Age group	**≤ 5**	**+5**	**≤ 5**	**+5**	**≤ 5**	**+5**	**≤5**	**+5**
N	58	44	182	180	99	86	86	33
Positivity	52	39	70	49	67	56	40 – 129600	80 - 69200
Prevalence (%)	89.7%	88.6%	38.5%	27.2%	67.7%	65.1%		
Overall	**89.2% (91)**		**32.9% (119)**		**66.5% (123)**		**40 – 129600**	
Cramer's V	V=0.016; P=0.870		V=0.120; **P=0.023**		V=0.027; P=0.713		U=1407.000; P=0.096	

Legend: GM: Geometric Mean PD: Parasite Density
Cramer's V = 0.120; P= 0.023 – comparing malaria between ≤5 and >5 children using microscopy
Mann-Whitney U =1407.000; P= 0.096 - comparing parasitaemia between ≤5 and >5 children

Parasitaemia ranged from 40parasites/µL to 129600parasites/µL of blood, with a geometric mean parasite density (GMPD) of 1783. The GMPD showed a significant difference(P=0.036) between both sexes**.** (Table 20)

Table 20: Distribution of confirmed malaria in the study population by sex and according to the type of parasitological test that was used

	RDT (Confirmed malaria, T°C ≥37.5)		**Microscopy**		**PCR**		**Parasitaemia (GM ± SD ; Min-Max)**	
Sex	**Boy**	**Girl**	**Boy**	**Girl**	**Boy**	**Girl**	**Boy**	**Girl**
N	52	50	175	187	96	89	66	53
Positivity	46	45	66	53	71	52	40-129600	80- 78400
Prevalence (%)	88.5%	90.0%	37.7%	28.3%	74.0%	58.4%		
Overall	**89.2% (91)**		**32.9% (119)**		**66.5% (123)**		**40 – 129600**	
Cramer's V	V=0.025 P=0.802		V= 0.100 **P=0.058**		V=0.164 P=0.025		U=14617.500 **P=0.036**	

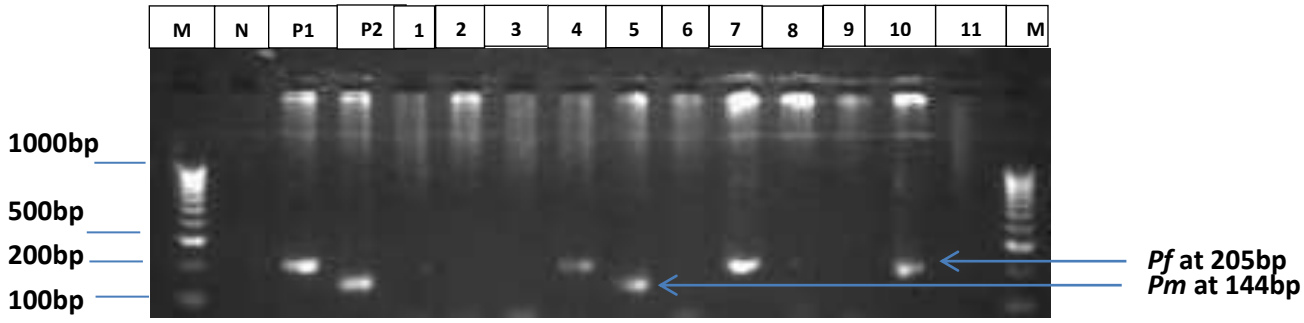
Legend: P= p-value; Cramer's V =0.100; P= 0.058 Comparing between boys and girls for microscopy
Mann-Whitney U = 14617.500; P= 0.036 – Comparing between boys and girls for parasitaemia

3.2.2.4. Molecular Identification of *Plasmodium* species by PCR

3.2.2.4.1. PCR positive samples

In order to confirm the *Plasmodium* species, PCR was performed. Of the 362 samples, 185 were run for PCR. Following microscopy, samples positive for microscopy and samples negative for microscopy were randomly selected, which was representative of the population. A total of 185 blood samples collected on filter paper were used for *Plasmodium* DNA extraction and amplification of the target DNA fragment by PCR. In PCR assay, for the filter-paper spot samples 123(66.5%) were PCR positive (95% CI: 62.4 - 75.9) when DNA was

extracted by Chelex Method. All microscopy positive samples were PCR positive. However, PCR detected 11 cases missed by microscopy. (Fig 16)



M = Molecular weight marker; **N** = Negative control; **P1**= *P. falciparum* positive control; **P2**= *P. malariae* Positive control; **4, 7**, **10** = *P.falciparum* positive sample; **5** = *P.malariae* positive sample; **1,3, 9** = *P. falciparum* negative sample; **2, 6, 8, 11** = *P. malariae* negative samples;

Figure 16 : Electrophoregram depicting *Plasmodium* species identified in North Region

3.2.2.4.2. Co-infection by *Plasmodium* species

With regards to the different *Plasmodium* species identified in the study population, using microscopy as the standard, *Plasmodium falciparum* and *Plasmodium malariae* were the parasite species found, with *Plasmodium falciparum* representing 86.6% (103/119) of single infections. There were only 8.4 % (10/119) of single infections of *Plasmodium malariae*, while mixed infections of *Plasmodium falciparum* and *Plasmodium malariae* represented 5% (6/119). No *Plasmodium ovale* or *Plasmodium vivax* infection was found in any sample. (Table 21) This disagrees with findings by Russo *et al.* (2017) in Dschang, West Cameroon who reported that *Plasmodium vivax* was found in 5.6% patients, representing 38.6% of all *Plasmodium* infections as well as by Fru Cho *et al.* (2014); Ngassa and Das, (2014) in Bolifamba, South-West Cameroon, who observed that *Plasmodium vivax* represented 14.9% of *Plasmodium* infections as cited by Antonio- Nkondjio *et al.* (2019) on the recent review of

the malaria situation in Cameroon. Nevertheless, our results are similar to those of Moyeh *et al.* (2019) in coastal Cameroon who also pointed out that, no cases of vivax were seen. However, our findings are in line with those of Moyeh *et al.* (2019) in Mutengene, coastal Cameroon who equally reported no cases of *vivax*. Moyeh *et al.* found cases of monoinfection with *Plasmodium ovale* (5.14%), mixture of *falciparum* and *malariae* (7.41%), and even mixed infections of *ovale* and *falciparum* 94.67%). Whereas in our study, our findings on mixed infections were limited to falciparum and malariae only (5%).

Table 21: Prevalence of the different *Plasmodium* species and cases of Co-infection in the study population (N=119)

Plasmodium specie	**Frequency(n)**	**Prevalence (%)**
Plasmodium falciparum	103	86.6
Plasmodium malariae	10	8.4
Plasmodium falciparum and *Plasmodium malariae*	6	5.0
Total	**119**	**(100%)**

3.2.2.4.3. Prevalence of malaria in the different SES levels

Based on the comparisons, amongst the malaria positive children (32.9%), malaria prevalence was significantly (p=0.045) highest (42.4%) in the average SES quintile and lowest (18.0%) in the very rich SES quintile. (Table 22)

Table 22: Prevalence of malaria in the different Socio-economic levels in the study population

Socio-economic status	Frequency (n)	Prevalence of malaria (%)	N
Very low	37	34.9	106
Low (poor)	17	29.8	58
Average	**39**	**42.4**	**92**
High (rich)	16	28.6	56
Very high	**9**	**18.0**	**50**
Total	**119**	**32.9**	**362**

Cramer's V= 0.197 P=0.045

3.2.2.4.4. Predictors of Malaria

For Malaria, the logistic regression model below demonstrated that village [OR=1.62, (95% CI: 1.28-2.05); (p<0.001)] and nutritional status [OR=2.07, (95% CI: 1.22-3.53); (p=0.007)] were significant predictors of malaria (Table 23). This result signifies that a child residing in Bala is 1.62 times more likely to become infected with malaria, than one living in Mayo-oulo. Therefore, village (location) correlated with malaria status. Also, malnourished children were 2.07 times more likely to habour the malaria parasite compared to well- nourished children.

Overall, malaria prevalence in malnourished children was 37.8%. Considering the different forms of malnutrition, malaria parasitaemia prevalence was observed to be higher in both stunted (54.3%) and underweight (58.6%) children while wasting (31.4%) was lower compared to their normal mates. The manifestation of chronic and acute nutritional deficits which result in stunting and wasting respectively influenced the prevalence of malaria parasite in the population. Overall, while children who were stunted and underweight had a higher prevalence of malaria parasite, those wasted had a lower prevalence. This is in line with earlier studies by Sumbele *et al.* (2015) in the Mount Cameroon Region, as well as Fillol *et al.* (2009) in Rural Senegal.

Table 23: Logistic regression model predicting risk factors of [a]malaria

Predictors	B	P	OR	95% C.I.for EXP(B)	
				Lower	Upper
[b]Villages	**.484**	**<0.001**	**1.622**	**1.280**	**2.055**
[c]Religion	.378	0.226	1.460	.791	2.692
[d]Gender of family head	1.146	0.122	3.147	.736	13.457
[e]Family head relationship with child	.195	0.105	1.216	.960	1.540
[f]Care giver relationship to family head	-.886	0.290	.412	.080	2.130
[g]Marital status of family head	6.247	0.999	516.599	.000	.
[h]Level of education of care giver	**-1.153**	**<0.001**	**.316**	**.169**	**.588**
[i]Level of education of family head	.375	0.263	1.455	.754	2.808
[j]Type of housing	-.025	0.770	.975	.825	1.153
[k]Household size	.298	0.135	1.347	.912	1.990
[l]Gender of child	.292	0.268	1.339	.799	2.243
[m]Development index	**-.282**	**0.042**	**.755**	**.576**	**.989**
[n]Nutritional status	**.729**	**0.007**	**2.073**	**1.218**	**3.528**
[o]Child age	.267	0.310	1.306	.780	2.186
[p]Family head employment status	**-.393**	**<0.001**	**.675**	**.573**	**.796**

Legend: B= Significant correlation ; P=P-value; OR=Odds Ratio; C.I= Confidence Interval

P is significant at p<0.05

Other risk factors for malaria were; level of education of care giver [OR=0.32, (95% CI: 0.17-0.59); (p<0.001)], development index [OR=0.75, (95% CI: 0.58-0.99); (p=0.042)], and family head employment status [OR=0.68, (95% CI: 0.57-0.79); (p<0.001)].

Implying that children from mothers with a low level of education were 0.32 times more likely to become infected by malaria parasite compared to those whose mothers were well educated.

Based on the Logistic Regression Model, malaria status also correlated with development index. [OR= 0.75, (95%C.I: 0.58-0.99); p=0.042]. This result shows that children in the low socio-economic class were 0.75 times more likely to become infected by the malaria parasite compared to children in the high socio-economic class. Socioeconomic factors may influence malaria morbidity, although their respective roles are somewhat controversial (Luckner *et al.,* 1998).

Nutritional status (r = 0.73) showed a strong positive correlation with malaria. However, level of education of the care givers (r = -1.2), development index (r = -0.28) and family head employment status (r = -0.39) were all negatively correlated with malaria. (Table 23).

The key to effective management of malaria is prompt and accurate diagnosis. The WHO recommends that malaria case management where possible should be based on parasitological

diagnosis, except when considering young children in endemic areas where lack of resources or urgency of response temporarily limits its application (Hassan *et al.,* 2010; Sousa-Figueiredo *et al.,* 2010). Malaria control in Cameroon relies principally on anti-vector intervention using long lasting insecticide nets (LLIN) (Bigoga *et al.,* 2012) and mainly through large scale campaign and free distribution of the nets. Therefore, the Ministry of public health of Cameroon should continue to implement serious plans to scale- up control strategies for malaria parasite nation- wide

3.2.2.5. Relationship between Malaria and Nutritional Status

Out of the 119 children who had malaria parasitaemia, 86(23.8%) were ≤5years old while 33(9.1%) were >5years. Unlike O'brien *et al.* (2018) who found no effect of anthropometric

indices on occurrence of malaria in the Niger**,** our results are consistent with those of Nyakeriga *et al.* (2004) in children living in the coast of Kenya who reported a controversial synergistic relationship between malaria and malnutrition. Deribew *et al.* (2010) in South Western Ethiopia also reported no association between malaria and malnutrition, however, our study clearly showed that there was a bi-directional association between malaria and malnutrition which is similar to findings by Ehrhardt *et al.* (2006) in Ghana, who also identified malnutrition as a major underlying cause of malaria.

The relationship between the presence of malaria parasite and malnutrition in our study was 2 ways; a synergistic relationship with malnourished children being 2.07 times more likely to habour malaria parasite compared to well-nourished children [OR= 2.07, (95% CI: 1.22 – 3.53)] (Table 23) while malaria positive children were 1.89 times more likely to be malnourished compared to the malaria negative children [OR=1.89, (95% CI: 1.12 – 3.19)]. This implies that on one hand, malaria may have caused malnutrition, whereas on the other hand, malnutrition itself may have modulated susceptibility to malaria (Nyakeriga *et al.,* 2004). The observed result therefore confirms the controversial synergistic relationship between malaria and malnutrition as reported by Nyakeriga *et al.* (2004) in children living in the coast of Kenya**.**

3.2.3. Prevalence of Anaemia

3.2.3.1. Prevalence of Anaemia in the study population

Overall, 74 children were anaemic making a proportion of 20.6% (95% CI: 16.7-25.0).

With regards to anaemia, anaemia is a symptom of severe malaria found in children (Ehrhardt *et al.*, 2006), an indicator of poor nutrition and poor health (Sumbele *et al.*, 2020) and is a key public health challenge in Cameroon. The overall prevalence as revealed by our study was 20.6%. This is unexpected in a peri-urban community where malaria is hyper endemic. The

DHS in 2011 revealed a prevalence of 62.2% in the North region as opposed to 69.5% in the 2004 DHS. However, the most recent DHS in 2018 revealed a prevalence of 56.5%, indicating a drop in about 6% in this region (WHO, 2019). This result was by far lower than earlier studies by Jourdan *et al.* (2008), who reported a prevalence as high as 82% in children attending a clinic in Northern Cameroon. It is also much lower than 77.3% reported by Asoba *et al.,* (2019) in the Mount Cameroon area as well as the 84% observed by Sumbele *et al.* (2020) in conflict-hit Mount Cameroon. It's lower than 70% observed by Desai *et al.* (2005) in Kenya; 87.1% by Anumudu *et al.* (2008) in Nigeria; 41.1% by Gebreweld *et al.* (2019) in North Western Ethiopia and 70.9% by Nambiema *et al.* (2019) in Togo. Interestingly, our result is comparable to findings in Mexico (20.6%) and Honduras (29%), (Meija Torres *et al.,* 2014) among 6-59-year olds. The differences in our study may be explained by the Vit A supplementation intervention programme in children, which was on-going in the study area, during the study period. This may have contributed a great deal in lowering the prevalence of anaemia. Other factors such as improved sanitation may also have been responsible; coupled to the fact that our study was a community- based household survey and most of the children examined were apparently healthy and not necessarily sick children in hospital. According to the WHO classification, the overall prevalence of 20.6% for anaemia (>20) revealed by our study, shows that anaemia is considered a moderate public health problem in the Pitoa and Mayo-Oulo Health Districts, of the North Region of Cameroon.

3.2.3.1.1. Prevalence of Anaemia by Health District

Pitoa Health District generally had a significantly higher (p=0.001) prevalence of anaemia than Mayo-oulo, with the highest seen in Kirambo 22(32.8%) and the lowest in Dourbeye 1(3.6%). (Table 24)

3.2.3.1.2. Prevalence of Anaemia by age group

Although not significantly different, children < 5years 40(22.2%) had a higher (p=0.45) anaemia prevalence when compared to those ≥ 5 years 34(19.0%) (Table 24**).** Furthermore, the trend observed for anaemia was higher in the <5 age group compared to the ≥5 age group probably due to the fact that the <5 age group were more malnourished than their counterparts.

3.2.3.1.3. Prevalence of Anaemia by sex

Anaemia in this study was comparable between males 37(21.3%) and females 37(20.0%), although no significant difference (p>0.05) was observed. (Table 24) Akin to studies by Sumbele *et al.* (2020) in the Owe, Mpundu and Meanja villages at the foot of Mount Cameroon, which revealed a similar trend, showing similar rates in both males (74.6%) and females (75.9%). The higher anaemia prevalence observed in the male children could probably be explained by the high rates of malnutrition in the male sex.

Table 24: Prevalence of anaemia in the study population by health district, age group and sex.

Category		Anaemia		
Health district	**Villages**	**Frequency(n)**	**Percent (%)**	**N**
Mayo-Oulo.	Bala	10	12.7	79
	Mayo-Oulo	12	18.2	66
	Dourbeye	1	3.6	28
Overall Mayo-oulo		**23**	**13.3**	**173**
Pitoa	Guizigaré	21	26.6	79
	Kirambo	22	32.8	67
	Boussa	8	20.0	40
Overall Pitoa		**51**	**27.4**	**186**
Total		**74**	**20.6**	**359**
Age group	**≤5**	40	22.2	180
	>5	34	19.0	179
Total		**74**	**20.6**	**359**
Sex	Males	37	21.3	174
	Females	37	20.0	185
Total		**74**	**20.6**	**359**

Comparing between health disticts **Cramer's V= 0.174; P=0.001**
Comparing between age groups **Cramer's V= 0.040; P=0.450**
Comparing between sex **Cramer's V= 0.016; P=0.767**

3.2.3.1.4. Prevalence of anaemia in the different SES levels

Overall anaemia prevalence was (20.6%), (Table 25) being highest (p=0.085) in the low socio-economic class (26.8%) and least in the high class (7.1%), though the difference was not significant (0.085). (Table 25)

Table 25: Prevalence of anaemia in the different Socio-economic levels in the study Population

Socio-economic status	Frequency(n)	Prevalence of anaemia %	N
Very low	25	23.6	106
Low (poor)	**15**	**26.8**	**56**
Average	20	22.0	92
High (rich)	**4**	**7.1**	**56**
Very high	10	20.4	49
Total	74	**74 (20.6)**	**359**

Comparing between SES levels Cramer's V= 0.248 ; P= 0.085

3.2.3.2. Anaemia categorized

Anaemia was categorized according to WHO, 2014 classification. Our study revealed mild, moderate and severe anaemia rates to be 8.1%, 9.2% and 3.3%, respectively, with moderate anaemia being the highest (9.2%), and severe anaemia (3.3%) the least. This indicates that severe anaemia was rare in the study area. Meanwhile the WHO prevalence rates for mild, moderate and severe anaemia stood at 25%, 33% and 3% in 2017, (WHO, 2017) confirming the same trend in our study. The Cameroon Demographic Health Survey report (DHS, 2018) equally showed a similar trend. However, findings on anaemia by Behera and Bulliyya, (2016) in Odisha, India, showed a contrary trend where mild anaemia was higher than moderate anaemia and severe anaemia in that order respectively, with severe anaemia being the least. (Fig 17)

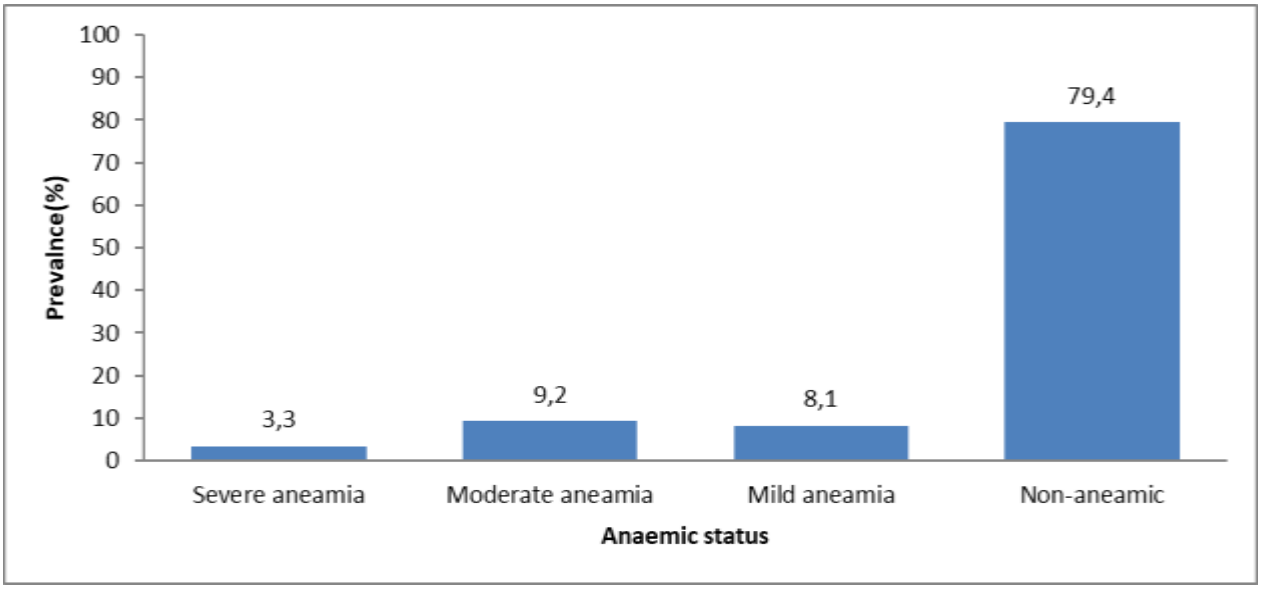


Figure 17: Anaemic status of study population categorized

3.2.3.3. Predictors of Anaemia

The Logistic Regression Model significantly identified development index [OR=1.45, (95% CI: 1.05-2.00); (p=0.023)] and family head relationship with child [OR=1.41, (95% CI: 1.1-1.8); (p=0.006)] as significant risk factors for anaemia (Table 25). Meaning, anaemia correlated with socio-economic status (p=0.023). Children with a low SES were 1.45 times more likely to be anaemic [OR=1.45, (95% CI: 1.05 – 2.0)] compared with children in the high socio-economic level. This result is in line with earlier findings which state that anaemia constitutes a major public health problem in young children in the developing world with wide social and economic implications (Le Cornet *et al.*, 1998).

Family head relationship with child (r= 0.34) and development index (r=0.37) were both positively correlated to anaemia.

Table 26: Logistic regression model predicting risk factors of [a]anaemia

Predictors	B	P	OR	95% C.I.	
				Lower	Upper
[b]Villages	-.043	0.753	.958	.734	1.251
[c]Religion	.555	0.103	1.742	.893	3.399
[d]Gender of family head	-.196	0.815	.822	.160	4.228
[e]Family head relationship with child	**.344**	**0.006**	**1.411**	**1.102**	**1.807**
[f]Care giver relationship to family head	.486	0.512	1.626	.380	6.962
[g]Marital status of family head	.050	0.914	1.051	.424	2.608
[h]Level of education of care giver	-.238	0.494	.788	.399	1.558
[i]Level of education of family head	-.038	0.919	.963	.469	1.978
[j]Type of housing	.075	0.467	1.078	.881	1.319
[k]Household size	-.100	0.654	.905	.584	1.402
[l]Gender of child	-.052	0.854	.949	.545	1.652
[m]Development index	**.373**	**0.023**	**1.452**	**1.053**	**2.001**
[n]Malaria status	.233	0.416	1.262	.720	2.215
[o]Child age	.363	0.263	1.438	.761	2.714
[p]Family head employment status	.344	0.220	1.411	.814	2.444

Legend: B= Significant correlation; P=P-value; OR=Odds Ratio; C.I= Confidence Interval
P is significant at P<0.05

3.2.3.4. Relationship between Anaemia, Malaria and Malnutrition.

Anaemia prevalence was higher (p=0.422) in malaria positive children 27(23.1%) than malaria negative children 47(19.4%) and it was also higher (p=0.599) in malnourished children 42(21.6%) than well-nourished children 32(19.4%) (Table 27).

Table 27: Prevalence of anaemia in the study population by nutritional and malaria status, and age group

	Nutritional status				**Malaria status**				**Age range**	
	Malnourished		**Normal**		**Malaria positive**		**Malaria negative**			
Age range	**≤ 5**	**>5**	**≤ 5**	**>5**	**≤ 5**	**>5**	**≤ 5**	**>5**	**≤ 5**	**>5**
N	100	94	80	85	69	48	111	131	180	179
Anaemic	24	18	16	16	15	12	25	22	40	34
Prevalence	24.0%	19.1%	20.0%	18.8%	21.7%	25.0%	22.5%	16.8%	22.2	19.0
Overall	21.6% (42)		19.4% (32)		23.1% (27)		19.4% (47)		20.6 (74)	
Cramer's V	V=0.059; P=0.412		V=0.015; P=0.849		V=0.038; P=0.680		V=0.072; P=0.262		V=0.040 P=0.450	
Cramer's V	V=0.028; P=0.599				V=0.042; P=0.422					

Comparison of anaemia between malnourished and well-nourished children ; p=0.059
Comparison of between malaria positive and malaria negative children; p=0.422
Comparison of anaemia between <5 and ≥5 age groups; p=0.450
Cramer's V test

In relation to malaria, anaemia prevalence was 23.1% in the malaria positive group of children, while it was 19.4% in the malaria negative group (p=0.422). With regards to malnutrition, anaemia prevalence was 21.6% in the malnourished group and 19.4 in the well-nourished group of children (p=0.599). Meaning that anaemia was prevalent in all the categories of children, although the differences were not statistically significant (p>0.05). Therefore, our study showed no significant or apparent associations between anaemia and

malaria or malnutrition. Our findings on anaemia are similar to those of Osazuwa and Ayo, (2010) in rural communities of Edo state, Nigeria, who reported that there was no association between malaria infection and anaemia. Also, Stoltzfus *et al.* (2000) in Zanzibar as well as Jeremiah *et al.* (2007) in Nigeria, did not detect any association between malaria and anaemia. However, our results were contrary to findings by Maketa *et al.* (2015) in the Democratic Republic of Congo as well as Deribew *et al.* (2010) in South Western Ethiopia, who both reported that malaria or *Plasmodium falciparum* infection was strongly correlated with anaemia. Our results were also contrary to findings by Sumbele *et al.* (2015), in the Mount Cameroon region and Ehrhardt *et al.* (2006) in Ghana, who also reported that malnutrition was a fundamental factor contributing to anaemia. These differences in our study may be attributed to the poor socio-economic status of our study population, considering the fact that the children played around naked and moved around bare-footed

Anaemia was observed in our study to be higher in malnourished children than well-nourished children. This trend is consistent with findings by Ullah *et al.* (2014) in Pakistan. It was also higher in malaria positive children than the negative children.

Age wise, further observations also highlighted anaemia to be higher in <5's than the ≥5's, though the difference was not significant. Most findings (in Malaysia and Western Kenya) also reported correlation between education level and anaemia. But our study failed to find such an association.

Anthropometry has become a practical tool for evaluating the nutritional status of populations, particularly of children in developing countries (Mahgoub *et al.*, 2006) and nutritional status is the best indicator of the global well-being of children (Kandala *et al.,* 2011). Despite the fact that our study on nutritional status was limited to anthropometry, that iron deficiency adversely affects human health is widely recognized. Iron plays a critical role in the transport of oxygen throughout the body and in cellular processes of growth and division. Iron

deficiency results in a decrease in the haemoglobin concentration, which when sufficiently low is identified as anaemia.

In contrast, malaria causes anaemia through cytokine-mediated suppression of haematopoiesis, and in addition, when infected with *P. falciparum*, the erythrocyte changes and becomes vulnerable to clearance (Caulfield *et al.*, 2004). Hookworm and other infections which cause blood loss also contribute to iron deficiency and anaemia, often severe anaemia. All types of anaemia, regardless of cause, reduce the oxygen transported throughout the body, and this leads to decreased productivity and increased risk of cardiovascular events (Caulfield *et al.*, 2004).

It is expected that malaria causes chronic anaemia, impaired growth and delayed development in young children (Holding and Kitsao-Wekulo., 2004), however, in this study, no statistically significant association between anaemia and malaria ($p=0.422$) was found. Although no significant differences were observed in the prevalence of anaemia between the different sexes ($p>0.05$), anaemia was higher in males than in females (Table 25). Similar observations were made in Tanzania. This may be explained by the fact that males were significantly more malnourished than females ($p=0.018$). . In this study, the fact that anaemia may have been brought about by other causes is not completely ruled out.

In settings where iron deficiency is the most frequent cause, additional iron intake is usually provided through iron supplementation at low doses to vulnerable goups; young children and pregnant women in particular.

Also, food-based approaches to increase iron intake through food fortification and dietary diversification are important, sustainable strategies for preventing IDA in the general population. In settings where iron deficiency is not the only cause of anaemia, approaches that combine iron interventions with other measures are needed. Strategies should include addressing other causes of anaemia (WHO, 2006; Crompton *et al.*, 2003).

SPECIFIC OBJECTIVE 2

3.3. Influence of nutritional status on malaria parasitaemia, malaria parasitaemia on anaemia severity, nutritional status on anaemia status in the study population.

3.3.1. Means ± SD Z-scores for HAZ, WAZ, and WHZ in children affected with malaria parasite by age group

For the children infected with malaria parasite, the mean±SD Z-scores for HAZ, WAZ and WHZ in the <5 age group were -1.580±2.100, -1.504±2.121 and -0.231±2.729 respectively while for the ≥5age group, -1.245±1.925, -1.732±1.224 and -1.355±1.566, respectively. (Table 28) with mean WHZ within the two age groups being significantly different (p=0.004).

This result indicates that there were more wasted children (lower WHZ) in the <5 age group when compared to the ≥5 age group.

Table 28: Means ± SD Z-scores for HAZ, WAZ, and WHZ in children affected with malaria parasite summarized by age group

Indicators	**Categories**	**Children with malaria**		
		≤5 (n=182)	5+ (n=180)	
Nutritional status (mean±SD)	HAZ	-1.580±2.100	-1.245±1.925	U=1644.000 P=0.701
	WAZ	-1.504±2.121	-1.732±1.224	U=1538.500 P=0.341
	WHZ	-0.231±2.729	-1.355±1.566	U=-2.859 **P=0.004**

Legend : HAZ = Sunting; WAZ = Underweight; WHZ = Wasting P= p-value
Comparing between HAZ, WAZ and WHZ within the ≤5 and >5 age groups in malaria- infected children.
Mann-Whitney U test

3.3.2. Influence of nutritional status on malaria parasitaemia in the study population.

The type and level of malnutrition in a child could be linked to parasitaemia/ parasite density

(Table 29). Geometric mean density for parasitaemia was not significantly different (p>0.05) in stunted (r= -0.010; p=0.851), underweight (r= -0.005; p=0.922) or wasted (r= -0.002; p=0.973) children compared to their counterparts. This result show that even though they were negatively correlated, there was no significant association between parasitaemia and nutritional status (stunting, underweight or wasting, (Table 29) suggesting that malaria parasitaemia was not influenced by nutritional status. Our results are in agreement with Fillol *et al.,* (2009) in rural Senegal whose findings showed no significant difference (p>0.05) in the geometric mean of parasite density between stunted and wasted children when compared to their normal counterparts. Juster *et al.,* (2017) also reported no correlation between parasitaemia and wasting in Kenyan children. However, Sumbele *et al.,* (2015) in the Mount Cameroon area observed that clinical malaria parasitaemia was significantly higher (p=0.01) in stunted and underweight children compared to their counterparts.

Table 29: Influence of nutritional status on malaria parasitaemia in the study population

Parameter		**N=362**
	Parasitaemia	
Stunting	R= -0.010	P=0.851
Underweight	R= -0.005	P=0.922
Wasting	R= -0.002	P=0.973

Correlation is significant at the 0.01 level (2-tailed). P values not statistically significant (p>0.05)
Correlation is significant at the 0.05 level (2-tailed).
Spearman rank test

3.3.2.1. Influence of the severity levels of nutritional status on malaria parasitaemia in the study population.

For varying severity levels of stunting, although the difference was not significant, severely stunted children presented a higher geometric mean parasite density than those who were moderately or mildly stunted compared to the normal (well-nourished). (Table 30) However, for varying severity levels of underweight, moderately underweight children presented a higher parasite density compared to their counterparts. In the case of severity levels of wasting, mild wasting showed a significantly higher ($p=0.031$) level of parasitaemia when compared to the moderately and severely wasted counterparts. Indicating that, mild wasting significantly ($p=0.031$) influenced malaria parasitaemia.

Our result is similar to previous findings by Juster *et al.* (2017) in Kenyan children who observed that severely malnourished children both stunted and wasted, presented a higher parasite density than those moderately or mildly malnourished. Although, for wasting, it was the contrary in our study.

A higher mean parasite density was equally observed in normal (well-nourished) children compared to the malnourished children for all the forms of malnutrition. This also was not in agreement with ealier findings. (Juster *et al.*, 2017)

Table 30: Influence of the severity levels of nutritional status on malaria parasitaemia in the study population

Variable/ Category		Parasitaemia		
Stunting (HAZ)	**N**	**Geometric Mean**	**Min**	**Sum**
Normal	42	2699	80	3234040
Mildly stunted	20	1004	80	52760
Moderately stunted	16	1127	40	65000
Severely stunted	31	1831	80	499880
Underweight(WAZ)				
Normal	38	2701	80	70400
Mildly underweight	27	1100	40	24960
Moderately underweight	35	2234	80	129600
Severely underweight	18	852	80	19200
Wasting (WHZ)				
Normal	64	2419	40	97200
Mildly wasted	21	1070	80	19200
Moderately wasted	15	964	120	46200
Severely wasted	13	669	80	8040

Comparing Parasitaemia between different categories of stunting. Kruskal Wallis H= 5.531 ; P=0.137
ComparingParasitaemia between different categories of underweight.Kruskal Wallis H=7.193;P= 0.066
Comparing Parasitaemia between different categories of wasting. Kruskal Wallis H= 8.875 ; P= 0.031

Parasitaemia- Parasites/µL of blood

3.3.3. Influence of nutritional status on anaemic status in the study population.

With regards to haemoglobin, in varying levels of nutritional status, although the differences were not significant ($p>0.05$), mean haemoglobin was higher in moderately stunted, mildly underweight and moderately wasted children compared to the normal (well nourished) children as well as the other respective levels of nutritional status (Table 31). This result means that generally, malnourished children (stunted, underweight and wasted) had higher haemoglobin than those who were well nourished. Suggesting that nutritional status may not have influenced anaemic status. This is surprising and is not in agreement with studies by Tesema *et al.* (2021) whose findings instead revealed that stunting, underweight and wasting were significantly associated with increased odds of higher levels of anaemia compared to normal children in sub-Saharan Africa. However, our observations are similar to those of Antonio and San Sebastien, (2014) in Brazil who reported that no associations were found between anaemia and stunting or underweight. The possible explanation may be that other factors may have accounted for the anaemia in these malnourished children. Presumably helminthes.

Table 31: Influence of nutritional status on anaemic status in the study population

Variable/ Categ ry		Haemoglobin		
Stunting				
(HAZ)	**N**	**Mean**	**Min**	**Maximum**
Normal	131	12.2003	2.17	6.67
Mildly stunted	64	12.1554	4.67	16.50
Moderately stunted	53	12.7843	3.33	18.67
Severely stunted	86	12.0783	2.00	18.33
Underweight (WAZ)				
Normal	126	12.1882	2.17	16.83
Mildly underweight	100	12.7755	6.17	18.67
Moderately underweight	75	11.8685	2.00	17.33
Severely underweight	52	12.1008	6.67	18.33
Wasting (WHZ)				
Normal	212	12.2773	2.00	18.67
Mildly wasted	58	11.6420	3.00	17.33
Moderately wasted	31	12.8870	9.33	16.33
Severely wasted	35	12.6118	8.17	16.67

Comparing Haemoglobin between different categories of stunting. Kruskal Wallis H= 1.653; P=0.648
Comparing Haemoglobin between different categories of underweight. Kruskal Wallis H= 4.473; P= 0.215
Comparing Haemoglobin between different categories of wasting. Kruskal Wallis H= 2.540; P= 0.468
Haemoglobin- g/Dl

3.3.4. Influence of malaria parasitaemia on anaemic status in the study population.

On the other hand, looking at density of malaria parasitaemia in varying severity levels of anaemia (Table 33), our findings showed that again, although the difference was not significant ($p>0.05$), geometric mean density of parasitaemia was higher in mildly anaemic children compared to normal (non-anaemic) as well as the moderately and severely anaemic children. This observation is inconsistent with findings by Achidi *et al.* (2012) in three regions and three ethnic groups in Cameroon, who stated that the geometric mean density of parasitaemia was significantly higher in severely anaemic children than their counterparts. It also differs from reports by Teh *et al.* (2021), who showed that parasitaemia negatively correlated with haemoglobin levels ($p=0.04$) in Batoke-Limbe, Mount Cameroon area. Our results also contradict those of Kateera *et al.* (2015) who observed a strong association between malaria parasitaemia and anaemia in Rwandan children. However, in our study, we failed to find any such correlations.

Table 32: Influence of malaria parasitaemia on the severity of anaemia in the study population

Category		**Parasitaemia**		
Hb levels	**N**	**Geometric Mean**	**Minimum**	**Maximum**
Non-anaemic	90	1551	40	129600
Mild anaemia	14	3895	120	24960
Moderate anaemia	10	2751	80	78400
Severe anaemia	3	1750	200	6560

Comparing Parasitaemia between different Haemoglobin levels
Kruskal Wallis H=4.182; P=0.242
Parasitaemia-Parasites/µL of blood

SPECIFIC OBJECTIVE 3

3.4. Effects of malaria, nutritional status and its varying severity levels on anti-malarial IgG1 and IgG3 antibody responses to crude *Plasmodium falciparum* 3D7 antigen amongst the children.

3.4.1. Total IgG, IgG1 and IgG3 responses to malaria and nutritional status

Total IgG and IgG subclasses 1 and 3 responses were elicited in the Pitoa and Mayo-oulo health districts of Cameroon against the 3D7 *Plasmodium falciparum* antigen. Generally, these immune responses were low. As for Total IgG, 155 children, making a proportion of 46.4% (95% CI: 41.1-51.8) were lower producers of anti-malaria antibody response.

Concerning IgG1, 121 children making a proportion of 36.2% (95% CI: 31.2-41.5) were also lower producers whereas for IgG3 the number of lower producers was 66, making a proportion of 19.8% (15.7-24.3) at 95% CI. Overall, there were no high producers seen (**Figure 18.)**

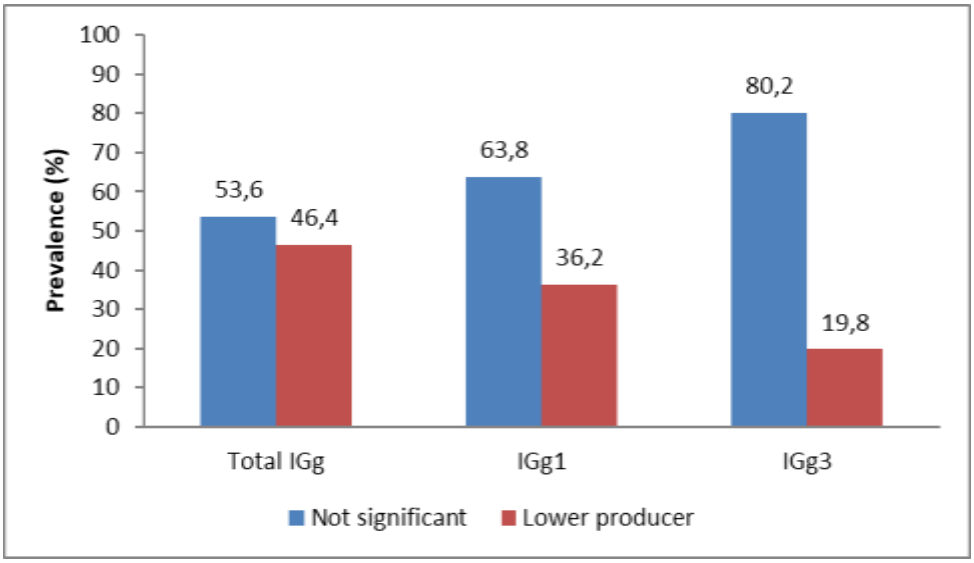


Figure 18: IgG status of children in the study population

3.4.2. Predictors of Immunity

3.4.2.1. Predictors of Total IgG

Based on the ORs, Anaemia surfaced as the highest predictor of Total IgG. Based on the Log-Likelihood Ratio statistics, Anaemia was a significant predictor of Total IgG.

[OR= 1.756 (95% CI:1.0-3.1) ; (p=0.048)] (Table 33). Meaning that anaemic children were 1.76 times more likely to be producers of Total IgG compared to non-anaemic children.

Table 33: Logistic Regression depicting significant predictors of Total IgG

Variable		B	P-value	OR	95% C.I.	
					Lower	Upper
	MalarStat	.332	0.185	1.394	.853	2.279
	Nutrition	.121	0.602	1.128	.717	1.777
	Age	.369	0.112	1.447	.917	2.281
	Anaemia	.563	**0.048**	1.756	1.	3.108
	Constant	-2.453	0.002	.086		

B- significant correlation OR- Odds Ratio C.I. - Confidence Interval

3.4.2.2. Predictors of IgG1

As for IgG1, none of the indicators appeared as significant predictor (for all variables). (Table 34)

Table 34: Logistic Regression depicting significant predictors of IgG1

		B	P	OR	95% C.I.	
					Lower	Upper
	MalarStat	.054	0.833	1.055	.640	1.741
	Nutrition	-.383	0.108	.682	.428	1.088
	Age	-.199	0.403	.819	.513	1.307
	Anemia	.331	0.268	1.392	.775	2.499
	Constant	-.352	0.652	.703		

B- significant correlation OR- Odds Ratio C.I. - Confidence

Age was a significant predictor of IgG3 (P<0.05). (Table 35) [OR=0.56 (95% CI: 0.315 - 0.979); (p=0.042)]. This result means that children of the ≥5 age group had a lower IgG3 response compared to the <5 age group. And age had a negative correlation with IgG3 (r = -0.59).

Table 35: Logistic Regression depicting significant predictors of IgG3

		B	P	OR	95% C.I.	
					Lower	Upper
	MalarStat	.023	0.940	1.023	.562	1.863
	Nutrition	.046	0.870	1.048	.599	1.831
	Age	-.588	**0.042**	.556	.315	.979
	Anaemia	.636	0.107	1.889	.872	4.090
	Constant	-1.752	0.077	.173		

B- significant correlation OR- Odds Ratio C.I. - Confidence Interval

3.4.3. Effect of Malaria, Malnutrition and Anaemia on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

3.4.3.1. Effect of malaria on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

The prevalence of anti-malarial IgG immune responses was lower in malaria negative children compared to malaria positive children for both IgG1 and IgG3 antibodies although the difference was not significant. (Table 36)

The generally low IgG responses observed in our study (Fig. 19) differs from findings by Anong *et al.* (2016) who reported a significantly strong IgG response in parasitized individuals (without fever). Apparently, in this study, only Total IgG seemed to show a significantly weak association (r=0.110; p=0.045) to children with parasiatemia**.** (Fig. 20) Both IgG1 and 3 responses showed no significant associations (r=0.010; p=0.855) and (r=0.024; p=0.658) with parasiatemia respectively.

Considering that, protection against the blood forms of *P. falciparum* seem to rely largely on cytophilic antibodies of the IgG1 and /or IgG3 isotypes that target the surface antigens expressed on the surface of merozoites and /or schizonts (Cavanagh *et al.,* 2001), hence it is important that any molecule that is a marker of protective immunity to malaria should prefentially elicit strong immune responses mainly of IgG1 or IgG3 antibody subclasses (Anong *et al.,* 2016). However, our study showed the contrary. A possible explanation may be due to the fact that other factors may have been implicated in the IgG responses observed.

Table 36: Effect of malaria on anti-malarial IgG response to the crude 3D7 antigen amongst the children

		Total IgG		**IgG1**		**IgG3**		
Malaria status		Not significant	Lower producer	Not significant	Lower producer	Not significant	Lower producer	**Total**
Positive	N	62	41	65	38	82	21	103
	%	60.2%	**39.8%**	63.1%	**36.9%**	79.6%	**20.4%**	100.0%
Negative	N	117	114	148	83	186	45	231
	%	50.6%	**49.4%**	64.1%	**35.9%**	80.5%	**19.5%**	100.0%
Cramer's V		V=0.088; **P=0.106**		V=0.009; **P=0.866**		V=0.011; **P=0.847**		
Total	N	179	155	213	121	268	66	334
	%	53.6%	46.4%	63.8%	36.2%	80.2%	19.8%	100.0%

Comparison between malaria positive and negative for IgG1: Cramer's V=0.009; p=0.866
Comparison between malaria positive and negative for IgG3: Cramer's V=0.011; p=0.847
P is significant at 0.05

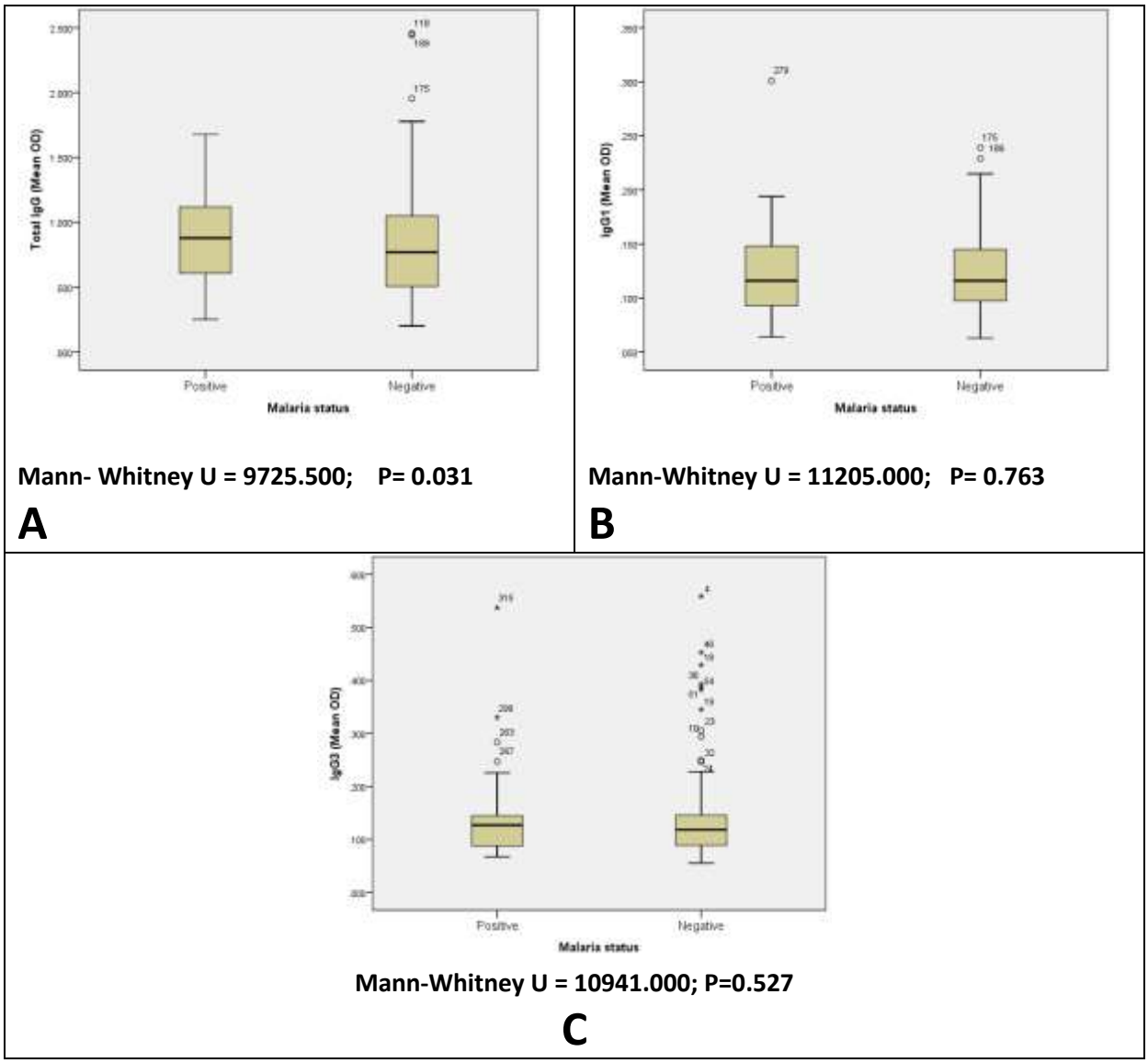


Figure 19: The impact of malaria on IgG antibody responses. IgG responses in groups of chidren with or without malaria in the North Region of Cameroon. Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Malaria positive children (n=119) and malaria negative children (n=143). Mean individual OD's are presented. Statistical significance between groups is indicated (Mann-Whitney U test).

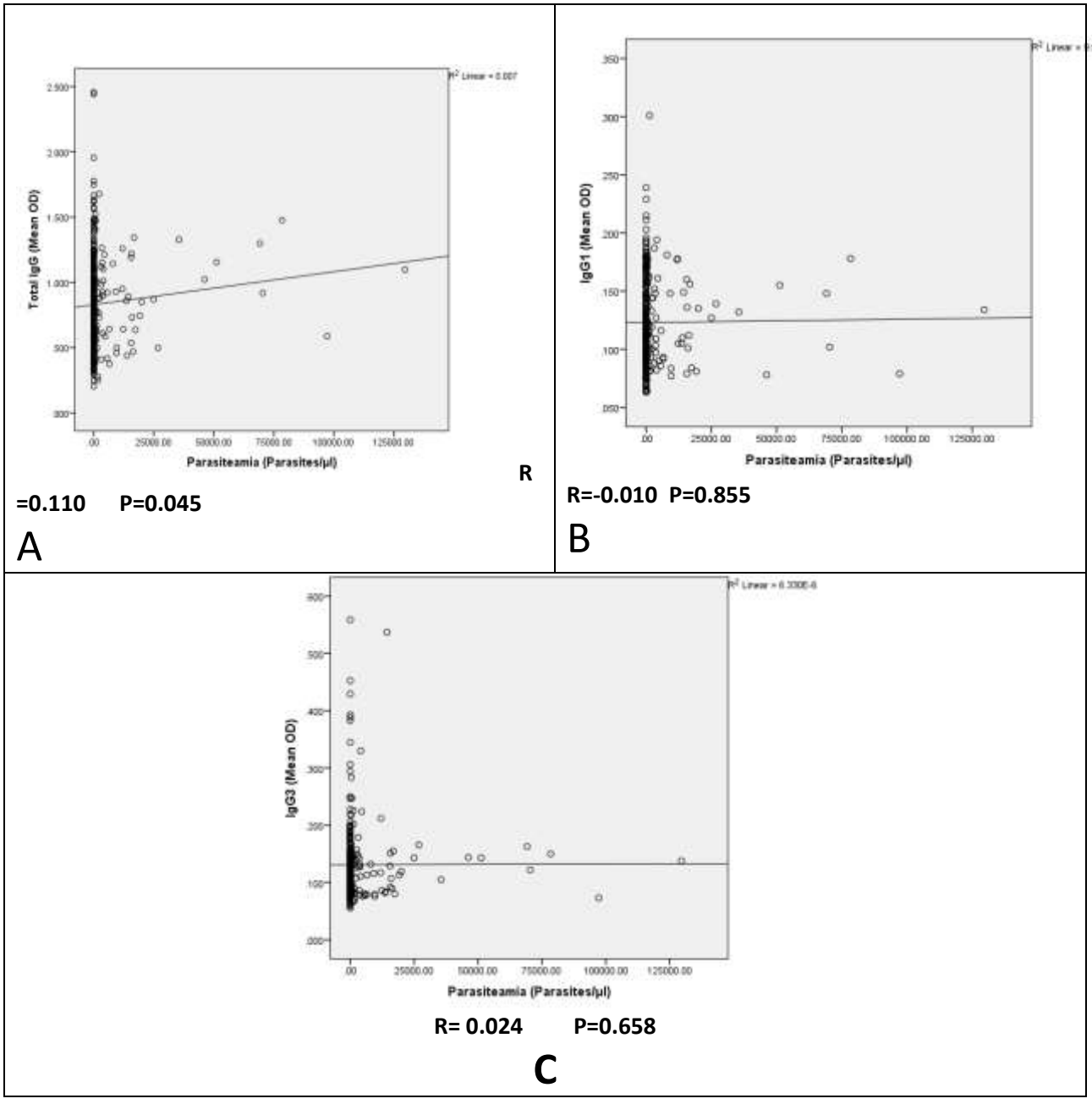


Figure 20: The impact of parasitaemia on IgG antibody responses. IgG responses in groups of children with or without parasitaemia. Bars indicate the median values for each group. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Parasitized children (n=119) and non-parasitized children (n=143). Mean individual OD's are presented. Statistical significance between groups is indicated (Spearman's Rank test).

This result (Fig. 20) differs from the pattern of IgG subclass response reported in subjects in Bolifamba to the UB05 antigen (Anong *et al.,* 2016). Previous studies by Titanji *et al.,* (2002), also showed lowered levels of IgG 1-3 to 3D7 in children residing in this area. Nevertheless, prevalence of Total IgG responses was observed to be significantly higher (p=0.031) in malaria positive children compared to those negative, implying that malaria status may have influenced Total IgG response. The lowered prevalence levels of IgG1 and IgG3 responses to 3D7 as observed in our study may be associated with lowered resistance to malaria, suggesting that the IgG1 and IgG3 subclasses may have had a weakened role in 3D7 mediated immune protection against malaria in children in the Pitoa and Mayo-Oulo health districts, of the North Region of Cameroon.

3.4.3.2. Effect of malnutrition on anti-malarial IgG responses to the crude 3D7 antigen amongst the children

The prevalence of anti-malarial IgG1 and 3 responses against crude *P. falciparum* 3D7 antigen was not significantly different in malnourished children compared to well-nourished children. (Table 37) This result is suggestive that nutritional status may have had no impact on the anti-malarial IgG1 and 3 responses to the 3D7 antigen (Fig. 21).

With regards to malnutrition in this study, generally the IgG responses observed were low. A probable explanation may be that, majority (54.1%) of the children in the study population were malnourished, probably suffering from stunting, underweight and wasting and so may have been unable to mount up appropriate immune responses to the malaria antigen. This confirms earlier findings that nutrition is a critical determinant of immune responses and malnutrition the most common cause of immune-deficiency worldwide (Chandra, 1997). It is clear that maintaining a good nutritional status and adequate micronutrient stores in the body are essential for mounting an effective immune response to opportunistic infections

(Friedman, 2005). Well-nourished individuals are better prepared immunologically to fight microorganisms.

The lowered antibody production observed in these malnourished children may also be explained by the elevated levels of Zn deficiency earlier reported by Ngwa-Akonwi *et al.* (2015), in similar localities within the study area. This may possibly have contributed to the lowered IgG levels, since it has earlier been reported that Zn compromises antibody formation especially the IgGs as well as the development of the B lymphocytes (Shankar, 1998). Suggesting that lack or insufficient dietary of foods rich in Zn, may greatly contribute to lowered antibody production**.**

Furthermore, from observations made by the research team on the field, most of the staple foods grown in the study area (maize, sorghum, millet, rice) are mostly carbohydrates, implying that protein-rich foods are rarely consumed. Considering that antibodies are the body's disease fighting proteins which neutralize foreign invading antigens, it is thus conceivable that the lowered antibody production observed in these malnourished children may be associated with the scarce or rare sources of proteins and aminoacids in the diet. This is in line with earlier findings by Scrimshaw *et al.* (1997) who reported that severe protein deficiency bore a definite relationship to antibody formation and the development of the immune systems in infants and children. This is therefore suggestive that their lack or insufficient dietary intake may have resulted in the lowered antibody production observed in these malnourished children.

Table 37: Effect of malnutrition on anti-malarial IgG response to the crude 3D7 antigen amongst the children

		Total IgG		**IgG1**		**IgG3**		
Nutritional status		Not significant	Lower producer	Not significant	Lower producer	Not significant	Lower producer	**Total**
Malnourished	N	88	70	93	65	127	31	103
	%	55.7%	**44.3%**	58.9%	**41.1%**	80.4%	**19.6%**	100.0%
Normal	N	91	85	120	56	141	35	231
	%	51.7%	**48.3%**	68.2%	**31.8%**	80.1%	**19.9%**	100.0%
Cramer's V		V=0.040; **P=0.465**		V=0.097; **P=0.077**		V=0.003; **P=0.951**		
Total	N	179	155	213	121	268	66	334
	%	53.6%	46.4%	63.8%	36.2%	80.2%	19.8%	100.0%

Comparison between malnourished and well-nourished for IgG1: Cramer's V=0.097; p=0.077
Comparison between malnourished and well-nourished for IgG3: Cramer's V=0.003; p=0.951
P is significant at 0.05

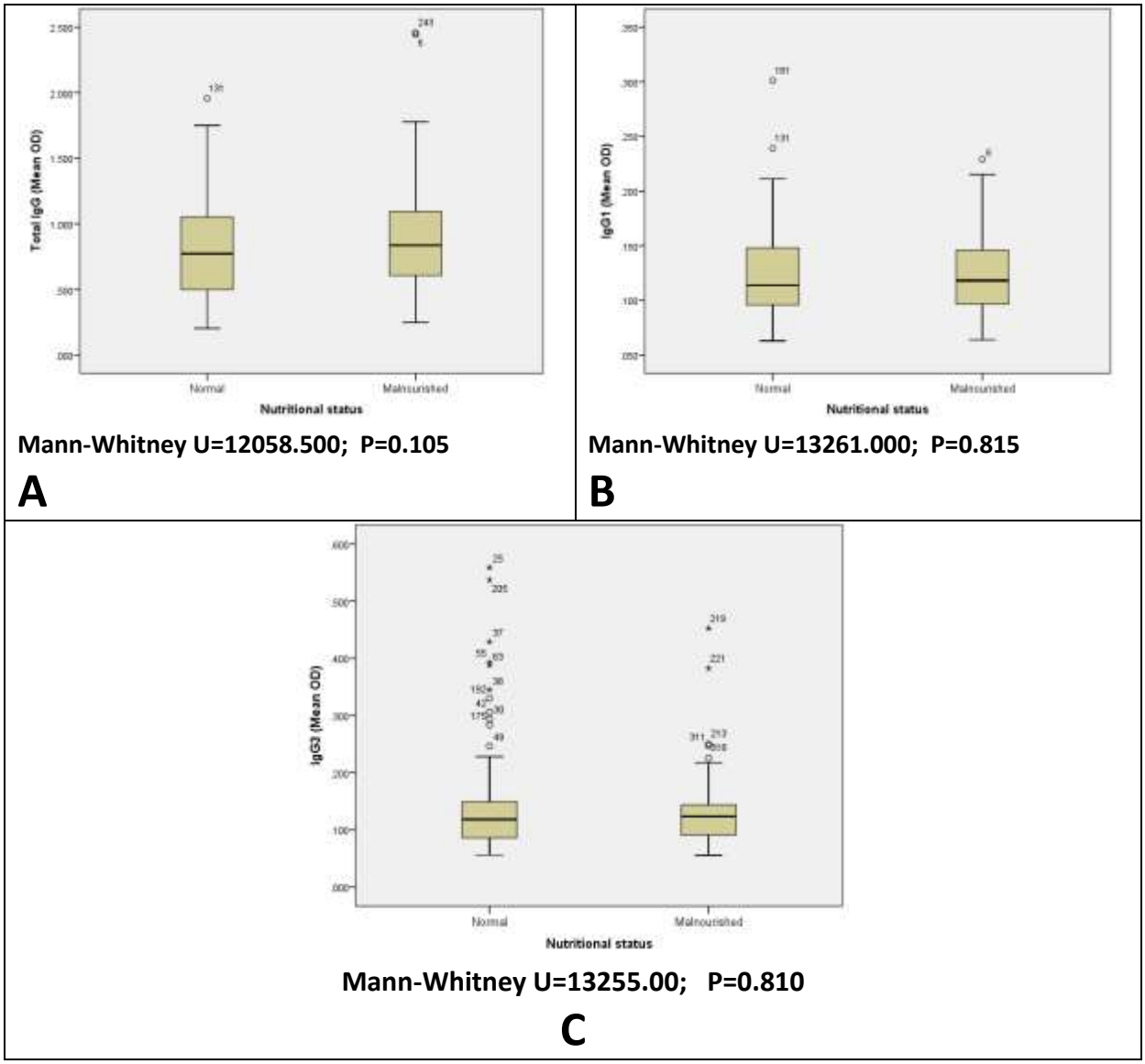


Figure 21: The impact of malnutrition on IgG antibody responses. IgG responses in groups of malnourished or well-nourished children. Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Malnourished children (n=196) and well-nourished children (n=166). Mean individual OD's are presented. Statistical significance between groups is indicated (Mann-Whitney U test).

3.4.3.3. Effect of the different forms of nutritional status on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

3.4.3.3.1. Effects of stunting, underweight and wasting on anti-malarial IgG responses to the crude 3D7 antigen amongst the chikdren.

This study investigated stunting, underweight and wasting forms of malnutrition in relation to their impact on antibody responses. Similar to findings by Fillol *et al.* (2009), the IgG responses were explored according to the type of child malnutrition. In this study, stunting showed no significant ($p>0.05$) associations to Total IgG ($r= -0.073$; $p=0.185$), IgG1($r= -0.075$; $p=0.171$) or IgG3 ($r= -0.13$; $p=0.816$), respectively, although all IgG responses showed a negative trend in association to stunting. The same was observed for wasting, which equally showed no significant ($p>0.05$) associations to the IgG responses. All IgGs showed a positive trend in association to wasting; Total IgG ($r=0.008$; $p=0.889$), IgG1 ($r=0.096$; $p=0.079$), or IgG3 ($r=0.062$; $p=0.260$). However, in the case of underweight, although no significant association was observed either with Total IgG ($r= -0.072$; $p=0.187$) or IgG1 ($r= -0.023$; $p=0.678$), they both showed negative trends in association to underweight. Nevertheless, for IgG3 ($r=0.018$; $p=0.749$), although not significant, showed a positive correlation to underweight. Implying that IgG3 responses increased with increase in underweight. (Figs. 22, 23, 24). This result indicates that underweight may have had a positive effect on IgG3 antibody response to the crude 3D7 *P.falciparum* antigen. Contrarily, earlier studies by Fillol *et al*, (2009) in rural Senegalese pre-school children, stated that malnutrition, especially stunting may down-regulate the anti-*P. falciparum* antibody response, both in terms of prevalence of immune responders and specific IgG antibody levels. In Kenya, Juster *et al.* (2017) equally showed that both wasting and stunting reduce IgG responses in children below 5 years to *P. falciparum* infection and predisposes them to malaria. These conflicting results may be explained by the differences in the definitions and methods used in the evaluation or

determination of malnutrition levels. While our study used the WHO/NCHS 2006 child growth standard curves reference to classify children as stunted, underweight and wasted, Fillol *et al.* (2009) and Juster *et al.* (2017), and Tepa *et al*. (2020) used other classification methods.

With respect to Total IgG, our findings agree with studies by Tepa *et al.* (2020) in Eastern Cameroon, who reported that neither stunting, underweight nor wasting had any significant influence on children's anti-*P. f* IgG Total levels to 3D7. Although their study was limited to Total IgG, ours went further to investigate the effect of nutritional status on the cytophilic subclasses 1 and 3.

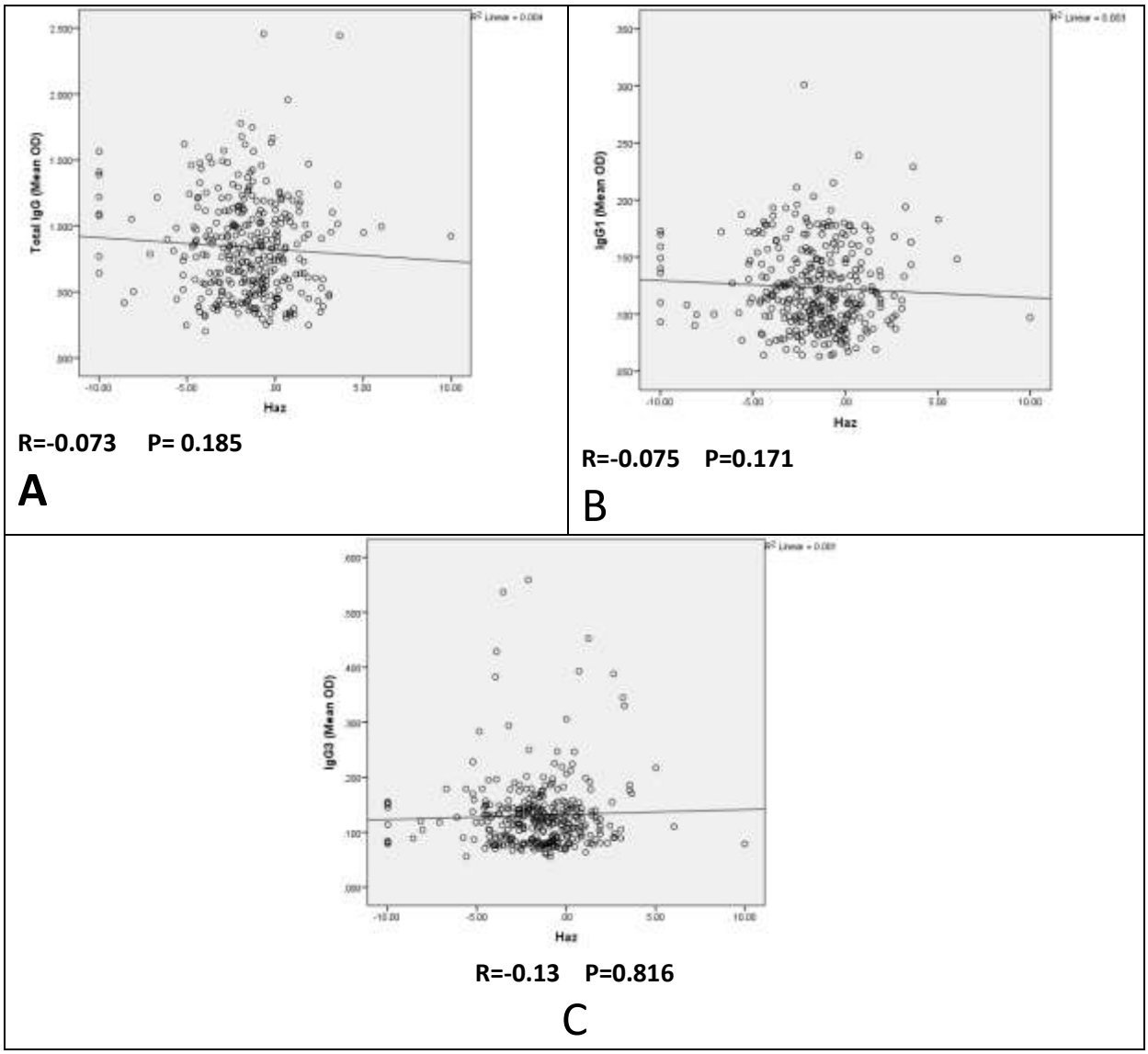


Figure 22: The impact of stunting on IgG antibody responses. IgG responses in stunted chidren. Bars indicate the median values for each group. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Spearman's Rank test).

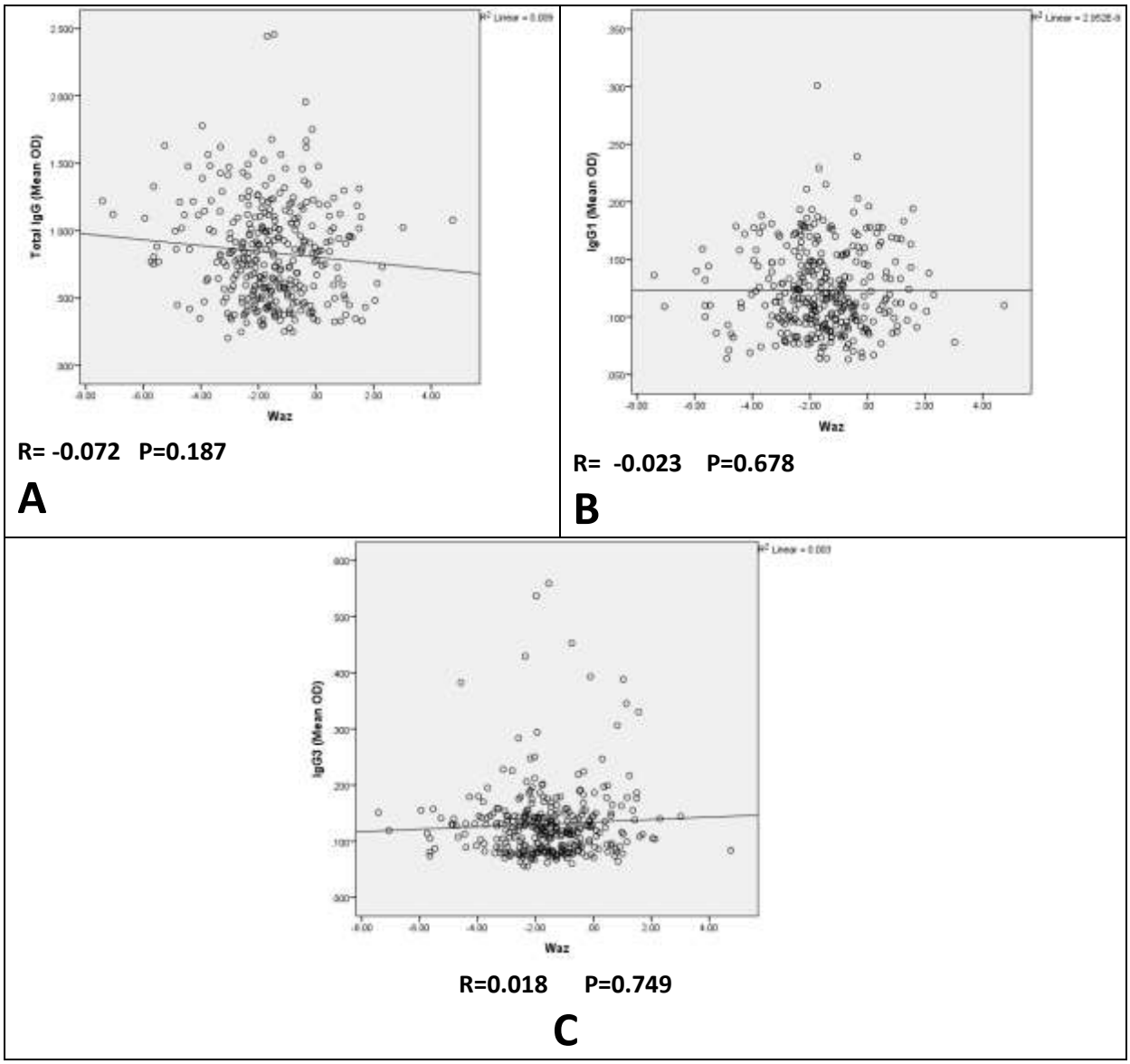


Figure 23: The impact of underweight on IgG antibody responses. IgG responses in underweight chidren. Bars indicate the median values for each group. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Spearman's Rank test).

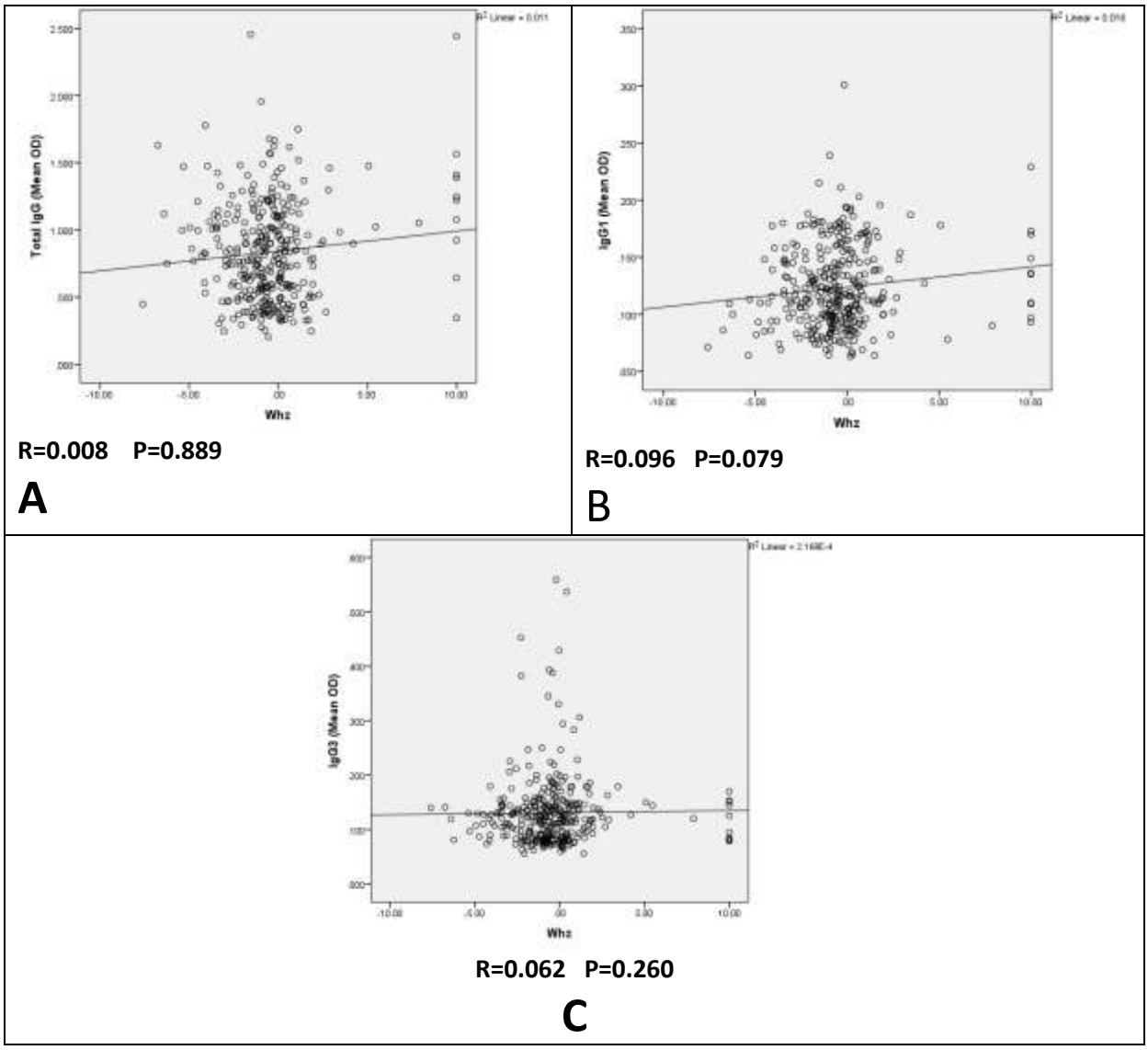


Figure 24: The impact of wasting on IgG antibody responses. IgG responses in wasted chidren. Bars indicate the median values for each group. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Spearman's Rank test).

3.4.3.3.2. Effect of varying severity levels of nutritional status on IgG responses to the crude 3D7 antigen amongst the children.

3.4.3.3.2.1. Effect of varying severity levels of stunting (HAZ) on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

Depending on the value of the Z-score, a child was classified as mildly, moderately or severely malnourished. In this study, -1SD was mildly malnourished; -2SD was moderately malnourished while -3SD was severely malnourished. Studies by Tepa *et al.* (2020) in Eastern Cameroon, were limited to the type of malnutrition only. Our study not only reports the IgG responses in stunted, underweight and wasted children, but further highlights the relationship between the IgG responses and varying severity levels of all these forms of malnutrition, to estimate whether severity levels of stunting, underweight or wasting influenced the anti-malarial IgG responses.

With regards to varying severity levels of stunting in this study, no significant difference was found between Total IgG, IgG1 and IgG3 responses in mildly, moderately and severely stunted children when compared to their normal (well-nourished) counterparts.(Fig. 25) Although the differences may not have been significant for Total IgG **(**H=3.160; P=0.368), IgG1 (H=4.562; P=0.207) and IgG3 (H=3.395; P=0.335) , our result indicates that Total IgG, IgG1 and IgG3 responses were higher in the severely stunted children when compared to their normal counterparts. This result differs from those of Fillol *et al.,* (2009) in pre-school Senegalese children who reported that the prevalence of antibody responses was significantly lower (p=0.026) in severely stunted children when compared to their controls, whereas no significant difference was seen in mildly stunted children compared to their controls as was the case in our study.

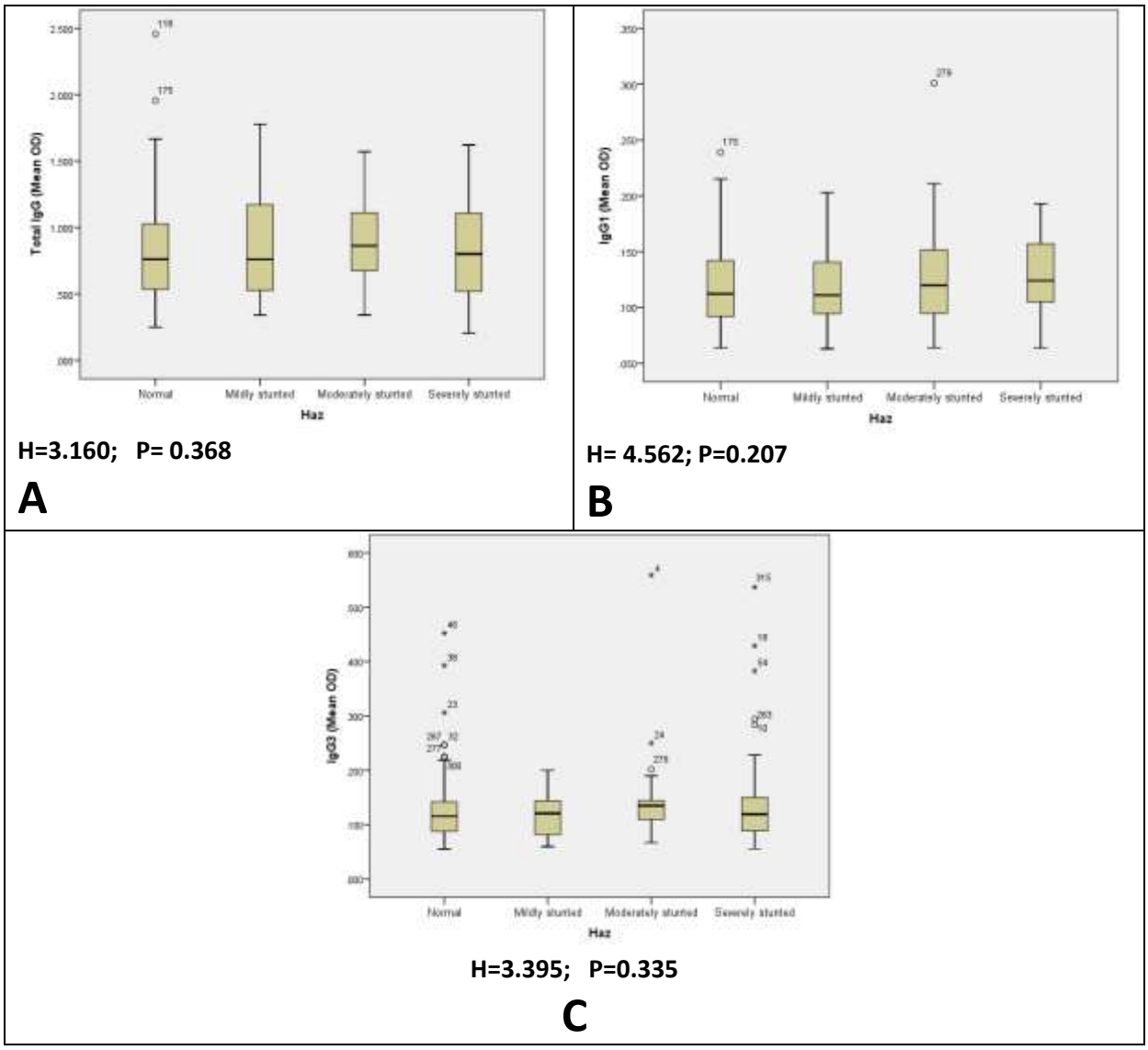


Figure 25: The impact of varying severity levels of stunting on IgG antibody responses.
IgG responses in groups of normal (well-nourished), mildly, moderately and severely stunted children.
Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Krukal Wallis test).

3.4.3.3.2.2. Effect of varying severity levels of underweight (WAZ) on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

For underweight, except for Total IgG which showed a significant difference (p=0.034) between normal, mild, moderate and severe underweight, no significant difference was observed for the subclasses IgG1 and IgG3 responses in mildly, moderately and severely underweight children when compared to normal (well-nourished) children. (Fig. 26)

In varying severity levels of underweight, Total IgG (H=8.675; P=0.034), IgG1 (H=1.636; P=0.651) and IgG3 (H= 2.530; P=0.470), (Fig. 26) only Total IgG seemed to show a significant difference (p=0.034) in responses in severely underweight children compared to the normal (well nourished) children and their counterparts. IgG1 and IgG3 responses showed no significant differences (p>0.05) in varying severity levels of underweight. Our results indicate that, just like the case of stunting, IgG1 and IgG3 responses may not have been influenced by severity levels of underweight. However, Total IgG was influenced.

With respect to Total IgG, those who were severely underweight had significantly higher mean IgG concentrations based on the OD. Implying that, severe underweight significantly elevated Total IgG responses to the crude 3D7 antigen amongst the children when compared to their counterparts.

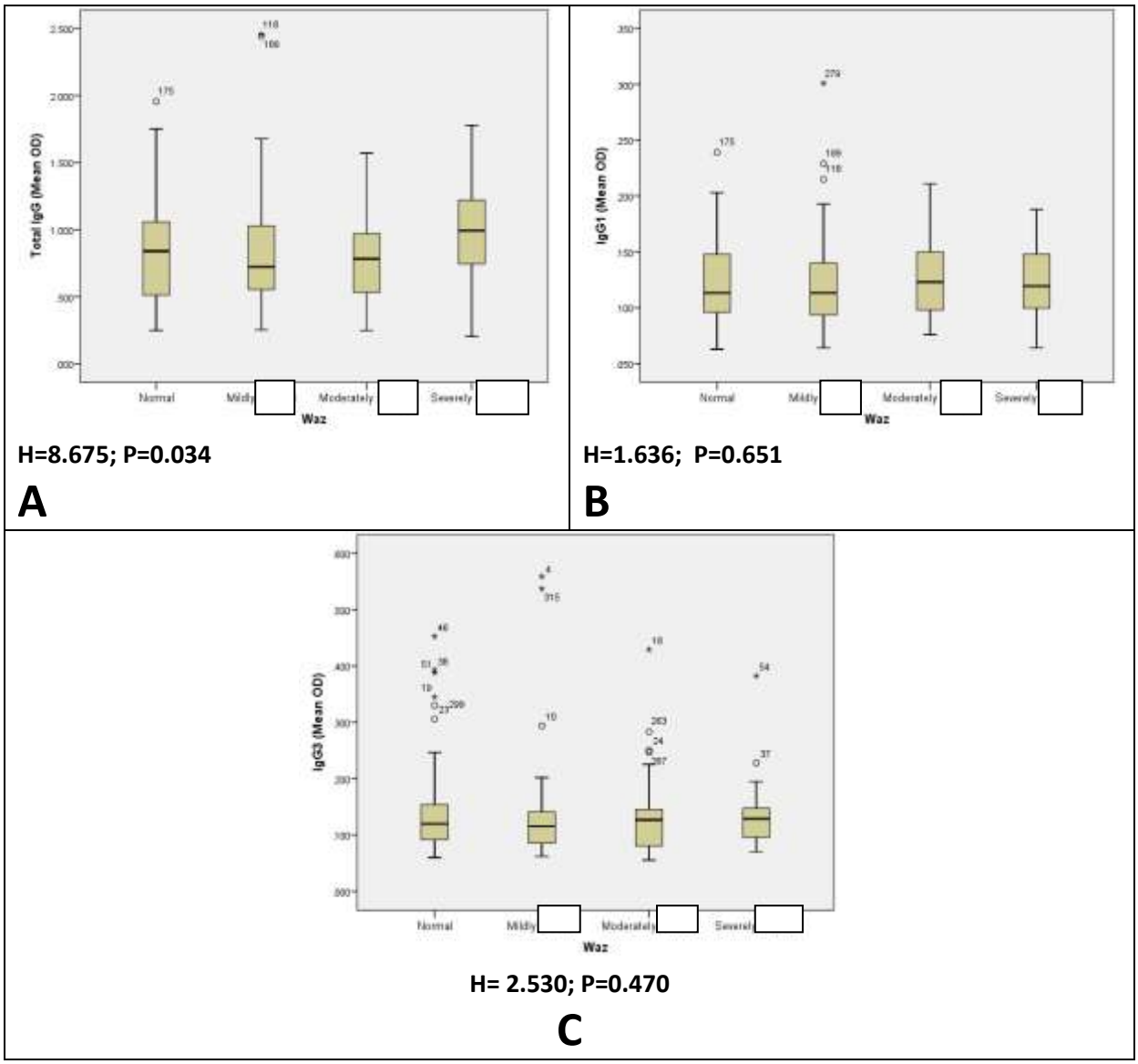


Figure 26: The impact of varying severity levels of underweight on IgG antibody responses. IgG responses in groups of normal (well-nourished children), mildly, moderately and severely underweight children. Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Kruskal Wallis test).

3.4.3.3.2.3. Effect of varying severity levels of wasting (WHZ) on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

For wasting, no significant difference was found between Total IgG, IgG1 and IgG3 responses in mildly, moderately and severely wasted children when compared to normal (well-nourished) children (Fig. 27)

In varying severity levels of wasting, Total IgG (H=5.746; P=0.125), IgG1 (H=1.563; P=0.668) and IgG3 (H=0.014; P=1.000) (Fig. 27). Total IgG, IgG1 and IgG3 responses in mildly, moderately and severely wasted children were not significantly different ($p>0.05$) compared to the normal (well nourished) children. Although the differences may not have been significant, Our results therefore indicate that, IgG responses may not have been influenced by severity levels of wasting.

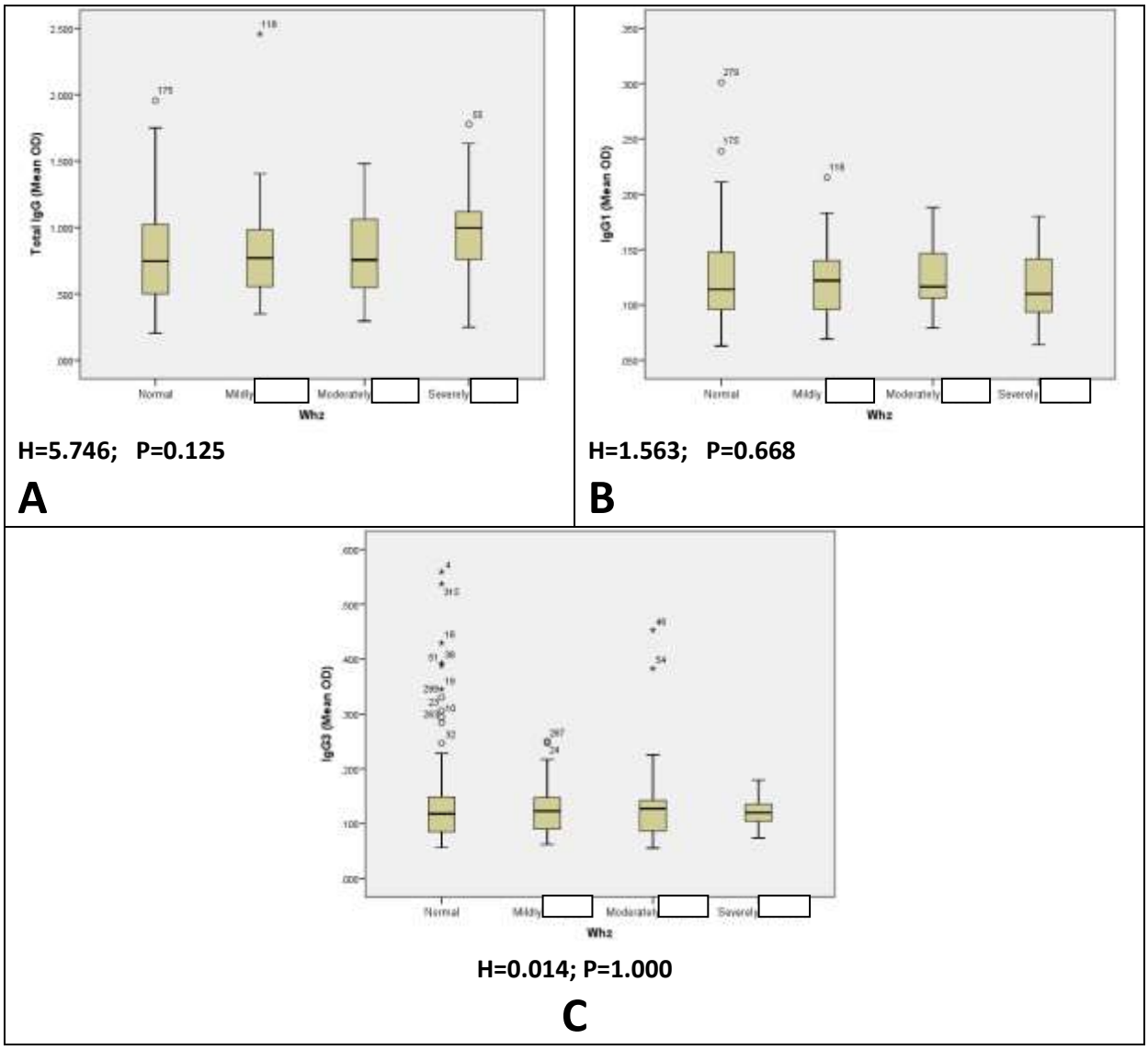


Figure 27: The impact of varying severity levels of wasting on IgG antibody responses. IgG responses in groups of normal (well-nourished children), mildly, moderately and severely wasted children. Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Kruskal Wallis test).

3.4.3.3. Effect of age on the anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

With regards to age, the prevalence of anti-malarial immune responses for both IgG1 and IgG3 were observed to be lower in ≥5 age group of children compared to < 5 group, but the difference was not significant for IgG1**.** Suggesting that IgG1 and IgG3 responses were inversely related to age. The reverse was seen for Total IgG (Table 38). The lowered prevalence observed in the older age group (≥5), reveal a different trend to findings by Titanji *et al.,* (2002) in the Bolifamba village in South-Western Cameroon, who showed that IgG levels to 3D7 *P. falciparum* antigens were lower in young children (1-5years) than adults. Our result also contradicts previous studies by Thorarinsdottir *et al.* (2005) who reported that younger children are more vulnerable to infection due to their poorly developed immune systems. However, it Sarr *et al.* (2007) is consistent with findings by Aka *et al.* (2020) who observed that the level of different antibody responses was not significantly influenced by age. It also confirms findings by in Senegal who also reported that malaria specific antibody responses increased with age in children <5 years old and then stays high until 8. (Fig. 28)

Table 38: Effect of age on anti-malarial IgG antibody response to the crude 3D7 antigen amongst the children

		Total IgG		**IgG1**		**IgG3**		
Age group		Not significant	Lower producer	Not significant	Lower producer	Not significant	Lower producer	**Total**
<5	N	90	62	93	59	114	38	103
	%	59.2%	**40.8%**	61.2%	**38.8%**	75.0%	**25.0%**	100.0%
≥5	N	78	84	105	57	136	26	231
	%	48.1%	**51.9%**	64.8%	**35.2%**	84.0%	**16.0%**	100.0%
Cramer's V		V=0.112; **P=0.049**		V=0.038; P=0.505		V=0.111; **P=0.048**		
Total	N	168	146	198	116	250	64	334
	%	53.5%	46.5%	63.1%	36.9%	79.6%	20.4%	100.0%

Comparison between <5 and ≥5 for IgG1: Cramer's V=0.038; p=0.505
Comparison between <5 and ≥5 for IgG3: Cramer's V=0.111; p=0.048
P is significant at 0.05

Our findings are similar to previous studies by Anong *et al.* (2016) who stated that levels of IgG subclasses 1 and 3 anti- malarial antibody response to UB05 also increased with age.

Prevalence tend to be even lower in older children who are supposed to be better protected agaist infection and disease. This differs from findings by Aucan *et al.* (2000).

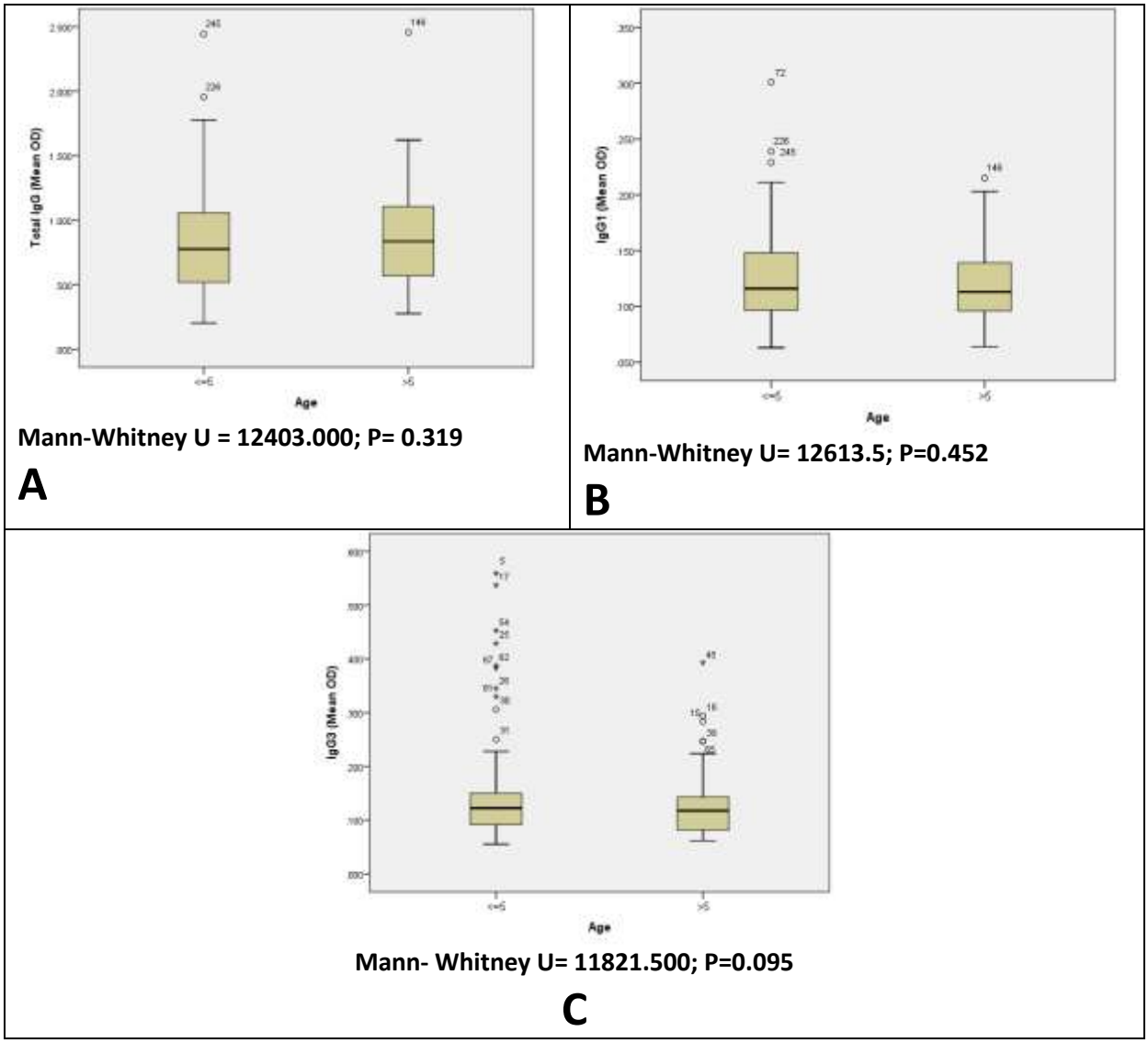


Figure 28: The impact of age on IgG antibody responses. IgG responses in groups of <5 and ≥5 children. Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Mean individual OD's are presented. Statistical significance between groups is indicated (Mann-Whitney U test).

3.4.3.4. Effect of anaemic status on anti-malarial IgG responses to the crude 3D7 antigen amongst the children.

This result show that the prevalence of Total IgG antibody response was significantly lower (p=0.033) in the anaemic children when compared to the non-anaemic and equally lower in anaemic children than non-anaemic children for both IgG 1 (p= 0.339) and IgG 3 (p=0.119), but the differences were not significant. (Table 39) However, based on the box plots, (Fig. 29) IgG1 showed a significantly higher (p=0.024) response in the non-anaemic children when compared to their anaemic counterparts. Implying that anaemia may result to depressed immune function. This confirms earlier findings by Ullah *et al.* (2014) in Pakistan, who reported that in children, the presence of anaemia decreases or weakens immunity. Llanos *et al.*, (2016) also reported that iron deficiency anaemia affects the growth and development of children by decreasing their immune system resistance against infections (Baker, 2010). Also, considering that all the categories of children were anaemic, it is understandable that generally the immune responses observed were low.

Table 39: Effect of anaemic status on anti-malarial IgG antibody responses to the crude 3D7 antigen amongst the children

		Total IgG		**IgG1**		**IgG3**		
Anaemic status		Not significant	Lower producer	Not significant	Lower producer	Not significant	Lower producer	**Total**
Anaemic	N	43	23	45	21	57	9	66
	%	65.2%	**34.8%**	68.2%	**31.8%**	86.4%	**13.6%**	100.0%
Non-anaemic	N	124	122	152	94	191	55	246
	%	50.4%	**49.6%**	61.8%	**38.2%**	77.6%	**22.4%**	100.0%
Cramer's V		V=0.121; **P=0.033**		V=0.054; **P=0.339**		V=0.088; **P=0.119**		
Total	N	167	145	197	115	248	64	312
	%	53.5%	46.5%	63.1%	36.9%	79.5%	20.5%	100.0%

Comparison between anaemic and non-anaemic for IgG1: Cramer's V=0.054; p=0.339
Comparison between anaemic and non-anaemic for IgG3: Cramer's V=0.088; p=0.119
P is significant at 0.05

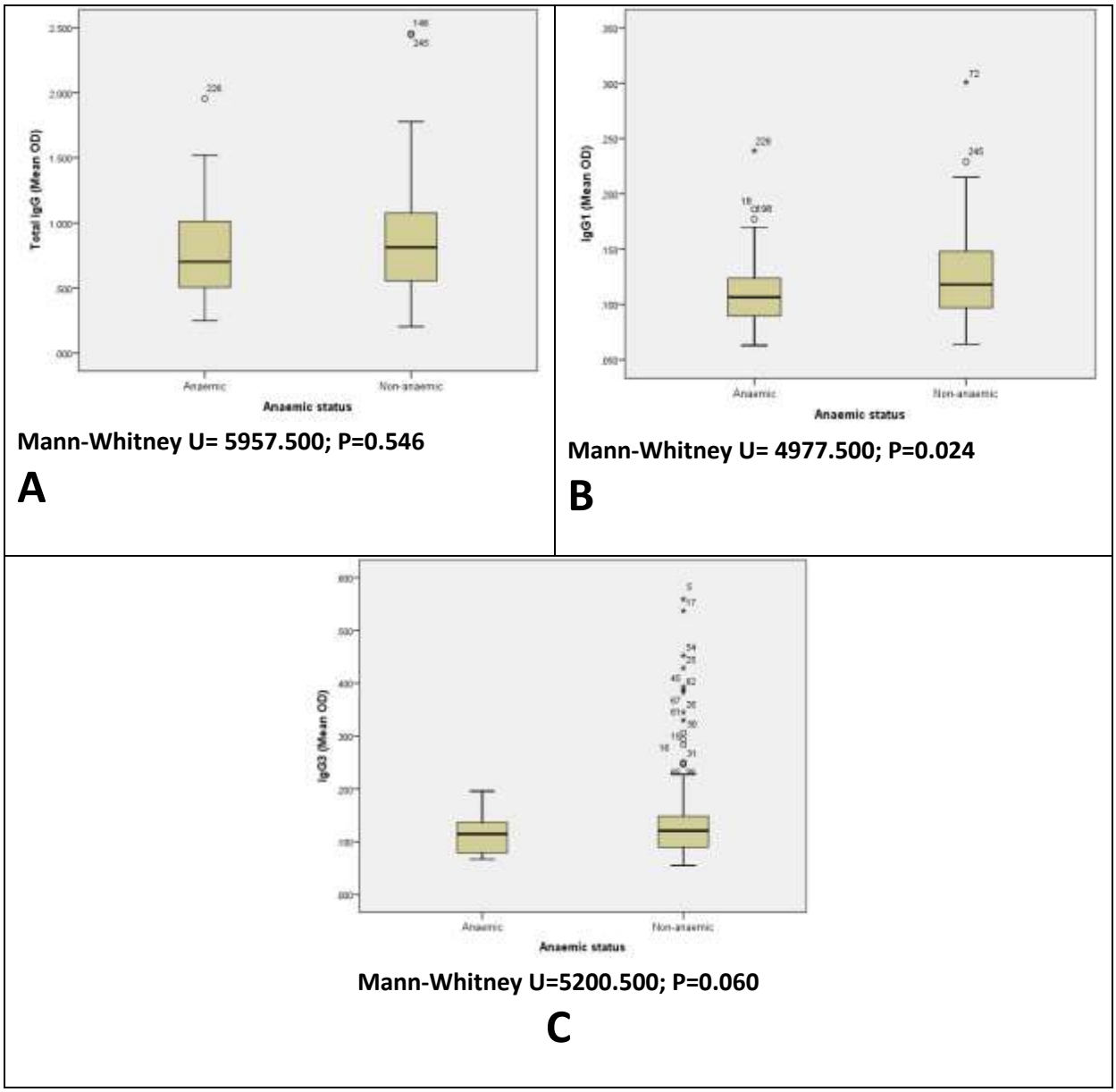


Figure 29: The impact of anaemia on IgG antibody responses. IgG responses in groups of anaemic and normal (non-anaemic children). Box plots represent IgG responses. The dots represent outliers. Total IgG **(A),** IgG1 **(B)** and IgG3 **(C)** responses to 3D7 *Plasmodium falciparum* antigen. Anaemic children (n=74) and non-anaemic children (n=288). Mean individual OD's are presented. Statistical significance between groups is indicated (Mann-Whitney U test).

CHAPTER FOUR: CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

CHAPTER FOUR: CONCLUSIONS, RECOMMENDATIONS AND PERSPECTIVES

4.1. CONCLUSIONS

We have investigated the prevalence of malaria, malnutrition, anaemia and the relationship between malaria, malnutrition, anaemia, IgG responses and socio-economic status using 362 under-ten children and the following conclusions can be drawn;

1. Overall, our study demonstrates that one in every three children was positive for malaria, one in two was malnourished and one in five anaemic with the < 5 age group and the male children being the most affected. Significant predicting risk factors of malaria were nutritional status and village of location, while those of malnutrition were malaria status, level of education of the care-giver and sex of child.

2. This study generally highlights a synergistic relationship between malaria and malnutrition, and reveals that, specifically mild wasting significantly influenced malaria parasitaemia, implying that the presence of mild wasting in the community significantly elevated the prevalence of *Plasmodium* infection.

3. Our results also showed that IgG1 and IgG3 subclasses elicited a weakened role in 3D7 mediated immune protection against malaria, suggesting lowered resistance to malaria. Underweight positively correlated to IgG3 response, while severe underweight significantly increased Total IgG response when compared to the midly, moderately underweight as well as their normal counterparts.

This study addresses the Global Nutrition Targets (GNT's) 1 and 6 of 2025 as well as Sustainable Development Goal (SDG) 3 of 2030. It elucidates the interplay between the various health determinants and generates preliminary baseline information needed by policy

makers for strategic planning and implementation of prompt nutritional interventions as well as improved malaria parasite control measures in the Pitoa and Mayo-oulo health districts of the North Region of Cameroon. Meanwhile, further detailed investigations are needed to complete this study.

4.2. RECOMMENDATIONS

- The Ministry of Public Health, through the National Malaria Control Programme, should ensure effective collaboration between nutrition intervention and malaria control programmes for better/proper case management and reduced socio-economic burden.
- In order to meet up with the Global Nutrition Targets (GNTs 1 and 6) of 2025 as well as Sustainable Development Goal (SDG 3) of 2030, improved nutritional practices are recommended to reduce childhood stunting by 40% and wasting to <5%, as part of the country's national development agenda; while conducting appropriate deworming interventions to ameliorate the anaemic status of the children.
- Children with pale mucosae should also be provided with iron supplementation at low doses as well as Vit A.
- Consumption of protein rich diets should be encouraged to help in the proper production of antibodies.
- Intake of foods rich in or fortified with Zn, Fe and Vit A should be increased to boost immunity.

4.3. PERSPECTIVES

All these results indicated that they deserve to be further investigated for proper case management and improved socio-economic status. So, we propose to;

- Conduct a larger morbidity study to determine the possible effects of malaria, malnutrition and helminthes infection on anaemia.

- ❖ Carry out further research to evaluate the impact of malnutrition on anti-malarial IgG antibody responses in children from different geographical areas in Cameroon.
- ❖ Further studies to determine the effects of micronutrient deficiencies and diets and their influence on malaria parasitaemia and antibody responses.

REFERENCES

REFERENCES

Abanyie AF, Arguin PM, and Gutman J (2011). *State of malaria diagnostic testing at clinical laboratories in the United States: a nationwide survey.* Malaria Journal **10**(1): 340

Achidi EA, Apinjoh TO, Anchang-Kimbi JK, Mugri RN, Ngwai AN, Yafi CN (2012). *Severe and uncomplicated falciparum malaria in children from three regions and three ethnic groups in Cameroon: a prospective study.* Malaria Journal **11:** 215

Adair LS, Fall CHD, Osmond C, Stein AD, Martorell R, Ramirez-Zea M, Sachdev HS, Dahly DL, Bas I, Norris SA, Micklesfield L, Hallal P, Victora CG (2013). *COHORTS Group. Associations of linear growth and relative weight gain during early life with adult health and human capital in countries of low and middle income: findings from five birth cohort studies.* Lancet Journal; **382**: 525–34

Agho KE, Dibley MJ, D'Este C, Gibberd R (2008). *Factors associated with haemoglobin concentration among Timor-Leste children aged 6-59 months.* Journal of Health Population and Nutrition; **26**: 200-9

Aka KG, Traore DF, Sagna AB, Zoh DD, Assi SB, Tchieko BN, Adja AM, Remoue F, Poinsignon A (2020). *Pattern of antibody responses to Plasmodium falciparum antigens in individuals differently exposed to Anopheles bites.* Malaria Journal **19:** 83

Akonwi NF, Ngondi JL, Edoun FL, Dibacto KRE, Oben JE (2015). *Multiple Micronutrient Deficiencies are related to the Nutritional Status of Children Living in North and Far North Regions of Cameroon.* American Journal of Pharmacy & Health Research **3**: 2321–3647

Anong DN, Fru-Cho J, Mbandi KS, Amambua NA, Nkuo-Akenji T, Titanji VPK (2016). Journal of Biological Sciences and Pharmaceutical Research **4**(3): 11-16

Ansong D, Osei-Akoto A, Owusu S, Boakye I, Blay Nguah S, Sambian D, Sylverken J, Arhin B (2013). *Haematological indices, nutritional assessment and mortality outcome of children presenting with severe malaria to a tertiary hospital in Ghana.* Journal of Pediatric Sciences **5**: e189

Antonica C and San Sebastien M (2014). *Anaemia and malnutrition in indigenous children and adolescents of the Peruvian Amazon in a context of lead exposure: a cross-sectional study*. Global Health Action **7(1):** p.22888

Antonio-Nkondjio C, Ndo C, Njiokou R, Bigoga J, Awono-Ambene P, Etang J, Ekobo AS, Wondji CS (2019). *Review of malaria situation in Cameroon: technical viewpoint on challenges and prospects for disease elimination.* Journal of Parasites and Vectors **12:** 501

Antonio-Nkondjio C, Atangana J, Ndo C, Awono-Ambene P, Fondjo E, Fontenille D (2008). *Malaria transmission and rice cultivation in Lagdo, Northern Cameroon.* Transactions of the Royal Society of Tropical Medicine and Hygiene **102:** 52-359

Anumudu C, Afolami M, Igwe C, Nwagwu M, Keshinro O (2008). *Nutritional anaemia and malaria in pre-school and school age children.* Annals of African Medicine 7(1): 11-17

Aribot G, Rogier C, Sarthou JL, Trape JF (1996). *Patterns of immunoglobulin isotype response to Plasmodium falciparum blood stage antigens in individuals living in a holoendemic area of Senegal (Dielmo, West Africa).* The American Journal of Tropical Medicine and Hygiene **54**(5): 449- 57

Asim M, Nawaz Y (2018). *Child Malnutrition in Pakistan: Evidence from Literature.* Journal of Children **5:** 60

Asoba GN, Sumbele IUN, Anchang-Kimbi JK, Metuge S, Teh RN (2019). *Influence of infant feeding practices on the occurrence of malnutrition, malaria and anaemia in children ≤5 years in the Mount Cameroon area: A cross-sectional study.* PLoS ONE **14**(7): e0219386

Asobayire FS, Adou P, Davidsson L, Cook JD, Hurrell RF (2001). *Prevalence of iron deficiency with and with-out concurrent anemia in population groups with high prevalence of malaria and other infections: a study in Côte d'Ivoire.* American Journal of Clinical Nutrition **74**: 776-82

Aucan C, Traore Y, Tall F, Nacro B, Traore- Leroux T, Fumoux F, Rihet P (2000). *High immunoglobulin G2 (IgG2) and low IgG4 levels are associated with human resistance*

to plasmodium falciparum malaria. Journal of infection and immunology **68:** 1252-1258

Awono-Ambene PH, Etang J, Antonio-Nkondjio C, Ndo C, Eyisap WE, Piameu MC,Mandeng ES, Mbakop RL, Toto JC, Patchoke S, Mnzava AP, Knox TB, Donnelly M, Fondjo E, Bigoga JD (2018). *The bionomics of the malaria vector Anopheles rufipes Gough, 1910 and its susceptibility to deltamethrin insecticide in North Cameroon*. Journal of Parasites and Vectors **11:** 253

Ayoya MA, Ngnie-Teta I, Séraphin MN, Mamadoultaibou A, Boldon E, Saint-Fleur JE, Koo L, Bernard S (2013). *Prevalence and risk factors of anemia among children 6–59 Months Old in Haiti*. Anemia Journal, 2013: 502968

Baig- Ansari N, Rahbar MH, Bhutta ZA, Badruddin SH (2006). *Child's gender and house-hold food insecurity are associated with stunting among young Pakistani children residing in urban squatter settlements.* Food and Nutrition Bulletin **27:** 114-127

Baker RD (2010). *Clinical Report Diagnosisand Prevention of Iron Deficiency and Iron Deficiency Anaemia in Infants and Young Children (0-3 years of age).* Pediatrics **126**: 1040-1051

Balarajan Y, Ramakrishnan U, Ozaltin E, Shankar AH, Subramanian SV (2011). *Anaemia in Low-income and Middle-income countries.* Lancet Journal **378:** 2123-2135

Barua P, Chandrasiri UP, Beeson JG, Dewey KG, Maleta K, Ashorn P, Rogerson SJ (2018). *Effect of nutrient supplementation on the acquisition of humoral immunity to Plasmodium falciparum in young Malawian children.* Malaria Journal **17:** 74

Basco L, Ngane V, Ndounga M, Same-Ekobo A, Youmba J, Abodo R, Soula G (2006). *Molecular Epidemiology of malaria in Cameroon. XXI. Baseline therapeutic effects of chloroquine, amodiaquine, and sulfadoxine-pyrimethamine monotherapies in children before national drug policy change.* American Journal of Tropical Medicine and Hygiene **75**: 388-95

Beard JL (2001). *Iron Biology in immune function, Muscle Metabolism, and Neuronal Functioning.* Journal of Nutrition **131**(2): 568-79

Beeson JG, Osier FH, Engwerda CR (2008). *Recent insights into humoral and cellular immune responses against malaria.* Trends in Parasitology **24:** 578-584

Behere S and Bulliyya G (2015). *Magnitude of anaemia and haematological predictors among children under 12 years in Odisha, India.* Anaemia Article ID 1729147

Benoist BD, McLean E, Egll I, and Cogswell M (2008). *Worldwide prevalence of anaemia 1993–2005: WHO global database on anaemia.* In WHO. Geneva, Switzerland: WHO Press; ISBN 978941596657.

Bigoga JD, Nanfack FM, Awono-Ambene PH, Patchoké S, Atangana J, Otia VS, Fondjo E, Moyou RS, Leke RGF (2012). *Seasonal prevalence of malaria vectors and entomological inoculation rates in the rubber cultivated area of Niete, South Region of Cameroon.* Parasite Vectors Journal, **5:** 197

Bigoga JD, Manga L, Titanji VPK, Coetzee M, Leke RGF (2007). *Malaria vectors and transmission dynamics in coastal South-Western Cameroon.* Malaria Journal **6**: 5

Black RE, Victora CG, Walker SP, Bhutta ZA, Christian P, de Onis M, Ezzati M, Grantham- McGregor S, Katz J, Martorell R, Uauy R, Maternal and Child Nutrition Study Group (2013). *Maternal and child undernutrition and overweight in low-income and middle-income countries.* Lancet Journal; **371:** 243–60

Blair TS, Alvarez SG, Villa RA, Carmona FJ, Rios OL (2003). *Nutritional status and immunoglobulin and cytokine concentrations in children with malaria.* An Pediatr (Barc); **58:** 418-424

Bouharoun- Tayoum H, Attanah P, Sabchareon A, Chongsuphajaisiddhi T, Druilhe P (1990). *Antibodies that protect humans against Plasmodium falciparum blood stages do not on their own inhibit parasite growth and invasion in vitro, but act in cooperation with monocytes.*J Exp Med **172** : 1633-1641

Bourke CD, Berkley JA, Prendergast AJ (2016). *Dysfunction as a cause and consequence of malnutrition.* Trends Immunol **37(6)** : 386-398

Breda Gavin- Smith (2020). *The Role of Nutrition in the Immune system. Should we pay more attention? Part I and II.* Global Publishing Health Nutrition, Sight and Life

Brown KH, Peerson JM, Rivera J, Allen LH (2002). *Effect of Supplemental Zinc on the Growth and Serum Zinc Concentrations of Prepubertal Children: A Meta-Analysis of Randomized Controlled Trials.* American Journal of Clinical Nutrition **75(6):** 1062–71

Calder PC (2020). *Nutrition Immunity and Covid-19.* BMJ Nutrition, Prevention & Health

0. doi : 10. 1136/bmjnph -2020-000085

Cameroon Academy of Sciences, *"Anti-malaria drug resistance in Cameroon,"* Work shop report, Cameroon Academy of Sciences, Yaounde, Cameroon, **(2014).**

Cape Verde. Ministério da Saúde. Inquérito Demográfico e de Saúde Reprodutiva (IDSR) 2005. Praia (2008). *Instituto Nacional de Estatística, Ministério da Saúde, República De CaboVerde*,.312p

Carswell F, Hughes AO, Palmer RI, Higginson J, Harland PS, Meakins RH (1981). *Nutritional status, globin titers, and parasitic infections of two populations of Tanzanian school children.* American Journal of Clinical Nutrition **34:** 1292-1299

Caulfield LE, Richard SA, Rivera JA, Musgrove P, Black RE (2006). *Stunting, Wasting and Micronutrient Deficiency Disorders. In: Jamison DT, Breman JG, Measham AR, et al., editors.*Disease Control Priorities in Developing Countries. 2nd edition. Washington (DC): The International Bank for Reconstruction and Development / The World Bank; 28. Oxford University Press, New York

Caulfield LE, Richard SA, Black RE (2004a). *Undernutrition as an underlying cause of malaria morbidity and mortality in children less than five years old.* American Journal of Tropical Medicine and Hygiene **71**(2 Suppl): 55–63

Caulfield LE, de Onis M, Blossner M and Black RE (2004b). *Undernutrition as an underlying cause of child deaths associated with diarrhoea, pneumonia, malaria and measles.* American Journal of Tropical Medicine and Hygiene **80**(1)**:** 193-98

Cavanagh DR, Dobano C, Elhassan IM, Marsh K, Elhassan A, Haviid L, Khalil EA, Theander TG, Arnot DE, McBride JS (2001). *Differential Patterns of human immunoglobulin G subclass responses to distinct regions of a single protein, the merozoite surface protein 1 of Plasmodium falciparum.* Journal of Infection and Immunology **69:** 1207-1211

CDC-Centers for Disease Control and Prevention (2013). Parasites - Malaria - Biology - Mosquitoes - Anopheles Mosquitoes **1**: 10. Assessed June 12, 2017. http://www.cdc.gov/parasites/sth/

CDC-Center for Disease Control and Prevention (1989*).**CDC criteria for anaemia in children and childbearing-aged women.*MMWR Morb. Mortal. Wkly. Rep **38**: 400-404

Chaparro CM and Suchdev PS (2019). *Anaemia epidemiology, pathophysiology, and etiology in low-and-middle-income countries.*Ann N Y Acad Sci **1450**(1): 15-31

Charan J, Goyal JP, Saxena D and Yadav P (2012). *Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis.* Journal of Pharmacology and Pharmacotherapy **3**(4): 300-303, Available at: http://www.jpharmacol.com/article.asp?issn=0976500X;year=2012;volume=3;issue=4;spage=300;epage=303;aulast=Charan (Assessed on 30th March 2020)

Cheesbrough M (2005). *District Laboratory Practice in Tropical Countries. Part I.* Second edition. Cambridge University Press. Low Price Edition.

Cogill B (2003). Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistance Project, Academy for Educational Development, Washington, DC

Cohen S, Danzaki K, Maclver NJ (2017). *Nutritional effects on T-cell immunometabolism.* European Journal of Immunology. **47**(2): 225-235

Committee on Military Nutrition Research (1999). *Military strategies for sustainment of nutrition and immune function in the field. ,* Food and Nutrition Board, Washington, DC: Institute of Medicine

Cotta RMM, **Oliveira FCC, Magalhães KA, Ribeiro AQ, Sant'Ana LF, Priore SE, Franceschini SC (2011).** *Social and Biological determinants of iron deficiency anaemia.* Cad. Saúde Pública, Rio de Janeiro Vol **27** supl. 2: S309-S320

Courtin D, Oesterholt M, Huismans H, Kusi K, Milet J, Badaut C, Gaye O, Roeffen W, Remargue E, Sauerwein R, Garcia A, Luty A (2009). *The Quantity And Quality of*

African Children's IgG Responses to Merozoite Surface Antigenens Reflect protection against Plasmodium falciparum malaria. PLoS ONE **4**(10): e7590

Crompton DWT, Montresor A, Nesheim MC, Savioli L (2003). *Controlling disease due to helminth infections.* World Health Organization, Geneva.

Crutcher JM and Hoffman SL (1996). *In: Baron S, editor. Medical Microbiology. 4th edition. Galvestone (TX):* University of Texas Medical Branch at Galveston: 1996**.** Chapter 83

Cuevas LE and Koyanagi A (2005). *Zinc and infection: a review.* Annual Tropical Paediatrics Journal **25**: 149–60

Cunningham-Rundles S, McNeeley DF, Moon A (2005). *Mechanisms of nutrient modulation of the immune response.* Journal of Allergy and Clinical Immunology **115** (6) : 1119-1128

Custodio E, Descalzo MA, Roche J, Sánchez I, Molina L, Lwanga M, Bernis C, Villamor E, Baylin A (2008). *Nutritional status and its correlates in Equatorial Guinean preschool children: Results from a nationally representative survey.* Food and Nutrition Bulletin 29(1)

Das D, Grais RF, Okiro EA, Stepniewska K, Mansoor R, Van der Kam S, Terlouw DJ, Tarning J, Barnes KI, Guerin PJ (2018). *Complex interactions between malaria and malnutrition : a systematic literature review.* BMC Medicine **16**: 186

De la Cruz-Góngora V, Villalpando S, Rebollar R, Shamah-Levy T, Méndez-Gómez Humarán I **(2012).** *Nutritional causes of anemia in Mexican children under 5 years. Results from the 2006 National Health and Nutrition Survey.* Salud Publica Mex **54**: 108-15

de Onis M, Yip R, Mei Z (1997). *The development of MUAC-for-age reference data recommended by a WHO Expert Committee.* Bull World Health Organ Journal, **75:** 11-18

Deen JL, Walraven GE, von Seidlein L (2002). *Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia.* Journal of Tropical Pediatrics **48:** 78–83

Deribew A, Alemseged F, Tessema F, Sena L, Birhanu Z, Zeynudin A, Sudhakar M, Abdo N, Deribe K, Biadgilign S (2010). *Malaria and Under-Nutrition: A Community Based Study Among Under-Five Children at Risk of Malaria, South-West Ethiopia.* PLoS ONE Journal **5 (5):** e10775

Desai MR, Terlouw DJ, Philips-Howard PA, Kariuki SK, Wannemuehler KA, Odhacha A, Hawley WA, Shi YP, Nahlen BL, Ter Kuile FO (2005). *Factors associated with hemoglobin concentration in pre-school children in Western Kenya: cross-sectional studies.* Am J Trop Med Hyg **72**(1): 47-59

Diamond TH, Levy S, Smith A, Day P (2002). *High bone turnover in Muslim women with vitamin D deficiency.* Medical Journal of Australia, **177**: 139–41

Djeutchouang S. C. (2010). *Intérêt de l'utilisation des tests de diagnostic rapide du paludisme sur le coût et l'efficacité de la prise en charge des patients fébriles à Yaoundé, Cameroun*. Thèse de Doctorat (Pathologie Humaine), Faculté de Médecine de Marseille, France, 162p.

Doolan DL, Dobano C, Baird JK (2009). *Acquired immunity to malaria.* Journal of Clinical Microbiology Review **22**(1): 13-36

Dominguez- Vazquez A, Alzate-Sanchez A (1990). *Nutritional status in children under 6 years of age and its relation to malaria and intestinal parasitism.* Salud Publica Mex **32:** 52-63

Ehrhardt S, Burchard GD, Mantel C, Cramer JP, Kaiser S, Kubo M, Otchwemah RN, Bienzle U, Mockenhaupt FP (2006). *Malaria, anemia, and malnutrition in african children–defining intervention priorities.* Journal of Infectious Diseases **194:** 108–114

Engle-Stone R, Ndjebayi A, Nankap M, Brown HK (2012). *Consumption of potentially fortifiable foods by women and young children varies by ecological zone and socio-economic status in Cameroon.* J Nutr. **142**(3): 555-65

Ewusie J, Ahiadeke C, Beyene J and Hamid JS (2014). *Prevalence of anemia among under-5 children in the Ghanaian population: estimates from the Ghana demographic and health survey.* BMC Public Health, **14**: 626

Eyong EEJ, Kegne-Ouafo AJ, Chounna PW, Datchou-Poutcheu FR, Wanji S (2016). *Altitudinal variation in the parasitological and entomological indices of malaria*

around Mount Cameroon, South West Region of Cameroon. J Parasitol Vector Biol. **8:** 74-85

Fawzi W, Msamanga G (2004). *Micronutrients and adverse pregnancy out-comes in the context of HIV infection.* Nutrition Review **62:** 269–75

Field CJ, Johnson IR, Schley PD (2002). *Nutrients and their role in host resistance to infection.* Journal of Leukocyte Biology **71:** 16-32

Fillol F, Sarr JB, Boulanger D, Cisse B, Sokhna C, Riveau G, Simondon KB, Remoué F (2009a). *Impact of child malnutrition on the specific anti-Plasmodium falciparum antibody response.* Malaria Journal **8:** 116

Fillol F, Cournil A, Boulanger D, Cisse B, Sokhna C, Targett G, Trape JF, Simondon F, Greenwood B, Simondon KB (2009b). *Influence of wasting and stunting at the onset of the rainy season on subsequent malaria morbidity among rural pre-school children in Senegal.* American Journal of Tropical Medicine and Hygiene **80:** 202-208

Friedman JF, Kwena AM, Mirel LB, Kariuki SK, Terlouw DJ, Philips- Howard PA, Hawley WA, Nahlen BL, Ping Shi Y, Kuile FOT (2005). *Malaria and nutritional status among pre-school children: results from cross-sectional surveys in western Kenya.* American Journal of Tropical Medicine and Hygiene, **73:** 698

Fru-Cho J, Bumah V, Safeukui I, Nkuo-Akenji T, Titanji V, Haldar K (2014). *Molecular typing reveals substantial Plasmodium vivax infection in asymptomatic adults in rural area of Cameroon.* Malaria Journal **13:** 170

Garner P and Brabin B (1994). *A review of randomized controlled trials of routine antimalarial drug prophylaxis during pregnancy in endemic malarious areas.* Bull World Health Organ Journal, **72:** 89–99

Gaston RT, Ramroop S, Habyarimana F (2021). *Joint modeling of malaria and anaemia in children less than five years of age in Malawi.*Heliyon **7**(5): eo6899

Gebreweld A, Ali N, Ali R, Fisha T (2019). *Prevalence of anaemia and its associated factors among children under five years of age attending at Guguftu health center, South Wollo, Northeast Ethiopia.* PLoS ONE **14**(7): e0218961

Gelu A, Edris M, Derso T, Abebe Z (2018). *Undernutrition and associated factors among children aged 6-59months living in slum areas of Gondar city, northwest Ethiopia: a cross-sectional study.* Journal of Pediatric Health, Medicine and Therapeutics **9**: 81-88

Genton B, Al-Yaman F, Ginny M, Taraika J, Alpers MP (1998). *Relation of anthropometry to malaria morbidity and immunity in Papua New Guinean children.* American Journal of Clinical Nutrition **68**: 734–41

Ghana Statistical Service (GSS) (2009), Ghana Health Services (GHS), ICF Macro: ***Ghana Demographic and Health Survey 2008.*** Accra: GSS, GHS, ICF Macro; 2009

Gombart AF, Pierre A and Maggini S (2020). *A Review of Micronutrients and the Immune System- Working in Harmony to Reduce the Risk of Infection*. Nutrients, Vol **12**(1), Available at: http://www.ncbi.nih.gov/pubmed/31963293. (Accessed on 29th March 2020).

Grantham-McGregor S, Cheung YB, Cueto S, Glewwe P, Richter L, Strupp B (2007). *International Child Development Steering Group. Developmental potential in the first 5 years for children in developing countries.* Lancet Journal; **369:** 60–70

Grantham-McGregor S, Ani C (2001). *A review of studies on the effect of iron deficiency on cognitive development in children*. Journal of Nutrition **131**: 649S–666S; 666S–668S

Green HK, Sousa-Figueiredo JC, Basáñez M-G, Betson M, Kabatereine NB, Fenwick A, Stothard JR (2011). *Anaemia in Ugandan preschool-aged children: the relative contribution of intestinal parasites and malaria.* Parasitology Journal **138**: 1534-45

Gysin DV, Dao D, Gysin CM, Lytvyn L, Loeb M (2016). *Effect of vitamin D3 supplementation on respiratory tract infections on healthy individuals: A systematic review and meta-analysis of randomized controlled trials.* PLoS one. **11**(9), Available at https://www.ncbi.nih.gov/pmc/articles/PMC5025082/ (Accessed on 1st April 2020).

Hassan MM, Magalhaes RJ, Ahmed S, Pervin S, Tariqujjaman M, Fatima Y, Mamun AA (2021). *Geographical variation and temporal trend in anaemia among children 6-59 months of age in low and middle-income countries during 2000-2018: forecasting the 2030 SDG target.* Public Health Nutr **9**: 1-20

Hassan Sel-D, Okoued SI, Mudathir MA, Malik EM (2010). *Testing the sensitivity and specificity of the fluorescence microscope (cyscope) for malaria diagnosis.* Malaria Journal, **9**: 88

Hassen HY and Ali JH (2016). *The association between chronicundernutrition and malaria amongEthiopian childrenaged 6-59 months:A facility-based case-control study.* Research **10**(1): 63-67

Helen Keller International, WFP (2013). ***What are the current issues in Cameroon?*** Assessed on the 31st October 2016. http//www.wfp.org/countries/Cameroon

Hemilä H, Louhiala P (2013). *Vitamin C for preventing and treating pneumonia.* Cochrane database of systematic reviews **(**Issue 8), Available at: https://www.cochranelibrary-com/cdsr/doi/10.1002/14651858.CD005532.pub3/abstract (Accessed 2nd April 2020)

Hess SY, Lonnerdal B, Hotz C, Rivera JA, Brown KH (2009). *Recent advances in knowledge of zinc nutrition and human health.* Food and Nutrition Bulletin. **30:** S5–11.

Hoddinott J, Alderman H, Behrman JR, Haddad L, Horton S (2013). *The economic rationale for investing in stunting reduction.* Maternal Child Nutrition; **9**(Suppl.2): 69–82

Hoddinott J, Maluccio JA, Behrman JR, Flores R, Martorell R (2008). *Effect of a nutrition intervention during early childhood on economic productivity in Guatemalan adults.* Lancet Journal **371**: 411–16

Holding PA, Kitsao-Wekulo PK (2004). *Describing the burden of malaria on child development: what should We Be measuring and How should We Be measuring It?* American Journal of Tropical Medicine and Hygiene, **71**(Suppl 2): 71–79

Huang Z, Liu Y, Qi G, Brand D, Zheng SG (2018a). *Role of Vitamin A in the immune system.* Journal of Clinical Medicine **7**(9): 258

Huang Z, Jiang F, Li J, Jiang D, Xiao T, Zeng J (2018b). *Prevalence and risk factors of anaemia among children aged 6-23 months in Huaihua, Hunan Province* .BMC Public Health **18:** 1267

Ireri RI, Nyanchoka AM, Mburu M, Ndungu J, Kiarie MS (2021). *Determinants of nutritional status in children aged 6-59 months, in Kiandutu informal settlement,*

Thika, Kenya. Proceedings of the Nutrition Society, **80** (OCEI), E3 Cambridge University Press

Irwin JJ, Kirchner JT (2001). *Anemia in children.* American Family Physician Journal, **64**(8): 1379

Jafarshad A, Dziegiel MH, Lundquist R, Nielsen LK, Singh S, Druilhe P (2007). *A novel antibody-dependent cellular cytotoxicity mechanism involved in defense against malaria requires costimulation of monocytes FcgammaRII and FcgammaRIII.* J Immunol **178**: 3099-3106

Jeremiah ZA, Uko EK, Buseri FI, Jeremiah TA (2007). *Malaria iron deficiency among asymptomatic Nigerian children.*J Nutri Env Med **163**: 232-241

Jourdan PM, Laoussou P, Lybie A, Kjetland EF (2008). *Indicators of anaemia in under-fives with malaria at a hospital in Northern Cameroon.* West African Journal of Medicine.**27:** 7-12

Juster M, Gicheru M, Kavili D, Mburu D (2017). *Association between malnutrition and immunoglobulin G responses to crude asexual P. falciparum schizont lysates in children attending ishiara hospital, Embu County, Kenya.* Journal of Applied Life Sciences International **11(1):** 1-14

Kamugisha ML, Crawford N, SavaelI Z, Msangeni H, Massaga JJ, Mmbando BP, Lemnge MM (2006). *Malaria and nutritional status in children living in the East Usambara Mountains, north-eastern Tanzania.* Tanzania Health Research Bull. **8**: 56–61

Kandala NB, Mandungu TP, Emina JBO, Nzita KPD (2011). *Malnutrition among children under the age of five in the Democratic Republic of Congo (DRC): does geographic location matter?* BMC Public Health; **11:** 261

Kassebaum NJ, Jasrasaria R, Naghavi M, Wulf SK, Johns N, Lozano R, Regan M, Weatherall D, Chou DP, Eisele TP, Flaxman SR, Pullan RL, Brooker SJ, Murray CJ (2014). *A systematic analysis of global anaemia burden from 1990 to 2010.* Blood Journal **123**(5): 615-624

Kateera F, Ingabire CM, Hakizimana E, Kalinda P, Mens PF, Grobusch MP, Mutesa L, van Vugt M (2015). *Malaria, anaemia and under-nutrition: Three frequently co-existing conditions among pre-school children in rural Rwanda.* Malaria Journal **14(1):** 440

Katona P and Katona–Apte J (2008). *The interaction between nutrition and infection.* Journal of Clinical Infectious Diseases **46**(10): 1582-1588

Kebede D, Getaneh F, Endalamaw K, Belay T, Fenta A (2021). *Prevalence of anaemia and its associated factors among under-five age children in Shaman gibe hospital, Southwest Ethiopia.* BMC Pediatr **21**: 542

Khara T, Dolan C (2014). *Technical briefing paper: Associations between wasting and stunting, policy, programming and research implications.* Oxford: Emergency Nutrition Network; 2014 (http://www.ennonline.net/waststuntreview2014, accessed 13 October 2014).

Khattak K and Shah JS, (2010). *Malnutrition and associated risk factors in pre-school children (2-5Years) in district Swabi (NWFP)- Pakistan.* Journal of Medical Science **10:** 34-39

Kimani-Murage EW, Norris SA, Pettifor JM, Tollman SM, Kilpstein K, Gomez-Olive XF, Dunger DB, Kahn K (2011). *Nutritional status and HIV in rural South African children.* BMC Pediatrics **11**: 23

Kimbi HK, Ajeagah HU, Keka FC, Lum E, Nyabeyeu HN, Tonga CF (2012a). *Asymptomatic Malaria in School Children and Evaluation of the Performance Characteristics of the Partec Cyscope® in the Mount Cameroon Region.* Journal of Bacteriology and Parasitology **3:** 153

Kimbi HK, Keka FC, Nyabeyeu HN, Ajeagah HU, Tonga CF, Lum E, Gah HA, Lehman LG (2012b). *An update of asymptomatic falciparum malaria in school children in Muea, Southwest Cameroon.* Journal of Bacteriology and Parasitology, **3**: 154

Kleinschmidt I, Bradley J, Knox TB, Mnzava AP, Kafy HT, Mbogo C (2018). *Implications of insecticide resistance for malaria vector control with long-lasting insecticidal nets: a WHO-coordinated, prospective, international, observational cohort study.* Lancet Journal of Infectious Diseases **18:** 640-9

Koller O (1982). *The Clinical Significance of Haemodilution during Pregnancy.* Journal of Obstetrical and Gynaecological Survey **37**(11): 649-652

Koram KA, Owusu-Agyei S, Utz G, Binka FN, Baird JK, Hoffman SL, Nkrumah FK (2000). *Severe anemia in young children after high and low malaria transmission seasons in the Kassena-Nankana district of northern Ghana.* American Journal of Tropical Medicine and Hygiene **62**(6): 670–674

Korenromp EL, Armstrong-Schellenberg JR, Williams BG, Nahlen BL, Snow RW. (2004). *Impact of malaria control on childhood anaemia in Africa— a quantitative review.* Tropical Medicine and International Health Journal **9**: 1050–65

Kwenti TE, Kukwah AT, Kwenti BDT, Nyassa RB, Dilonga HM, Enow-Orock G, Tendongfor N, Anong DN, Wanji S, Njunda AL, Nkuo- Akenji T (2019). *Comparative analysis of IgG and IgG subclasses against Plasmodium falciparum MSP-1*$_{19}$ *in children from five contrasting bio-ecological zones of Cameroon.* Malaria Journal **18:** 16

Kwenti ET, Kwenti TDB, Njuda LA, Latz A, Nkuo-Akenji T (2017). *Epidemiological and clinical profile of pediatric malaria: a cross-sectional study performed on febrile children in five epidemiological strata of malaria in Cameroon.* BMC Infectious Diseases **17:** 499

Laghari ZA, Soomro AM, Tunio SA, Lashari K, Baloach FG, Baig NM, Bano S (2015). *Malnutrition among children under five years in district Sanghar, Sindh, Pakistan.* Gomal Journal of Medical Sciences **13:** 314-318

Lau YL, Lai MY, Fong MY, Jelip J, Mahmud R (2016). *"Loop-mediated isothermal amplification assay for identification of the five human plasmodium* species *in Malaysia,"* American Journal of Tropical Medicine and Hygiene **94**(2): 336-339

Le Cornet M, Hesran JUL, Fievet N, Personne P, Gounoue R, Beyeme M, Deloron P (1998). *Prevalence of and risk factors for anaemia in young children in southern Cameroon.* American Journal of Tropical Medicine and Hygiene **58**: 606-611

Lee GY and Nim Hang S (2018). *The Role of vitamin E in Immunity.* Nutrients **10**(11): 1614 Available at : https://www.ncbi.nim.nih.gov/pmc/articles/PMC6266234/ (Accessed on 2nd April 2020.

Leilei Pei, Lin Ren, and Hong Yan.(2014). *A survey of undernutrition in children under three years of ages in rural Western China***.** BMC Public Health **14**: 121

Lengeler C. (2004). *Insecticide-treated bed nets and curtains for preventing malaria.* Cochrane database System Reviews (online) 2(2)

Llanos G, Zamudio MJG, Reyes-Garcia JLL (2016). *Significance of anaemia in the different stages of life.*Entermeria Global **43**: 419-430

Lobo V, Patil A, Phatak A, Chandra N (2010). *Free radicals, anti-oxidants and functional foods: Impact on human health.* Pharmacognosy Reviews **4**(8): 118-126

Lozoff B, Smith JB, Clark KM, Perales CG, Rivera F, Castillo M (2010). *Home Intervention Improves Cognitive and Social-Emotional Scores in Iron Deficient Anemic Infants.*Journal of Pediatrics 126 **(4):** e884-e894

Luckner D, Lell B, Greve B, Lehman LG, Schmidt-Ott RJ, Matousek P, Herbich K, Schmid D, Mba R, Kremsner PG (1998). *No influence of socioeconomic factors on severe malarial anaemia, hyperparasitaemia or reinfection.* Transactions of the Royal Society of Tropical Medicine and Hygiene Journal, **92**: 478–81

Magalhaes RJS and Clements ACA(2011). *Mapping the risk of anaemia in pre-school children: The contribution of malnutrition , malaria and helminth infection in West Africa.* PLoS Medicine Journal **8**(6): e1000438

Mahgoub SEO, Nnyepi M, Bandeke T (2006). *Factors affecting prevalence of malnutrition among children under three years of age in Botswana.* African Journal of Food, Agriculture, Nutrition and Development, **6**: 1. Available at http://www.ajfand.net/

Maitland K, Williams TN, Bennett S, Newbold CI, Peto CEA, Viji J, Timothy R, Clegg JB, Weatherall DJ, Bowden DK (1996). *The interaction between Plasmodium falciparum and P. vivax in children on Espiritu Santo island, Vanuatu.*Transactions of the Royal Society of Tropical Medicine **90**(6): 614-620

Maketa V, Mavoko HM, da Luz RI, Zanga J, Lubiba J, Kalonji A, Lutumba P, Van geertkuyden JP (2015). *The relationship between Plasmodium infection, anaemia and nutritional status in asymptomatic children aged under 5years living in stable*

transmission zones in Kinshasa, Democratic Republic of Congo. Malaria Journal **14:** 83

Maleta K, Virtanen SM, Espo M, Kulmala T, Ashorn P (2003). *Seasonality of growth and the relationship between weight and height gain in children under three years of age in rural Malawi.* Acta Paediatrics Journal; **92**(4): 491–7

Manly BF (1992). *The Design and Analysis of Research Studies, UK.* University of Otago, Cambridge University Press, 1992.

Martineau RA, Newton SM, Wilkinson KA, Kampmann B, Hall BM, Nawroly M, Packe GE, Davidson RN, Griffiths CJ, Wilkinson RJ (2007). *Neutrophil-mediated innate immune resistance to mycobacteria.* Journal of Clinical Investigations **117**(7): 1988-94

Martorell R, Horta BL, Adair LS, Stein AD, Richter L, Fall CH, Barghava SK, Biswas DKS, Perez L, Barros FC, Victora CG and Corsortium on Health Oriented Research in Transitional Societies Group (2010). *Weight gain in the first two years of life is an important predictor of schooling outcomes in pooled analyses from five birth cohorts from low- and middle-income countries.* Journal of Nutrition; **140:** 348–54.

Mboya IB, Mamseri R, Leyaro BJ, George J, Msuya SE, Mgongo M (2020). *Prevalence and factors associated with anaemia among children under five years of age in Rombo district, Kilimanjaro region, northern Tanzania.* F1000Research **9**(1102): 1102

McCann JC, Ames BN (2007). *An overview of evidence for a causal relation between iron deficiency during development and deficits in cognitive or behavioural function.* American journal of Clinical Nutrition **85:** 931-45

McDonald CM, Olofin I, Flaxman S, Fawzi WW, Spiegelman D, Caulfield LE, Black RE, Ezzati M, Goodarz D (2013). *Nutrition Impact Model Study. The effect of multiple anthropometric deficits on child mortality: meta-analysis of individual data in 10 prospective studies from developing countries.* American Journal of Clinical Nutrition; **97**(4): 896–901

Mejia Torres RE, Franco Garcia DN, Fontecha Sandoval GA, Hernandez Santana A, Singh P, Mancero Bucheli ST, Saboya M, Paz MY (2014). *Prevalence and Intensity*

of Soil-Transmitted Helminthiasis, Prevalence of Malaria and Nutritional Status of School Going Children in Honduras. PLoS Neglected Tropical Diseases Journal, **8**(10): e3248

Mens PF, Schoone GJ, Kager PA, Schallig DFH (2006). *"Detection and Identification of human Plasmodium* species *with real time quantitative nucleic-acid sequence-based amplification,"* Malaria Journal **5:** 80

Meremikwu M, Donegan S, Sinclair D, Esu E, Oringanje C (2012). Intermittent preventive treatment for malaria in children living in areas with seasonal transmission. Cochrane Database of systemic reviews 2012, Issue2. Article. No.: CD003756

Meydani SN, Han SN, Wu D (2005). *Vitamin E and immune response in the aged: molecular mechanisms and clinical implications.* Immunological Review **205**: 269-284, Available at: https://www.ncbi.nlm.nih.gov/pubmed/15882360 (Accessed on 8th April 2020).

Mfonkeu JB, Gouado I, Kuate HF, Zambou O, Grau G, Combes V, Zollo PH (2008). *Clinical Presentation, haematological indices and management of children with severe and uncomplicated malaria in Douala, Cameroon.* Pakistan Journal of Biological Sciences **11**(20)**:** 2401-2406

Mfuh KO, Achonduh-Atijegbe OA, Bekindaka ON, Esemu LF, Mbakop CD, Ghandi K, Leke RGF, Nerurkar VF (2019). *A comparison of thick-film microscopy, rapid diagnostic test, and polymerase chain reaction for accurate diagnosis of Plasmodium falciparum malaria.* Malaria Journal **18:** 73

Mikkelsen K and Apostolopoulos V (2019). *Vitamin B12, Folic acid and the Immune System. In Mahmoudi M, Rezaei N (eds).* Nutrition and Immunity, Springer, Cham.

Minsante (2018). *Xlième Journée mondiale de lutte contre le paludisme "prêt à vaincre le paludisme" Nous sommes la generation qui peut éliminer le paludisme. Dossier de Presse. Minsante.* 2018: 1-20

Mogensen CB, Soerensen J, Bjorkman A, Montgomery SM (2006). *Algorithm for the diagnosis of anaemia without laboratory facilities among small children in malaria endemic area of rural Tanzania.* Acta Tropica **99**(2-3): 119-125

Moody A (2002). *Rapid diagnostic tests for malaria parasites.* Clinical Microbiology Review **15**(1): 66-78

Moriguchi S and Muraga M (2000). *Vitamin E and Immunity.* Vitamin Horm. Vol **59**pp: 305-336, Available at: https://www.ncbi.nlm.nih.gov/pubmed/10714244 (Accessed on 1st April 2020)

Moyeh MN, Ali IM, Njimoh DL, Nji AM, Netongo PM, Evehe MS, Atogho-Tiedeu B, Ghogomu SM, Mbacham WF (2019). *Comparison of the Accuracy of Four Malaria Diagnostic Methods in a High Transmission setting in Coastal Cameroon.* Journal of Parsitology Research.Article ID 1417967

Mulenga M, Malunga P, Bennett S, Thuma PE, Shulman C, Fielding K, Greenwood BM (2005). *Factors associated with severe anaemia in Zambian children admitted with Plasmodium falciparum malarial anaemia. Annals of Tropical Paediatr*ic Journal; **25:** 87–90

Mu¨ller O, Traore C, Jahn A, Becher H (2003a). *Severe anaemia in west African children: malaria or malnutrition?* Lancet Journal **361**: 86–7

Mu¨ller O, Garenne M, Kouyate´ B, Becher H (2003b). *The association between protein-energy malnutrition, malaria morbidity and all-cause mortality in West African children.* Tropical Medicine of International Health Journal, **8:** 507–11

Murray CK, Gasser RA Jr, Magill AJ, Miller RS (2008). *Update on rapid diagnostic testing for malaria.* Clinical Microbiology Review **21**: 97-110

Nambiema A, Robert A, Yaya I (2019). *Prevalence and risk factors of anaemia in children aged from 6 to 59 months in Togo: analysis from Togo demographic health survey data, 2013-2014.* BMC Public Health **19:** 215

Nankabirwa V, Tylleskar T, Nankkunda J, Engebretsen I, Sommerfelt H, Tumwine J (2011). *Malaria parasitaemia among infants and its association with breast-feeding peer counseling and vitamin A supplementation.* PLoS One 6(70):e21862

Ndiabamoh CM-c, Ekali GL, Esemu L, Llyod YM, Djontu JC, Mbacham W, Bigoga J, Taylor DW, Leke RGF (2020). *The immunoglobulin G antibody response to malaria*

merozoite antigens in asymptomatic children co-infected with malaria and intestinal parasites. PLoS ONE **15**(11): e0242012

Ngassa Mbenda HG and Das A (2014). *Molecular evidence of Plasmodium vivax mono and mixed malaria parasite infections in Duffy-negative native Cameroonians.* PLoS ONE **9**: e103262

Ngnie-Teta I, Receveur O, Kuate-Defo B (2007). *Risk factors for moderate to severe anaemia among children in Benin, and Mali: Insights from a multilevel analysis.* Food and Nutrition Bulletin **28** (1): 76-89

Njunda AL, Ngouadjeu DT, Nsagha D, Nyanjoh EM, Kwenti T, Assob NJ (2016). *Haematological profile of children with malaria in Kumba Health District, South West Region Cameroon.* Afr J Integrated Health. **6:** 23-29

Nkuo-Akenji TN, Sumbele I, Mankah EN, Njunda AL, Samje M, Kamga L (2008). *The burden of malaria and malnutrition among children less than 14 years of age in a rural village of Cameroon.* African Journal of Food, Agriculture, Nutrition and Development JFAND; **8:** 252–64

Nord M, Andrews M, Carlson S (2006). *Household food security in the United States, 2005 [report ERR-29]. Washington, DC: US Department of Agriculture*

Nussenblatt V and Semba RD (2002). *Micronutrient malnutrition and the pathogenesis of malarial anaemia.* Acta Tropica Journal, **82**: 321- 337

Nyaaba DA, Brown PK, Agbenorevi JK (2017). *Assessing the Association between Nutritional Status and Asymptomatic malaria parasitaemia of children under-five years in Ghana.* Pyrex J nutrition Metabolism. 2017;**2:**1-14

Nyakeriga A, Troye-Blomberg M, Dorfman JR, Alexander ND, Ansong D, Osei-Akoto A, Owusu SK, Boakye I, Nguah SB, Sambian DJ, Sylverken J, Arhin B (2013). *Haematological indices, nutritional assessment and mortality outcome of children presenting with severe malaria to a tertiary hospital in Ghana*. Journal of Pediatric Sciences; **5:** e189

Nyakeriga AM, Troye-Blomberg M, Chemtai AK, Marsh K, Williams TN (2004a). *Malaria and nutritional status in children living on the coast of Kenya.* American Journal of Clinical Nutrition, **80:** 1604-1610

Nyakeriga AM, Troye-Blomberg M, Dorfman JR, Alexander ND, Ba¨ck R, Kortok M, Chemtai AK, Marsh K and Williams TN (2004b). *Iron deficiency and malaria among children living on the coast of Kenya.* Journal of Infectious Diseases, 190**(3).** pp.439-447

Nyasa RB, Zofou D, Kimbi HK, Kum MK, Ngu RC, Titanji VPK (2015). *The current status of malaria epidemiologyin Bolifamba, a typical Cameroonian rain forest zone: an assessment of intervention strategies and seasonal variations.* BMC Public Health **15**: 1105

Nzefa LD, Monebenimp F, Äng Christofer (2019). *Undernutrition among children under five in the Bandja village of Cameroon, Africa.*South African Journal of Clinical Nutrition **32:** 2

O'Brien KS, Amza A, Kadri B, Nassirou B, Cotter SY, Stoller NE, West SK, Bailey RL, Porco TC, Gaynor BD, Lietman TM, Oldenburg CE (2018). *Anthropometry and malaria among children in Niger: a cross-sectional study.* The American Journal of Tropical Medicine and Hygiene **99(3):** 665-669

Ojurongbe O, Adegbosin OO, Taiwo SS, Alli OAT, Olowe OA, Ojurongbe TA, Bolaji OS, Adeyeba OA (2013). *Assessment of Clinical Diagnosis, Microscopy, Rapid Diagnostic Tests, and Polymerase Chain Reaction in the Diagnosis of Plasmodium falciparum in Nigeria.*Malaria Research and Treatment Article ID 308069

O'Meara WP, Mangeni JN, Steketee R, Greenwood B (2010). *Changes in burden of malaria in sub-Saharan Africa.* Lancet Journal of Infectious Diseases **10**(8): 545-55

OMS (2012). *Directive: utilization d'association de micronutriments en poudre pour L'enrichessement à domicile de l'alimentation des nourrissons et des enfants de 6 à 23 mois.*

Oppenheimer S (2001). *Iron and its relation to immunity and infectious disease.* Journal of Nutrition **131:** 616S–35S.

Osazuwa F and Ayo OM (2010). *Contribution of malnutrition and malaria to anaemia in children in rural communities of Edo State, Nigeria*. North American Journal of Medical Sciences **2**(11): 532-536

Osei AK, Hamer DH (2008). *Management of Pediatric Malaria: Role of Nutritional Interventions.* Annales Nestlé **66:** 31-47

Özaltin E, Hill K, Subramanian SV (2010). *Association of maternal stature with offspring mortality, underweight, and stunting in low- to middle-income countries.* Journal of American Medical Association; **303**(15): 1507–16

Page AL, de Rekeneire N, Sayadi S, Aberrane S, Janssens AC, Rieux C, Djibo A, Manuguerra JC, Ducou-le-Pointe H, Grais RF, Schaefer M, Guerin PJ, Baron E (2013). *Infections in children admitted with complicated severe acute malnutrition in Niger.* PLoS One **8:** e68699

Patel SR, Malhotra A, GaoX, Hu FB, Neuman MI, Fawzi WW (2012). *A prospective study of sleep duration and pneumonia risk in women.* Sleep 1;**35**(1): 97-101, Available at: https://www.ncbi.nlm.nih.gov/pubmed/22215923 (Accessed on 8th April 2020)

Patterson AJ, Brown WJ, Roberts DC (2001). *Dietary and supplement treatment of iron deficiency results in improvements in general health and fatigue in Australian women of childbearing age.* Journal of American College of Nutrition **20:** 337–42

Pelletier D, Haider R, Hajeebhoy N, Mangasaryan N, Mwadime R, Sarka S (2013). *The principles and practices of nutrition advocacy: evidence, experience and the way forward for stunting reduction.* Maternal Child Nutrition Journal; **9** (Suppl. 2): 83–100.

Plebanski M, Locke E, Kazura JW, Coppel RL (2008). *Malaria vaccines: into a mirror, darkly?* Trends in parasitology **24:** 578-584

Plowe CV, Djimde A, Bouare M, Doumbo O, Wellems TE (1995). *Pyrimethamine and proguanil resistance-conferring mutations in Plasmodium falciparum dihydrofolate reductase : polymerase chain reaction methods for surveillance in Africa.* Am J Trop Med Hyg. 1995 Jun;**52**(6): 565-8

PNLP (2012). *Plan Strategique Nationale de lute contre le paludisme 2011-2015.* Rapport Minsante Cameroun. p.1-82

Programme National de Lutte contre le Paludisme-Cameroun (2018). *Plan stratégique national de lutte contre le paludisme – Cameroun : 2014- 2018.* Yaounde : Rapport Ministère Santé Publique ; 2018

Programme National de Lutte contre le Paludisme-Cameroun (2015). *Plan stratégique du Cameroun 2015,* Yaounde : Rapport Ministère Santé Publique Cameroun ; 2015

Programme National de Lutte contre le Paludisme-Cameroun (2015). *Annual report of activities for malaria control 2014.* Yaounde : Ministère de la Santé Publique ; 2015

Programme National de Lutte contre le Paludisme-Cameroun (2011). *Plan stratégique National de lutte contre le Paludisme au Cameroun, 2011.* Yaounde : Rapport Ministère Santé Publique Cameroun ; 2011

Rall LC and Meydani SN (1993). *Vitamin B6 and immune competence.* Nutrition Review Vol **51**(8) : 217-225, Available at : https://www.ncbi.nlm.nih.gov/pubmed/8302491 (Accessed on 2nd April 2020).

Resolution WHA65.6. Comprehensive implementation plan on maternal, infant and young child nutrition. *In: Sixty-fifth World Health Assembly Geneva, 21–26 May **2012**. Resolutions and decisions, annexes. Geneva: World Health Organization;* 2012:12–13 (http://www.who.int/nutrition/topics/WHA65.6_resolution_en.pdf?ua=1, accessed 6 October 2014).

Rice A L, West Jr KP, and Black RE (2004). *"Vitamin A Deficiency." In Comparative Quantification of Health Risks: Global and Regional Burden of Disease Attributes to Selected Major Risk Factors, Geneva: World Health Organization, **1**: 211-56*

Roberts DJ, Matthews G, Snow RW, Zewotir T, Sartorius B (2020). *Investigating the spatial variation and risk factors of childhood anaemia in four sub-Saharan African countries.* BMC Public Health **20**(1): 126

Rosa M.L. Semedo, Marta M.A.S. Santos, Mirian R. Baião Ronir R. Luiz, Gloria V. da Veiga (2014). *Prevalence of Anaemia and Associated Factors among Children below*

Five Years of Age in Cape Verde, West Africa. Journal of Health and Population Nutrition **32(**4): 646-657

Russo G, Faggioni G, Paganotti GM, Djeunang Dongho GB, Pomponi A, De Santis R, Tebano G, Mbida M, Sobze MS, Vullo V, Rezza G, Lista FR (2017). *Molecular evidence of Plasmodium vivax infection in Duffy negative symptomatic individuals from Dschang, West Cameroon.* Malaria Journal **16:**74

Rytter MJH, Kolte L, Biend A, Friis H, Christensen V B (2014). *The immune system in children with malnutrition-A systematic review.* PloS One **9(8):** el05017.

Sabchareon A, Burnouf T, Ouattara D, Attanath P, Bouharoun-Tayoun H, Chantavanich P, Foucault C, Chong suphajaisiddhi T, Druilhe P (1991). *Parasitologic and clinical human response to immunoglobulin administration in falciparum malaria.* Am J Trop med Hyg **45**: 297-308

Sama SO, Chiamo SN, Tiawe GS, Njume GE, Sumbele IUN (2021). *Microcytic and Malarial Anaemia Prevalence in Urban Children ≤15 Years in the Mount Cameroon Area: A Cross-Sectional Study on Risk Factors.*Anemia . Article ID 5712309

Sandeu MM, Bayibéki AN, Tchioffo MT, Abate L, Gimonneau G, Awono-Ambéné PH, Nsango SE, Diallo D, Berry A, Texier G, Morlais I (2017). *Do the venous blood samples replicate malaria parasite densities found in capillary blood ? A field study performed in naturally-infected assymptomatic children in Cameroon.* Malaria Journal **16 :** 345

Sarr JB, Remoue F, Samb B, Dia I, Guindo S, Sow C, Maiga S, Tine S, Thiam C, Schact AM, Simondon F, Konate L, Riveau G (2007). *Evaluation of antibody response to Plasmodium falciparum in children according to exposure of Anopheles gambiae s.l or Anopheles funestus vectors.* **6:** 117

Sazawal S, Black RE, Ramsan M, Chwaya HM, Stoltzfus JR, Dutta A, Dhingra U, Kabole I, Deb S, Othman MK, Kabole FM (2006). *Effects of routine prophylactic supplementation with iron and folic acid on admission to hospital and mortality in preschool children in a high malaria transmission setting: community-based, randomized, placebo-controlled trial.* Lancet Journal **367**: 133–143

Schaible UE and Kaufmann SH (2007). *Malnutrition and Infection: complex mechanisms and global impacts.* PLoS Med Journal **4:** e115

Schreir SL (2018). *Approach to the Adult Patient with Anaemia Mentzer WC, Ed. Waltham, MA: UpTo Date Inc.*

Scrimshaw NS and SanGiovanni JP (1997). *Synergism of nutrition, infection, and immunity: an overview.* American Journal of Clinical Nutrition **66**: 464S–77S.

Scrimshaw N, Taylor C, Gordon J (1968). *Interactions of nutrition and infection. Monograph series no. 37. Geneva, Switzerland:* World Health Organization, **57:** 3-329

Sema M, Alemu A, Bayih AG, Getie S, Getnet G, Guelig D, Burton R, LaBarre P, Pillai DR (2015). *Evaluation of non-instrumented nucleic acid amplification by loop mediated isoyhermal amplification (NINA-LAMP) for the diagnosis of malaria in Northwest Ethiopia,* Malaria Journal **14**(1): 44

Semedo RML, Santos MMAS, Baiao MR, Luiz RR, da Veiga GV (2014). *Prevalence of anaemia and associated factors among children below five years of age in Cape Verde, West Africa.* Journal of Health Population and Nutrition **32**(4): 646-657

Shah SM, Selwyn BJ, Luby S, Merchant A, Bano R (2003). *Prevalence and correlates of stunting among children in rural Pakistan.* International Journal of Paediatrics **45:** 49-53

Shankar A.H (2000). *Nutritional modulation of malaria morbidity and mortality.* Journal of Infectious Diseases **182:** S37-S53

Shankar A.H and Prasad A.S (1998). *Zn and immune function: the biological basis of altered resistance to infection.* American Journal of Clinical Nutrition **68:** 447S-63S

Shi YP, Udhayakumar V, Oloo AJ, Nahlen BL, Lal AA (1999). *Differential effect and interaction of monocytes, hyperimmune sera, and immunoglobulin G on the growth of asexual stage Plasmodium falciparum parasites.* Am J Trop Med Hyg **60**: 135-141

Simard F, Ayala D, Kamdem G, Pombi M, Etouna J, Ose K (2009). *Ecological niche partitioning* between *Anopheles gambiae molecular forms in Cameroon: the ecological side of speciation.* BMC Ecology **9**: 17

Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA (1999). *A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies.* American Journal of Tropical Medicine and Hygiene **60:** 687–692

Singh B, Cox-Singh J, Mille AO, Abdullah MS, Snounou G, Rahman HA (1996). *Detection of malaria in Malaysia by nested polymerase chain reaction amplification of dried blood spots on filter paper.* Trans R Soc Trop Med Hygiene **90**(5): 519-21

SMART Nutritional Survey (2021). *Preliminary result-SMART-SENS Nutritional Survey 2021-SENS 2021. Source: Action against hunger*

Snarill ZM, Bond UT, Johnson NE (2000). *Nutrition and educational achievement of urban primary school children in Malaysia.* Asian Pacific Journal of Clinical Nutrition; **9:** 264–273

Snirivasan S, Moody AH, Chiodini PL (2000). *Comparison of blood-film microscopy, the OptiMAL^{R} dipstick, Rhodamine-123 fluorescense staining and PCR, for monitoring anti-malarial treatment,* Annals of Tropical Medicine and Parasitology **94**(3): 227-232

Snounou G, Viriyakosol S, Jarra W, Thaitong S, Brown KN (1993a). *Identification of the four human malaria parasite species in field samples by polymerase chain reaction and detection of a high prevalence of mixed infections.* Journal of Molecular Biochemistry and Parasitology **58:** 283-92

Snounou G, Pinheiro L, Gonçalves A, Fonseca L, Dias F, Brown NK (1993b). *The importance of sensitive detection of malaria parasite in the human and insect hosts in epidemiological studies, as shown by the analysis of field samples from guinea Bissau.* Transactions of the Royal Society of Tropical medicine and Hygiene. **87:** 649-653

Snounou G, Viriyakosol S, Zhu XP, Jarra W, Pinheiro L, do Rosario VE, Thaithong S, Brown KN (1993c). *High sensitivity of detection of human malaria parasites by the use of nested polymerase chain reaction.* Journal of Molecular Biochemistry and Parasitology **61:** 315-20

Song H, Fall K, Fang F, Erlendsdóttir H, Lu D, Mataix- Cols D, Fernández de la Cruz L, D'Onofrio BM, Gottfreðsson M, Almqvist C, Unnur AValdimarsdóttir (2019). *Stress related disorders and subsequent risk of life threatening infections : population*

based sibbling controlled cohort study. Bio Med Journal **367** : I5784, Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6812608/ (Accessed 7th April 2020).

Songue E, Tagne C, Mbouyap P, Essomba P, Moyou-Somo (2013). *Epidemiology of malaria in three geo-ecological zones along the Tchad-Cameroon pipeline.* American Journal of Epidemiology and Infectious Diseases **1** (4), 27-33

Sousa-Figueiredo JC, Oguttu D, Adriko M, Besigye F, Nankasi A, Arinaitwe M, Namukuta A, Betson M, Kabatereine NB, Stothard JR (2010). *Investigating portable fluorescent microscope (cyscope) as an alternative rapid diagnostic test for malaria in children and women of child –bearing age.* Malaria Journal **9**: 245

Stevens, GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca f, Pena-Rosas JP, Bhutta ZA, Ezzati M (2013). *Global, regional and national trends in haemoglobin concentration and prevalences of total and severe anaemia in children and pregnant women for 1995-2011: a systematic analysis of population-representative data.* The Lancet Global Health **1**: 16-25

Stoltzfus RJ (2011). Iron interventions for women and children in low-income countries. Journal of Nutrition. **141**(4): 756S-62S

Stoltzfus RJ, Chwaya HM, Montressor A, Albonico M (2000*).* *Malaria, hookworms and recent fever are related to anaemia and iron status indicators in 0-to 5-year-old Zanzibari children and these relationships change with age.* J Nutr **130**: 24-33

Sumbele IUN, Nkain AJ, Anchang- Kimbi JK, Kimbi HK (2020a). *Influence of malaria, soil-transmitted helminthes and malnutrition on haemoglobin level among school-aged children in Muyuka, Southwest Cameroon: A cross-sectional study on outcomes.* PLoS ONE **15**(3):e0230882

Sumbele IUN, Asoba GN, Ning TR, Metuge S, Anchang- Kimbi JK, Nkuo-Akenji T (2020b). *Burden of moderate to severe anaemia and severe stunting in children ≤ 3 years in conflict-hit Mount Cameroon: a community based descriptive cross-sectional study.* BMC Pediatrics **20:** 396

Sumbele IUN, Sama SO, Kimbi HK, Taiwe GS (2016). *Malaria, moderate to severe anaemia, and malarial anaemia in children at presentation to hospital in the Mount Cameroon area: a cross-sectional study.* Anemia 2016: 5725634

Sumbele IUN, Bopda OSM, Kimbi HK, Ning TR, Nkuo-Akenji TN (2015). *Nutritional status of children in a malaria mesoendemic area: cross sectional study on prevalence, intensity, predictors, influence on malaria parasitaemia and anaemia severity.* BMC Public Health **15:** 1099

Sumbele IU, Ning TR, Bopda OS, Nkuo-Akenji T (2014). *Variation in malariometric and red cell indices in children in the Mount Cameroon area following enhanced malaria control measures: evidence from a repeated cross-sectional study.* Malar J. **13:** 334

Sumbele IUN, Samje M, Nkuo-Akenji TK (2013). *A longitudinal study on anaemia in children with Plasmodium falciparum infection in the Mount Cameroon region: prevalence, risk factors and perceptions by caregivers.* BMC Infectious Diseases, **13:** 123

Sylvetsky AC, Jefferds MED, De-Regil LM, Dowswell T (2011). *Intermittent iron supplementation for improvimg nutrition and development outcomes in children. In: The Cochrane Collaboration, editor. Cochrane Database of Systematic Reviews.* Chichester: John Wiley & Sons, Ltd; 2011

Tabue RN, Njeambosay BA, Zeukeng F, Esemu LF, Fodjo BAY, Nyonglema P, Awono-Ambene P, Etang J, Fondjo E, Achu D, Leke RGF, Kouambeng C, Knox TB, Mnzava AP, Bigoga JD (2019). *Case definitions of clinical malaria in children from three health districts in the North Region of Cameroon.* Journal of Biomedical Research International. Article ID 9709013

Tabue RN, Awono-Ambene P, Etang J, Atangana J, Antonio-Nkondjio C, Toto JC (2017). *Role of Anopheles (Cellia) rufipes (Gough, 1910) and other local anophelines in human malaria transmission in the northern savannah of Cameroon: a cross-sectional survey.* Journal of Parasites and Vectors **10:** 22

Tamure J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura Saitoh T, Kurabayshi H and Naruse T (1999). *Immunomodulation by Vit B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in Vit B12- deficient patients by methyl -B12 treatment.* Clinical Experimental Immunology Journal Vol **116**(1):28-32, Available at https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1905232/ (Accessed on 13th April 2020).

Taylor RR, Smith DB, Robinson VJ, McBride JS, Riley EM (1995). *Human antibody response to Plasmodium falciparum merozoite surface protein 2 is serogroup specific and predominantly of the immunoglobulin G3 subclass.* Journal of Infection and Immunology **63:** 4382-4388

Tebo AE, Kremsner PG, Luty AJ (2001). *Plasmodium falciparum: A major role for IgG3 in antibody-dependent monocyte-mediated cellular inhibition of parasite growth in vitro.* Journal of Experimental Parasitology **98:** 20-28

Teh RN, Sumbele IUN, Meduke DN, Nkeudem GA, Ojong ST, Teh EA, Kimbi HK (2021). *Insecticide-treated net ownership, utilization and knowledge of malaria in children residing in Batoke- Limbe, Mount Cameroon area: effect on malariometric and haematological indices.* Malar J. 2021 Jul 29; **20**(1): 333

Tepa A, Abame I, Makamta V, Fongang B, Donkeu J, Ayong L, Pierre CA (2020). *Nutritional status and humoral immune response to Plasmodium falciparum in children aged 6-59 months.* Journal of Tropical Medicine, Article ID 1843780

Tesema GA, Worku MG, Tessema ZT, Teshale AB, Alem AZ, Yeshaw Y, Alamneh TS, Liyew AM (2021). *Prevalence and determinants of severity levels of anaemia among children aged 6-59months in sub-Saharan Africa: A multi-level ordinal logistic regression analysis.* PLoS ONE **16(4):** e0249978

Thorarinsdottir HK, Ludviksson BR, Vikingsdottir T, Leopoldsdottir MO, Ardal B, Jonsson T, Valdimarsson H, Arason GJ (2005). *Childhood levels of immunoglobulins and mannan-binding lectin in relation to infections and allergy***.** Scandinavian Journal of Immunology, **61**: 466–74. 22

Thurnham D (2015). *Inflammation and Vitamin A.* Food and Nutrition Bulletin **36**(3): 290-298

Titanji VPK, Tamu VD, Nkuo-Akenji TK, Joutchop AS (2002). *Immunoglobulin G and subclass responses to Plasmodium falciparum antigens: a study in highly exposed Cameroonians.* Clin. Chem. Lab. Med., **40**: 937-940

The World Bank (2006). *Repositioning nutrition as central to development*: *a strategy for large-scale action. Washington DC: The World Bank;* 2006 (http://siteresources.

worldbank.org/NUTRITION/Resources/281846131636806329/NutritionStrategy.pdf, accessed 21 October 2014).

Tongren JE, Drakeley CJ, McDonald SL, Reyburn HG, Manjurano A, Nkya WMM, Lemnge MM, Gowda CD, Todd JE, Corran PH, Riley EM (2006). *Target antigen, age and duration of antigen exposure independently regulate immunoglobulin G subclass switching in malaria.* Infec Immun **74**: 257-264

Udoh EE, Oyo-Ita AE, Odey FA, Eyong KI, Oringanje CM, Oduwole OA, Okebe JU, Esu EB, Meremikwu MM, Asindi AA (2013). *Malariometric indices among Nigerian children in rural setting.* Malaria Research Treatment Journal 716805.

Ullah I, Zahid M, Alam Sthanadar A, Alam Sthanadar I, Asmat Ali P, Mudassirshah, Ismail Khan M, Kaleem M, Aslam M, Khayyam, Atiq-Ur-Rehman, Ullah W (2014). *Iron deficiency anaemia in school age children in District Karak Khyber Pakhtunkhwa Province, Pakistan.* Open Journal of Blood Diseases **4**: 9-15

UNICEF (2019). *The state of the world's children 2019. Children, food and nutrition: Growing well in a changing world. New York: United Nations Children's Fund;* (http://www.unicef.org.uk/Documents/Publication-pdfs/sowc-2019- children- food - and- nutrition .pdf, accessed 28 February 2021).

UNICEF (2013). *The state of the world's children 2013. Children with disabilities: From exclusion to inclusion. New York: United Nations Children's Fund;* (http://www.unicef.org.uk/Documents/Publication-pdfs/sowc-2013- children-with-disabilities.pdf, accessed 21 October 2014).

UNICEF (1998). *The state of the world's children 1998. Sound nutrition can change children's lives: New York: United Nations Children's Fund;* (http://www.unicef.org.uk/Documents/Publication-pdfs/sowc-1998- sound nutrition can change children's lives.pdf, accessed 21 October 2014).

UNICEF (1990). *The state of the world's children 1990. The great achievements and great setbacks of the 1980s. New York: United Nations Children's Fund;* (http://www.unicef.org.uk/Documents/Publication-pdfs/sowc-1998- The great achievements and great setbacks of the 1980s .pdf, accessed 21 October 2014).

United Nations Children's Fund, World Health Organization, World Bank Group (2019). *Joint child malnutrition estimates- levels and trends in child malnutrition.* 2019 edition. (WHO/NMH/NHD/19.20)

United Nations Children's Fund, World Health Organization, World Bank Group (2017). *Joint child malnutrition estimates- levels and trends in child malnutrition.* 2017 edition.

United Nations Children's Fund, World Health Organization, World Bank Group (2015). *Joint child malnutrition estimates- levels and trends in child malnutrition.* 2015 edition.

United Nations Administrative Committee on Coordination Nutrition (ACC/SCN) (2000). *4TH Report on the world nutrition situation: Nutrition Throughout the Life Cycle. Sub-Committee on Geneva:* ACC/SCN; 2000**.**

Van den Broek NR, Letsky EA, White SA, Shenkin A (1998). *Iron status in pregnant women: Which measurements are valid?* British Journal of Haematology **103**: 817

Villalpando S, Shamah-Levy T, Ram'ırez-Silva CI, Mej'ıa-Rodr'ıguez F, Rivera JA (2003). *Prevalence of anemia in children 1 to 12 years of age: results from a nationwide probabilistic survey in Mexico.* International Journal of Epidemiology **45**: 490–498

Walker SP, Chang SM, Powell CA, Simonoff E, Grantham-McGregor SM (2007). *Early childhood stunting is associated with poor psychological functioning in late adolescence and effects are reduced by psychosocial stimulation.* Journal of Nutrition; **137**: 2464–9

Wamani H, Astrom AN, Peterson S, TUMwine JK, Tylleskar T (2007). *Boys are more stunted than girls in sub-Saharan Africa: a meta-analysis of 16 demographic and health surveys.* BMC Pediatrics Journal **7**: 17

Warhurst DC and Williams JE (1996). *Laboratory diagnosis of malaria.* Journal of Clinical Pathology **49**(7): 533-538

Wongsrichanalai C, Barcus JM, Muth S, Sutamihardja A, Wernsdorfer WH (2007). *A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT).* American Journal of Tropical Medicine and Hygiene **77**(6): 119-127

World Health Organization (2002). *Reducing risks, promoting healthy life. The World Health Report* 2002. WHO Regions, Estimates for 2001. Geneva.

World Health Organization (2009). *Child growth standards and the identification of severe acute malnutrition in infants and children. A joint statement.* Geneva: World Health Organization;(http://apps.who.int/iris/bitstream/10665/44129/1/9789241598163_eng.pdf, accessed 19 November 2014).

World Health Organization (2020). *Food and Nutrition tips during self- quarantine.* Available at: https://www.euro.who.int/en/health-topics/health-emergencies/coronavirus-covid-19/novel-coronavirus-2019-ncov-technical-guidance/food-and-nutrition-tips-during-self-quarantine (Accessed on 2nd April 2020).

World Health Organization (2006). *Multicenter Growth Reference Study Group. WHO child growth standards. Length, height for-age, weight-for-age, weight-for-length and body mass index-for age. Methods and development*. Geneva: World Health Organization; (http://www.who.int/childgrowth/standards/Technical_report.pdf, accessed 13 October 2014).

World Health Organization (2005). World Health Organization Hand book: IMCI integrated management of childhood illness, Geneva, Switzerland.

World Health Organization (2014) *Global nutrition targets 2025: childhood overweight policy brief* (WHO/NMH/NHD/14.6). Geneva: World Health Organization; 2014

World Health Organization (2014) *Global nutrition targets 2025: stunting policy brief* (WHO/NMH/NHD/14.3). Geneva: World Health Organization; 2014

WHO/UNICEF/WFP (2014) *Global nutrition targets 2025: wasting policy brief* (WHO/NMH/NHD/14.8). Geneva: World Health Organization; 2014

Wilson AL, Bradley J, Kandeh B, Salami K, D'Alessandro U, Pinder M, Lindsay SW (2018). *Is chronic malnutrition associated with an increase in malaria incidence? A*

*cohort study in children aged under 5 years in rural Gambia.*Journal of Parasites and Vectors **11:** 451

Wilson AL, on behalf of the IPTc Taskforce (2011). *A Systematic Review and Meta-Analysis of the Efficacy and Safety of Intermittent Preventive Treatment of Malaria in Children (IPTc).* PLoS ONE **6**(2): e16976

Wondji C, Frederic S, Petrarca V, Etang J, Santolamazza F, Della TA (2005). *Species and populations of the Anopheles gambiae complex in Cameroon with special emphasis on chromosomal and molecular forms of Anopheles gambiae s.s.* Journal of Medical Entomology **42:** 988-1005

World Health Organisation (WHO), (2019). *World Malaria Report* Geneva, Switzerland.

World Health Organization (WHO), (2019). *WHO child growth standards: head circumference-for-age, arm circumference-forage, triceps skinfold-for-age and subscapular skinfold-for-age:* methods and development, **2007.**

World Health Organization (2012) *Global database on Child Growth and Malnutrition. Joint child malnutrition estimates – levels and trends. UNICEF-WHO-The World Bank project.* Geneva: World Health Organization; 2012(http://www.who.int/nutgrowthdb/estimates2012/en/, accessed 8 October 2014).

World Health Organization (2019*). Global Nutrition Report***.** Geneva, Switzerland.

World Health Organization (2020*). Global Nutrition Report***.** Geneva, Switzerland.

World Health Organization (2015) Global *database on Child Growth and Malnutrition. Joint child malnutrition estimates – levels and trends. UNICEF-WHO-The World Bank project.* Geneva: World Health Organization; 2015(http://www.who.int/nutgrowthdb/estimates2015/en/,2015 edition).

World Health Organization. *Global targets 2025. To improve maternal, infant and young child nutrition* (www.who.int/nutrition/topics/nutrition_globaltargets2025/en/, accessed 6 October 2014).

World Health Organization (2001*). Iron deficiency anaemia: assessment, prevention and control; a guide for programme managers.*Geneva: World Health Organization, 114 p.

World Health Organization (1968). *Nutritional anaemias: Report of a WHO Scientific Group.* Geneva: World Health Organization; 1968

World Health Organization (1995). *Physical Status: the Use and Interpretation of Anthropometry: Technical Report Series no. 854***.** Geneva: World Health Organization, 1995

WHO. The Global Prevalence of Anaemia 2011. Geneva, Switzerland: World Health Organization; 2015.

WHO (2017). Nutritional anaemias 2017, Geneva, Switzerland: World Health Organization; 2017

World Bank. *Prevalence of anaemia among children (% of children under five)/ data. 2020.https://data.worldbank.org/indicator/SH.ANM.CHLD.ZS.*

World Health organization (2016). Global Health Observatory data repository: prevalence of anaemia in children.

World Health Organization (2011). Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity.

World Health Organization (2000). *Severe falciparum malaria.* Transactions of the Royal Society of Tropical Medicine and Hygiene; **94**(Suppl 1): S1–90

World Health Organization (2015). World Malaria Report 2021. Geneva, Switzerland

World Health Organization (2015). World Malaria Report 2019. Geneva, Switzerland

World Health Organization (2015). World Malaria Report 2015. Geneva, Switzerland

World Health Organization (2008). Worldwide prevalence of anaemia 1993-2005: *WHO global database on anaemia.* Geneva: World Health Organization, 40 p.

Zhou Jian MD, Du Juan MD, Huang Leting MD, Wang Youcheng MD, Shi Yimei MD (2018). *Preventive Effects of Vitamin D on Seasonal Influenza A in Infants- A Multi-Center, Randomized, Open, Controlled Clinical Trial.* The Paediatric Infectious Disease Journal **37**(8): 749-754, Available at: https://journals.lww.com/pidj/fulltext/2018/08000/Preventive_Effects_of_Vitamin D_on_Seasonal.5.aspx (Accessed on 2nd April 2011).

APPENDICES

APPENDICES

Appendix 1:

1.1. Ethical Clearance

COMITÉ NATIONAL D'ETHIQUE

NATIONAL ETHICS COMMITTEE

N° d'enregistrement : FWA IRB00001954 BP 1937, Yaoundé, Tel: (237)-221 12 84
Arrêté N° 079 /A/MSP/DS du 22 OCTOBRE 1987 portant création et organisation d'un Comité d'Ethique sur la recherche impliquant les êtres humains
cnecprot@yahoo.fr

Yaoundé le, 29 Juillet 2009

AUTORISATION N° 102/CNE/SE/09

CLAIRANCE ETHIQUE

Le Comité National d'Ethique a réexaminé ce 17 juillet 2009, la demande de clairance éthique du projet de recherche intitulé : ***"IMPACT OF INSECTICIDE RESISTANCE ON THE EFFECTIVENESS OF LONG LASTING INSECTICIDAL TREATED NET IN NORTH CAMEROON"*** introduit par le **Dr. NDONG A BESSONG Prosper,** Investigateur Principal local du projet.

Ce projet présente une valeur sociale certaine. Sa mise en œuvre serait une contribution significative à la prévention primaire du paludisme en Afrique. Le protocole de recherche présenté présume un projet scientifiquement valide. Le projet comporte les risques de non respect de confidentialité sur les données et de l'autonomie des participants. Les procédures décrites de prévention de ces risques permettraient d'en minimiser la probabilité de survenue. Les CVs des investigateurs les décrivent capables de mettre en œuvre le protocole. Le dossier de soumission est complet. Le projet est ainsi en ligne avec la réglementation de la recherche sur les êtres humains au Cameroun et les principes éthiques protégeant les droits et la dignité des potentiels participants à la recherche. De ce fait, le comité a approuvé pour une période d'un an, la mise en œuvre de la présente version du protocole.

L'investigateur principal est responsable du respect scrupuleux du protocole approuvé et ne devra y apporter aucun amendement aussi mineur soit-il sans avis favorable du comité national d'éthique. Il devra informer au premier cas et le plus tôt possible le Comité National d'Ethique, l'Autorité Sanitaire du Cameroun, et le promoteur du présent projet, de la survenue de tout incident inattendu lors de la mise en œuvre du protocole. Il est appelé à collaborer pour toutes descentes du Comité National d'Ethique pour suivi de la mise en œuvre dudit protocole. Les rapports annuels et le rapport final du projet devront être soumis au comité d'éthique et aux autorités sanitaires du Cameroun à la fin de l'étude.

La présente clairance peut être retirée en cas de non respect de la réglementation en vigueur et des recommandations sus-évoquées.

Le Président

Pr Lazare KAPTUE

1.2. Informed consent

CONSENTEMENT APRES INFORMATION

Bonjour, je m'appelle ….... …….. Je suis agent de santé communautaire et je travaille avec le centre de santé de …… (indiquer le nom du centre de santé auquel votre village /cluster est rattaché) .Nous effectuons en ce moment, une étude dans ce village concernant divers problèmes liés au paludisme.

Comme vous le savez, le paludisme est un grand problème de santé dans notre pays. Il tue chaque année des milliers de personnes, surtout les enfants de moins de 5ans et les femmes enceintes .Pour l'instant ,nous voulons avoir des informations sur la manière dont le paludisme sévit dans le Nord du Cameroun ,les différents moustiques qui véhiculent le parasite du paludisme et le comportement de ces moustiques ,quelques conditions humaines ,sociales et environnementales qui sous-tendent la transmission du paludisme .Ces informations nous aiderons à comprendre comment mieux lutter contre le paludisme dans cette zone du Nord du Cameroun .

Consentement éclairé

Cette notice d'information vous fournit des éclaircissements sur la recherche que nous menons. Lisez-la attentivement et posez nous autant de questions que vous voulez sur cette étude. Si vous avez besoin de plus de temps pour en parler à votre famille, à vos amis ou à votre médecin, il n'y a pas d'objection. Si vous acceptez de participer à cette étude, nous vous demanderons de signer un formulaire de consentement ou au moins de donner un consentement oral devant un témoin.

Donc ,dans le cadre de cette étude, nous travaillons uniquement avec les enfants de moins de 5 ans .Une fois que vous accepteriez la participation de votre enfants dans le projet , nous lui donnerons un traitement de paludisme et nous allons le suivre deux fois par mois (chaque deux semaines) jusqu'à décembre 2014.Au cours de l'une de ces 2 visites ,nous effectueront un test de diagnostic rapide (TDR) du paludisme et si ce test est positif ,nous traiterons l'enfant gratuitement et nous cesseront de le suivre .De même, si au cours d'une visite nous constatons que l'enfant a la fièvre ,nous lui effectuerons un TDR surplace et si ce test montre que c'est le paludisme simple ,nous le traiteront gratuitement puis nous cesserons de le suivre .Si c'est un cas grave , nous le référons au centre de santé le plus proche ou nous suivrons la

situation de l'enfant .L'enfant pourra sentir une légère douleur au moment de le piquer pour effectuer le test rapide ,il pourra également ressentir une fatigue due au médicament que nous lui donneront .Nous vous conseillons que l'enfant doit bien manger chaque fois avant de prendre son traitement .A chaque visite nous allons vous poser des questions au sujet de la santé de l'enfant .Toutefois les informations que vous fournirez resteront strictement confidentielles et ne seront transmises à personne en dehors de l'équipe de l'étude .La participation

de l'étude est totalement volontaire .Si vous décidez de ne plus participer cela ne portera aucun préjudice à votre relation avec l'équipe de recherche ou avec le personnel sanitaire de votre localité.

Nous souhaitons que votre enfant participe à cette étude qui est particulièrement importante pour la santé dans notre pays.

Avez- vous des questions à me poser sur l'étude ?

Si vous avez des questions à tout moment concernant l'étude ; vous pouvez contacter

Dr Djélé Sali (Tél : 77 47 30 28) ou M. **Moussa Souley (Tél : 77 54 34 72)** ou **Dr Kouambeng Célestin (Tél : 77 77 12 71)** ou le coordonnateur du projet, le **Dr Bigoga Jude (77 82 47 30).**

Puis-je commencer maintenant l'entretien pour recruter vos enfants ?

Je soussigné,……certifie avoir été bien informé du but et des procédures de l'étude ci-dessus .J'accepte librement la participation de mes enfants à l'étude en question .J'ai été informe du fait que je peux me retirer de l'étude à tout moment sans aucun préjudice.

Nom du parent /Tuteur : ……

Signature :

Nom du témoin : …….

Signature :

Appendix 2:

Preparation of Reagents/Solutions

2.1 Preparation of Giemsa Stain

-Prepare 1.5L of a one in ten dilution of Giemsa(10%).

-10% means 10ml Giemsa in 100ml water.

-For a 1500ml final volume, Quantity of Giemsa needed = ? (x)

$$X = \frac{1500 \times 10}{100} = \textbf{150 ml Giemsa}$$

-Therefore 10% will be 150ml Giemsa in **1350ml Water.**

Preparation of reagents for DNA Extraction

2.1.1 Preparation of Saponin

0.5% Saponin means 0.5g Saponin in 100ml autoclaved 1X PBS.

Prepare final volume of 150ml.

Quantity of Saponin needed = ? (x)

Where $x = \frac{150 \times 0.5}{100} =$ **0.75g of Saponin**

-Add autoclaved 1X PBS to 0.75g of Saponin, in a 150ml conical flask.

-Dissolve Saponin completely.

-Make up to the mark with PBS.

-Store at 4°C.

2.1.2 Preparation of 20% Chelex Solution

20% Chelex Solution means 20g Chelex in 100ml autoclaved 1X PBS.

For our reaction, we prepared a final volume of 15ml of 20% Chelex solution.

So the quantity of Chelex needed = ? (x)

Where $x = \frac{20 \times 15}{100} =$ **3g of Chelex**

Add 15ml of autoclaved 1X PBS to 3g of Chelex, in a 25ml falcon tube.

Shake the mixture well and allow the suspension to settle.

Carefully remove the supernatant.

Add PBS to make up the volume to 15ml.

Store the Chelex suspension at 4°C.

2.1.3 Preparation of 10X PBS (P^H 7.2)

Weigh Na_2HPO_4 (77.75g), NaH_2PO_4 (10.2g), and NaCl (190.84g) and dissolve in 300ml distilled H_2O.

Adjust the P^H to 7.2 using 1M HCL and complete the volume to 500ml with distilled water, in a reagent bottle.

Store the solution at room temperature (RT).

Preparation of reagents for Agarose Gel Electrophoresis21.2.1 Preparation of Gel

Gel is made up of Agarose in TBE buffer.

2% Agarose gel means 2g Agarose in 100ml TBE buffer.

Since the capacity of the gel mould is 300ml,

Therefore, the quantity of Agarose needed = ? (x)

$$X = \frac{2 \times 300}{100} = \textbf{6g Agarose}$$

Preparation of TBE Buffer 10X (from Ready Pack TM AMBRESCO ^{R})

A single strength (1X) solution contains 0.089M Tris base,

0.089M Borate and

0.002M EDTA.

To make 1Litre of 10X Liquid concentrate;

-Empty 1 packet into container and add distilled water to 1L final volume.

-This kit contains 2 x 1L powder pouch.

To make 1X;

-Dilute 500mL of 10X in 5L of distilled water.

-Store at room temperature.

2.2.3 Preparation of TE Buffer 10X

Reagents: Tris - EDTA BUFFER 100X SIGMA

Distilled water

Materials: Measuring cylinders (100mL)

Beaker (100mL)

Reagent bottle

Prepare 100mL of TE 10X concentrate from TE100X.

Quantity of TE 100X needed = ? (x)

$$X = \frac{10 \times 100}{100} = \textbf{10mL of TE 100X}$$

and **90mL of Distilled water**

A one in ten dilution was done. Using a 100mL measuring cylinder, 10mL of Tris-EDTA was measured from the bottle. After which distilled water was added and mixed very well. Next, it was poured into a reagent bottle.

Preparation of the Dye Etidium Bromide (EtBr)

The standard is 5%.

We used 3% v/v meaning 3µl EtBr in 100ml gel

Therefore quantity of EtBr needed for our 300ml gel = X

$$X = \frac{3 \times 300}{100} = \textbf{9 µl EtBr}$$

Preparation of Reagents for ELISA

2.3.1 Coating Buffer (Carbonate/bicarbonate buffer) PH 9.5.

Prepare 250mL of 0.1M Carbonate buffer.

*Reagents: Standard for 100mL

Na_2Co_3 (0.16g) Sodium Carbonate Monohydrate Crystals - BAKER

$NaHCO_3$ (0.29g) Sodium Bicarbonate - SIGMA

NaN_3 (0.02g) Sodium Azide - SIGMA

*Procedure: For 250mL

Quantity of reagents needed;

0.16g Na_2CO_3…….....100mL Distilled H_2O

Xg needed……..250mL

$$X = \frac{250 \times 0.16}{100} = \textbf{0.4g } \mathbf{Na_2CO_3}$$

0.29g $NaHCO_3$……..100mL Distilled H_2O

Xg needed……. 250mL

$$X = \frac{250 \times 0.29}{100} = \textbf{0.725g } \mathbf{NaHCO_3}$$

0.02g NaN3…….100mL Distilled H2O

Xg needed……. 250mL

X = $\frac{250 \times 0.02}{100}$ = **0.05g NaN_3**

-In a 250ml flat bottomed flask, dissolve the above reagents with a small volume of Distilled water.

-Make up to the mark with distilled water.

-Adjust the P^H to 9.5

-Store at 4°C.

2**.3.2 Phosphate Buffered Saline** (PBS) P^H 7.2

Prepare 1L of PBS

*Reagents:

PBS tablets – SIGMA

Distilled H_2O

*Procedure:

Quantity of PBS needed;

1PBS tablet is dissolved in……200mL Distilled H_2O

X tablets ……..1000mL Distilled H_2O

X = $\frac{1000 \times 1}{200}$ = **5 Tablets of PBS**

-To 1000ml Distilled water in a reagent bottle, add 5 tablets of PBS.

-Shake gently and allow to dissolve.

-Adjust the P^H to 7.2 using 5N HCl

-Store at RT.

2.3.3 Washing Buffer (PBS 0.02% -Tween-20)

Prepare 1L of PBS-T

*Reagents:

PBS Solution

Tween- 20 FISHER

*Procedure

-To 1L of PBS in a reagent bottle add 200µL of Tween-20.

-Mix well by shaking the bottle.

- Store at 4°C or RT (1week)

2.3.4 Blocking Buffer (10% milk in PBS 0.02% - Tween 20)

Prepare 30mL of Blocking Buffer

*Reagents:

Milk (carnation non-fat dry milk) - CASINO

PBS-T

Quantity of Milk needed:

10% means

10g milk dissolved in 100mL PBS-T

Xg……30mL

$$X = \frac{30 \times 10}{100} = \textbf{3g of Milk}$$

*Procedure

-To 3g of dry milk in a 50mL beaker, add 30mL of PBS 0.02% Tween-20.

- Stir gently with a glass rod to homogenize the solution.

- Use freshly prepared.

.

2.3.5 Diluting Buffer (1% milk in PBS 0.02% - Tween 20)

Prepare 100mL of Diluting Buffer

*Reagents:

10% milk in PBS 0.02% - Tween 20

PBS-T

*Procedure

-Do a one in ten dilution of the blocking buffer.

-To 90mL of PBS –T , add 10mL of 10% Blocking Buffer and make up to the mark in a 100ml flat-bottomed flask.

-Use freshly prepared.

2.3.6 Alkaline Phosphatase Substrate Buffer PH 9.86

Prepare 1L of AP Substrate Buffer

*Regents:

Diethanolamine SIGMA

$MgCl_2,6H_2O$ SIGMA

*Procedure

-Dissolve 101mg of $MgCl_2,6H_2O$ in distilled water in a 1L reagent bottle.

-Add 97mL of Diethanolamine into the solution.

-Make up with distilled water to the mark.

-Shake well.

-Adjust the P^H with 12N HCl.

-Store at 4°C

2.3.7 P-Npp Elisa Substrate

Prepare 10ml

*Reagents:

Phosphatase substrate tablets SIGMA

AP Substrate buffer

*Procedure:

-Allow AP Substrate buffer to warm up to room temperature.

-Transfer 10mL into a 15mL falcon tube (sealed with tissue and foil).

-Add 2 p-npp tablets (1 tablet = 5mg ;conc. = 1mg/ml;)

-Shake well by inverting tube many times until tablets completely dissolve.

-Store in the dark.

Preparation of Antigen (Crude *Plasmodium falciparum* 3D7)

Crude Plasmodium falciparum 3D7 antigen

Prepare 5µg/mL from 2mg/mL stock of crude antigen.

Quantity of Coating Buffer needed……?

Total number of wells…….96wells

At 100µL …….. 1 well,

X µL……96 wells

$$X = \frac{96 \times 100}{1} = 9600\mu L \text{ of Coating Buffer.}$$

Approximately **10mL Coating Buffer**

Volume of coating buffer needed = 10mL

Volume of crude antigen needed = X

$C_iV_i = C_fV_f$

$V_i = \frac{C_fV_f}{C_i} = \frac{5\ \mu g/mL \times 10.000\ \mu L}{2000\ \mu g/mL}$

Volume needed = **25µL of Crude antigen**

Dilution of Serum samples and Controls

Dilute samples in Diluting buffer

Total volume required = 1mL (1000µL)

Volume of sample = ?

Volume of diluting buffer = ?

Dilution concentration is 1:50

meaning 1µL sample in 50µL diluent

so XµL …….in 1000µL (1mL)

$X = \frac{1000 \times 1}{50}$ = **20µL sample**

Therefore if vol. of sample needed = 20µL

Then, vol of diluent needed = 1000 - 20 = **980µL diluent**

-Pipette 980 µL diluting buffer into clearly labelled eppendorf tubes.

-Add 20 µL of serum sample into each corresponding tube.

-Vortex gently for about 5 seconds.

-Do same for positive and negative controls.

Dilution of Goat anti-human Whole IgG (enzyme-conjugated secondary antibody)

-Dilute Whole IgG in Diluting buffer

-Dilution Concentration 1 : 4000

-Prepare 10mL of $\frac{1}{}$ from $\frac{1}{}$ stock

4000 100

-What volume of IgG will be pipetted from the bottle?

$C_iV_i = C_fV_f$

$$V_i = \frac{C_fV_f}{C_i} = \frac{1}{4000} \times 10.000\ \mu L$$

$$\frac{\frac{1}{4000}}{\frac{1}{100}}$$

$$= \frac{1}{4000} \times 10.000\ \mu L \times \frac{100}{1} = \textbf{250 µL of Whole IgG}$$

and **9750 µL of Diluting buffer.**

-Pipette 9750µL of Diluent into a 15ml Falcon tube.

-Add 250µL of Whole IgG.

-Vortex gently for 5seconds.

- Do same for IgG1 (1: 6000) and IgG3 (1: 6500)

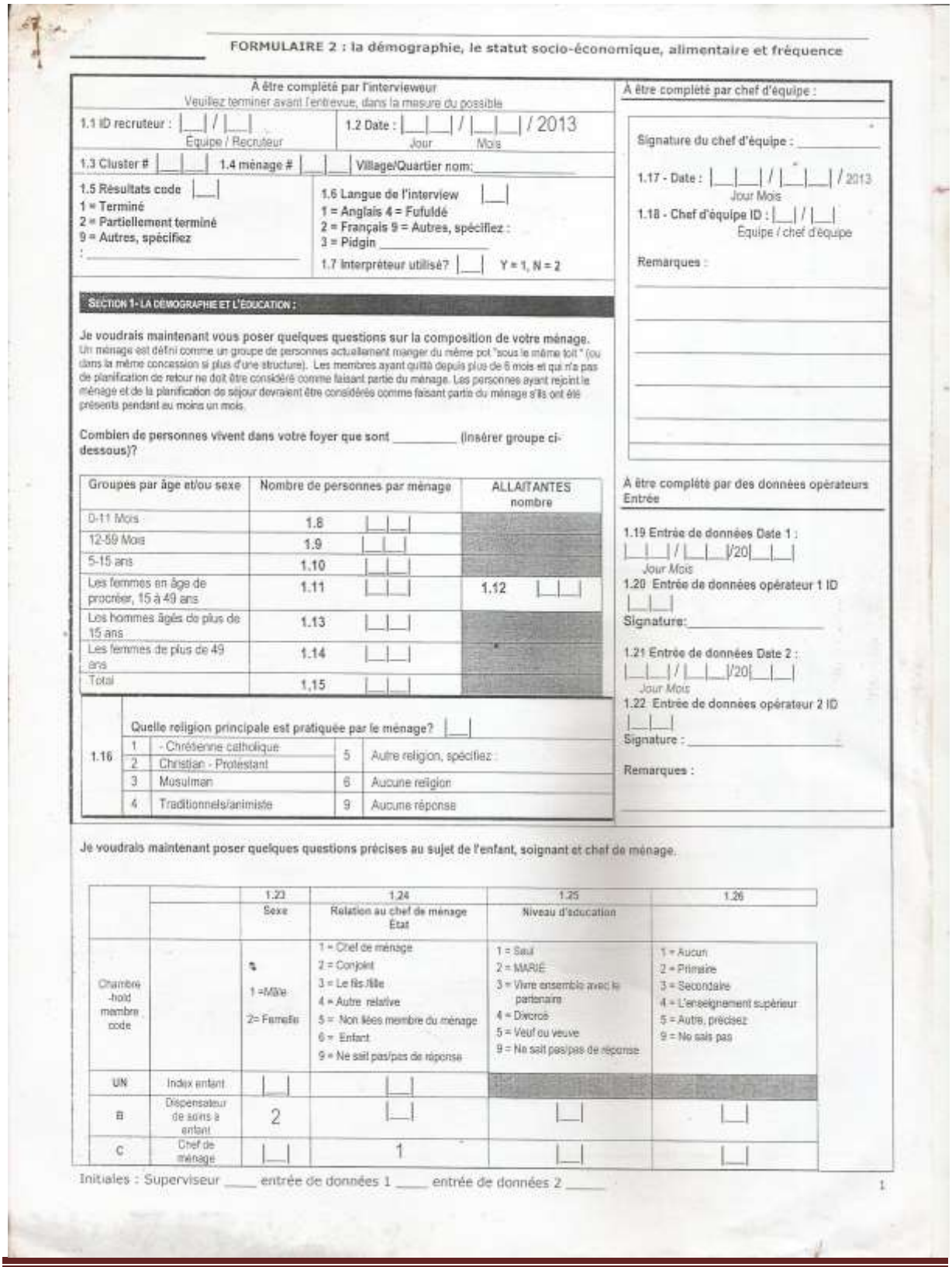
Primers for PCR

Primer	Sequence(5' ------- 3')	Base pairs	Specificity of primers
rPLU5 rPLU6	5'-CCT GTT GTT GCC TTA AAC TTC-3' 5'-TTA AAA TTG TTG CAT TTA AAA ACG -3'	1100 bp	Genus *Plasmodium*
rFAL1 rFAL2	5'-TTA AAC TGG TTT GGG AAA ACC AAA TAT ATT-3' 5'-ACA CAA TGA ACT CAA TCA TGA CTA CCC GTC-3'	205 bp	*P. falciparum*
rMAL1 rMAL2	5'-ATA ACA TAG TTG TAC GTT AAG AAT AAC CGC-3' 5'-AAA ATT CCC ATG CAT AAA AAA TTA TAC AAA-3'	144 bp	*P. malariae*
rOVA1 rOVA2	5'-ATC TCT TTT GCT ATT TTT TAG TAT TGG AGA-3' 5'-GGA AAA GGA CAC ATT AAT TGT ATC CTA GTG-3'	800 bp	*P. ovale*
rVIV1 rVIV2	5'-CGC TTC TAG CTT AAT CCA CAT AAC TGA TAC-3' 5'-ACT TCC AAG CCG AAG CAA AGA AAG TCC TTA-3'	120 bp	*P. vivax*

Appendix 3 :

Questionnaires

3.1. Nutritional and Socio-economic Status Questionnaire



FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

À être complété par l'intervieweur
Veuillez terminer avant l'entrevue, dans la mesure du possible

1.1 ID recruteur : |__|/|__| (Équipe / Recruteur)
1.2 Date : |__|__|/|__|__|/2013 (Jour / Mois)
1.3 Cluster # |__|__| 1.4 ménage # |__|__| Village/Quartier nom:_____
1.5 Résultats code |__|
1 = Terminé
2 = Partiellement terminé
9 = Autres, spécifiez :_____
1.6 Langue de l'interview |__|
1 = Anglais 4 = Fufuldé
2 = Français 9 = Autres, spécifiez :
3 = Pidgin _____
1.7 Interpréteur utilisé? |__| Y = 1, N = 2

À être complété par chef d'équipe :
Signature du chef d'équipe : _____
1.17 - Date : |__|__|/|__|__|/2013 (Jour Mois)
1.18 - Chef d'équipe ID : |__|/|__| (Équipe / chef d'équipe)
Remarques :

SECTION 1- LA DÉMOGRAPHIE ET L'ÉDUCATION :

Je voudrais maintenant vous poser quelques questions sur la composition de votre ménage.
Un ménage est défini comme un groupe de personnes actuellement manger du même pot "sous le même toit" (ou dans la même concession si plus d'une structure). Les membres ayant quitté depuis plus de 6 mois et qui n'a pas de planification de retour ne doit être considéré comme faisant partie du ménage. Les personnes ayant rejoint le ménage et de la planification de séjour devraient être considérées comme faisant partie du ménage s'ils ont été présents pendant au moins un mois.

Combien de personnes vivent dans votre foyer que sont _____ (insérer groupe ci-dessous)?

Groupes par âge et/ou sexe	Nombre de personnes par ménage	ALLAITANTES nombre
0-11 Mois	1.8 |__|__|	
12-59 Mois	1.9 |__|__|	
5-15 ans	1.10 |__|__|	
Les femmes en âge de procréer, 15 à 49 ans	1.11 |__|__|	1.12 |__|__|
Les hommes âgés de plus de 15 ans	1.13 |__|__|	
Les femmes de plus de 49 ans	1.14 |__|__|	
Total	1.15 |__|__|	

1.16 Quelle religion principale est pratiquée par le ménage? |__|

1	- Chrétienne catholique	5	Autre religion, spécifiez
2	Christian - Protestant		
3	Musulman	6	Aucune religion
4	Traditionnels/animiste	9	Aucune réponse

À être complété par des données opérateurs Entrée
1.19 Entrée de données Date 1 : |__|__|/|__|__|/20|__|__| (Jour Mois)
1.20 Entrée de données opérateur 1 ID |__|__|
Signature:_____
1.21 Entrée de données Date 2 : |__|__|/|__|__|/20|__|__| (Jour Mois)
1.22 Entrée de données opérateur 2 ID |__|__|
Signature : _____
Remarques :

Je voudrais maintenant poser quelques questions précises au sujet de l'enfant, soignant et chef de ménage.

		1.23 Sexe	1.24 Relation au chef de ménage Etat	1.25 Niveau d'éducation	1.26
Chambre -hold membre code		1 =Mâle 2= Femelle	1 = Chef de ménage 2 = Conjoint 3 = Le fils /fille 4 = Autre relative 5 = Non liées membre du ménage 6 = Enfant 9 = Ne sait pas/pas de réponse	1 = Seul 2 = MARIÉ 3 = Vivre ensemble avec le partenaire 4 = Divorcé 5 = Veuf ou veuve 9 = Ne sait pas/pas de réponse	1 = Aucun 2 = Primaire 3 = Secondaire 4 = L'enseignement supérieur 5 = Autre, précisez 9 = Ne sais pas
UN	Index enfant	|__|	|__|		
B	Dispensateur de soins à enfant	2	|__|	|__|	|__|
C	Chef de ménage	|__|	1	|__|	|__|

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____

1

FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

SECTION 2 - LOGEMENT, DES INSTALLATIONS ET DES BIENS

2.0	**Observer et noter. Ne demandez pas question!** Décrire la structure du logement. ⌊__⌋	1	Chambre séparée	5	PLUSIEURS huttes/bâtiments (MÊME composé)
		2	APPARTEMENT	6	PLUSIEURS huttes/bâtiments (DIFFÉRENTS composés)
		3	Boucarou	7	TENTE/Improvisé HOME
		4	CHAMBRE(S) autres (TYPE)	9	Autre, préciser :

Cluster : ⌊__⌋⌊__⌋ ménage : ⌊__⌋⌊__⌋

2.1	**Ne pas lire les réponses.** Est-ce que vous-même ou votre ménage propre ou de louer ce logement ? ⌊__⌋	1	Propre	5	Hypothèque
		2	Ne pas propre mais pour vivre libre	6	Autres, spécifiez :
		3	Payer le loyer	9	Je ne sais pas/pas de réponse
		4	Les squatters		

2.2	**Ne pas lire les réponses.** Quel genre de toilettes est-ce que les membres de votre ménage généralement utiliser? ⌊__⌋	1	Système d'eau toilettes modernes (toilettes)
		2	Latrines améliorées à fosse ventilée (VIP)
		3	Latrines à fosse avec dalle
		4	Latrines à fosse sans dalle/fosse ouverte
		5	La benne/Pan
		6	Le compostage toilettes
		7	Pas d'installations (bush, plage, etc.)
		8	Autres, spécifiez :
		9	Je ne sais pas/pas de réponse

2.3	**Ne pas lire les réponses.** Quelle est la <u>principale source</u> d'énergie pour la cuisine? ⌊__⌋	1	Électricité	5	Gaz
		2	Le Kérosène	6	Bois
		3	Charbon actif	7	Autre, précisez
		4	Déjections animales ou résidus agricoles	9	Je ne sais pas/pas de réponse

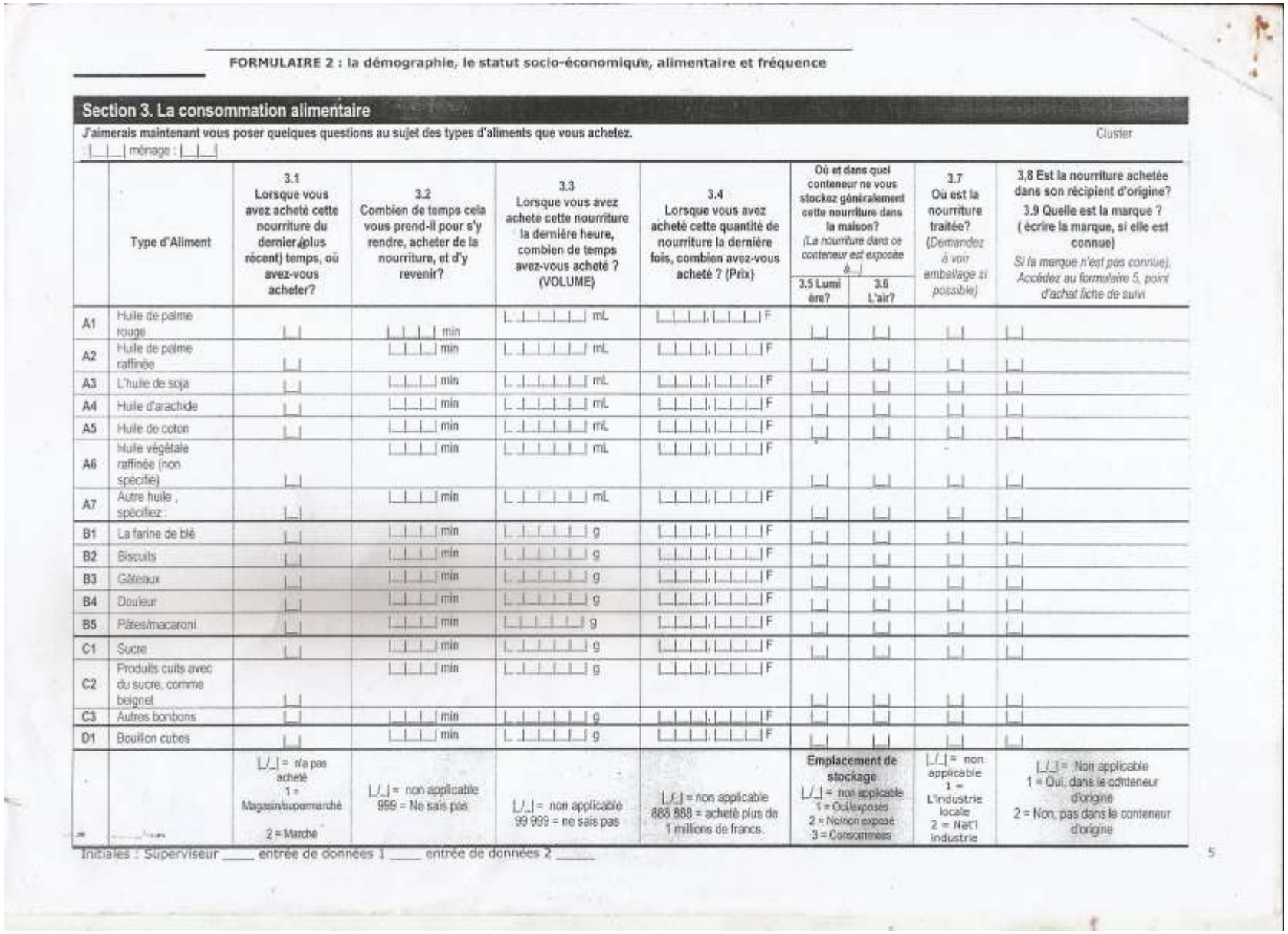
2.4	**Ne pas lire les réponses.** Quelle est la principale source de l'éclairage de la chambre? ⌊__⌋	1	L'huile et le kérosène ou de gaz lanterne	4	Bougies/bois	7	L'énergie solaire
		2	Batterie / lampes fluorescentes/éclairage du tube	5	Electric Company	8	Autres, spécifiez :
		3	Générateur électrique/inverteur	6	Pas d'éclairage	9	Je ne sais pas/pas de réponse

2.5	**Ne pas lire les réponses.** Quelle est la <u>principale source d'eau potable pour</u> les membres de votre ménage? ⌊__⌋	01	L'eau courante dans/hors du côté	07	Ressort protégé
		02	Tube bien/forage	08	Ressort non protégés
		03	**Protégées par l'Onu**creusé bien	09	L'eau de pluie
		04	Protégé creusé bien	10	Camion-citerne
		05	L'eau de surface (rivière, barrage, lac, etc.)	11	Eau minérale offerte
		06	Sachet d'eau	12	Autres, spécifiez :
				99	Je ne sais pas/pas de réponse

2.6	Où est la source d'eau potable situé?	1	Dans leur propre logement
		2	Dans sa propre courtracé

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____

2



FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

Section 3. La consommation alimentaire

J'aimerais maintenant vous poser quelques questions au sujet des types d'aliments que vous achetez. Cluster

|_|_| ménage : |_|_|

	Type d'Aliment	3.1 Lorsque vous avez acheté cette nourriture du dernier (plus récent) temps, où avez-vous acheter?	3.2 Combien de temps cela vous prend-il pour s'y rendre, acheter de la nourriture, et d'y revenir?	3.3 Lorsque vous avez acheté cette nourriture la dernière heure, combien de temps avez-vous acheté ? (VOLUME)	3.4 Lorsque vous avez acheté cette quantité de nourriture la dernière fois, combien avez-vous acheté ? (Prix)	Où et dans quel conteneur ne vous stockez généralement cette nourriture dans la maison? (La nourriture dans ce conteneur est exposée à...) 3.5 Lumière?	3.6 L'air?	3.7 Où est la nourriture traitée? (Demandez à voir emballage si possible)	3,8 Est la nourriture achetée dans son récipient d'origine? 3.9 Quelle est la marque ? (écrire la marque, si elle est connue) Si la marque n'est pas connue, Accédez au formulaire 5, point d'achat fiche de suivi
A1	Huile de palme rouge	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
A2	Huile de palme raffinée	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
A3	L'huile de soja	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
A4	Huile d'arachide	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
A5	Huile de coton	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
A6	Huile végétale raffinée (non spécifié)	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
A7	Autre huile , spécifiez :	|_|	|_|_|_| min	|_|_|_|_|_| mL	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
B1	La farine de blé	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
B2	Biscuits	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
B3	Gâteaux	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
B4	Douleur	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
B5	Pâtes/macaroni	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
C1	Sucre	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
C2	Produits cuits avec du sucre, comme beignet	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
C3	Autres bonbons	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
D1	Bouillon cubes	|_|	|_|_|_| min	|_|_|_|_|_| g	|_|_|_|,|_|_|_| F	|_|	|_|	|_|	|_|
		|_/_| = n'a pas acheté 1 = Magasin/supermarché 2 = Marché	|_/_| = non applicable 999 = Ne sais pas	|_/_| = non applicable 99 999 = ne sais pas	|_/_| = non applicable 888 888 = acheté plus de 1 millions de francs.	Emplacement de stockage |_/_| = non applicable 1 = Oui,exposés 2 = Noinon exposé 3 = Consommées		|_/_| = non applicable 1 = L'industrie locale 2 = Nat'l industrie	|_/_| = Non applicable 1 = Oui, dans le conteneur d'origine 2 = Non, pas dans le conteneur d'origine

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____

5

FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

	3 = Fournisseur Mobile 4 = Petit vendeur dans le quart 5 = Autre 9 = ne sait pas		999 999 = inconnu	immédiatement 9 = Ne sait pas	3 = Home 4 = Autre 9 = D/k		9 = Ne sais pas

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____

FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

Le soignant Cluster : |__|__| ménage : |__|__|

Demander au **personnel soignant:** Pourriez-vous me dire combien de jours dans les 7 derniers jours **vous** (le soignant) ont mangé les denrées alimentaires suivantes

	3.10 La nourriture Type d'huile Codes 0 = Ne pas mangé 1 = Rouge palm 2 = Raffiné palm 3 = Le soja, 4 = arachide 5 = Les graines 6 = Huile végétale 7 = Autre , précisez 9 = Ne sais pas	Préparation	3.11 Nombre de jours Mangée à passé 7 jours _/_ = non applicable 1 = 1 jour 2 = 2 jours 3 = 3 jours 4 = 4 jours 5 = 5 jours 6 = 6 jours 7 = 7 jours 9 = Ne sais pas	Quel a été le dernier jour que vous avez consommé cet aliment? 3.12 En ce jour vous avez consommé cet aliment, combien de fois par jour avez-vous consommer? _/_ = non applicable 99 = Ne sais pas	3.13 Si ce n'est pas consommé, pourquoi pas? (Ne pas lire les choix) _/_ = non applicable 1 = Trop cher 2 = Non disponible 3 = N'aime pas 4 = Ne pas préparer cette nourriture 5 = Pas de temps 6 = Autre, précisez 9 = Ne sais pas
A1	1 Huile Type __	Sauce/ragoût	__	___	__
B1		Aliments frits (eg, oeufs, légumes sautés, poisson, poulet, beignets, etc)	__	___	__
C1		Condré (mélange)	__	___	__
D1		D'autres avec de l'huile	__	___	__
A2	2 Huile Type __	Sauce/ragoût	__	___	__
B2		Aliments frits (eg, oeufs, légumes sautés, poisson, poulet, beignets, etc)	__	___	__
C2		Condré (mélange)	__	___	__
D2		D'autres avec de l'huile	__	___	__
A3	3 Huile Type __	Sauce/ragoût	__	___	__
B3		Aliments frits (eg, oeufs, légumes sautés, poisson, poulet, beignets, etc)	__	___	__
C3		Condré (mélange)	__	___	__
D3		D'autres avec de l'huile	__	___	__
E	La farine de blé	Pâte frit (beignets)	__	___	__
F		Pain	__	___	__
G		Pâtes - macaroni, spaghetti	__	___	__
H		Biscuits	__	___	__
J'AI		Gâteaux	__	___	__
J		D'autres aliments avec de la farine de blé	__	___	__
K	Sucre	Thé ou café	__	___	__
L		Le dessert (gâteaux et autres)	__	___	__
M		Haut, coke,	__	___	__
N		Jus (sucette)	__	___	__
O		Bonbon	__	___	__
P		Pap/bouillie	__	___	__
Q		D'autres aliments avec du sucre	__	___	__
R	Bouillon cube	Sauces et crèmes salade	__	___	__
S		Ajoutée à la viande	__	___	__
T		Plats mixtes	__	___	__

Remarques :

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____

7

FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

L'enfant Cluster : |__|__| ménage : |__|__|

Demander au **soignant :** Pourriez-vous me dire combien de jours dans le passé **7 jours l'enfant** a mangé les denrées alimentaires suivantes

	3.14 La nourriture **Type d'huile Codes** 0 = Aucun autre type mangé 1 = Rouge palm 2 = Raffiné palm 3 = Le soja, 4 = arachide 5 = Les graines 6 = Huile végétale 7 = Autre , précisez 9 = Ne sais pas	**Préparation**	**3,15 Nombre de jours Mangée à passé 7 jours** |_/_| = non applicable 1 = 1 jour 2 = 2 jours 3 = 3 jours 4 = 4 jours 5 = 5 jours 6 = 6 jours 7 = 7 jours 9 = Ne sais pas	*(Quel a été le dernier jour l'enfant consommé cet aliment?)* **3.16 En ce jour vous avez consommé cet aliment, combien de fois par jour l'enfant consommer?** |_/_| = non applicable 99 = Ne sais pas	**3.17 Si ce n'est pas consommé, pourquoi pas?** (Ne pas lire les choix) |_/_| = non applicable 1 = Trop cher 2 = Non disponible 3 = N'aime pas 4 = Ne pas préparer cette nourriture 5 = Pas de temps 6 = Autre, précisez 9 = Ne sais pas
A1	1 Huile Type |__|	Sauce/ragoût	|__|	|__|__|	|__|
B1		Aliments frits (eg, oeufs, légumes sautés, poisson, poulet, beignets, etc)	|__|	|__|__|	|__|
D1		Condré (mélange)	|__|	|__|__|	|__|
E1		D'autres avec de l'huile	|__|	|__|__|	|__|
A2	2 Huile Type |__|	Sauce/ragoût	|__|	|__|__|	|__|
B2		Aliments frits (eg, oeufs, légumes sautés, poisson, poulet, beignets, etc)	|__|	|__|__|	|__|
D2		Condré (mélange)	|__|	|__|__|	|__|
E2		D'autres avec de l'huile	|__|	|__|__|	|__|
A3	3 Huile Type |__|	Sauce/ragoût	|__|	|__|__|	|__|
B3		Aliments frits (eg, oeufs, légumes sautés, poisson, poulet, beignets, etc)	|__|	|__|__|	|__|
D3		Condré (mélange)	|__|	|__|__|	|__|
E3		D'autres avec de l'huile	|__|	|__|__|	|__|
E	La farine de blé	Pâte frit (beignets)	|__|	|__|__|	|__|
F		Pain	|__|	|__|__|	|__|
G		Pâtes - macaroni, spaghetti	|__|	|__|__|	|__|
H		Biscuits	|__|	|__|__|	|__|
J'AI		Gâteaux	|__|	|__|__|	|__|
J		D'autres aliments avec de la farine de blé	|__|	|__|__|	|__|
K	Sucre	Thé ou café	|__|	|__|__|	|__|
L		Le dessert (gâteaux et autres)	|__|	|__|__|	|__|
M		Haut, coke,	|__|	|__|__|	|__|
N		Jus (sucette)	|__|	|__|__|	|__|
O		Bonbon	|__|	|__|__|	|__|
P		Pap/bouillie	|__|	|__|__|	|__|
Q		D'autres aliments avec du sucre	|__|	|__|__|	|__|
R	Bouillon cube	Sauces et crèmes salade	|__|	|__|__|	|__|
S		Ajoutée à la viande	|__|	|__|__|	|__|
T		Plats mixtes	|__|	|__|__|	|__|

Remarques :

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____

8

FORMULAIRE 2 : la démographie, le statut socio-économique, alimentaire et fréquence

Cluster : |__|__| ménage : |__|__|

J'aimerais maintenant vous poser quelques questions à propos des changements dans la consommation de nourriture dans votre ménage tout au long de l'année.

3.18	Au cours des 12 derniers mois, au cours de quels mois a fait votre ménage mangent plus d'aliments contenant de l'huile ? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.19	Au cours des 12 derniers mois, au cours de quels mois a fait votre ménage manger moins d'aliments contenant de l'huile ? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.20	Au cours des 12 derniers mois, au cours de quels mois a fait votre ménage mangent plus de nourriture contenant **la farine de blé**? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.21	Au cours des 12 derniers mois, au cours de quels mois a fait votre ménage mangent moins de nourriture contenant **la farine de blé**? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.22	Au cours des 12 derniers mois, au cours de quels mois a votre ménage mangent plus de nourriture ou de boissons contenant du sucre **(excluant sodas et jus)**? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.23	Au cours des 12 derniers mois, au cours de quels mois a votre ménage manger moins d'aliments ou de boissons contenant du sucre **(excluant sodas et jus)**? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.24	Au cours des 12 derniers mois, quels sont les mois au cours des quels votre ménage a-t-il consommé de la nourriture contenant plus de **bouillon cube**? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas
3.25	Au cours des 12 derniers mois, quels sont les mois au cours des quels votre ménage a-t-il consommé de la nourriture contenant moins de **bouillon cube**? **(Entourez toutes les réponses qui s'appliquent)**													
	Oct 2012	Nov 2012	Déc 2012	Jan 2013	Fév 2013	Mars 2013	Avril 2013	Mai 2013	Juin 2013	Juillet 2013	Août 2013	Sept 2013	Aucun change ment	Je ne sais pas

Remarques :

Initiales : Superviseur ____ entrée de données 1 ____ entrée de données 2 ____ 9

3.2. Malaria Survey Questionnaire

ENQUETE PALUDOMETRIQUE TRANSVERSALE DE L'AN 4 DU PROJET « IMPACT OF INSECTICIDE RESISTANCE ON THE EFFECTIVENESS OF LLINS IN THE NORTH OF CAMEROON »

Les informations collectées au cours de cette enquête sont strictement confidentielles au terme de la loi N° 91/023 qui stipule en son article 5 que « les renseignements individuels d'ordre économique ou financier figurant sur tout questionnaire d'enquête statistique ne peuvent en aucun cas être utilisés à des fins de contrôle ou de répression économique ».

QUESTIONNAIRE MENAGE

NUMERO SEQUENTIEL DU MENAGE (ne pas remplir) : _____

Avez-vous utilisé des questionnaires ménages supplémentaires dans le ménage actuel (encercler)? Oui / Non

Si oui, le présent questionnaire est le numéro : /_____/ *sur* /_____/ *questionnaires ménages.*

District de santé: _____

Aire de santé : _____

Cluster (quartier/village) : _____

RENSEIGNEMENTS SUR LA COLLECTE

ENQUETEUR : _____ |__|__|

CONTROLEUR : _____ |__|__|

SUPERVISEUR : _____ |__|__|

DATE DE L'ENQUETE : _____ |__|__| |__|__|

DATE DE FIN D'ENQUETE : _____

RESULTAT DE LA COLLECTE : |__|

1 = Enquête complète (Toutes les sections renseignées)

2 = Enquête incomplète (Une ou plusieurs sections non renseignées)

3 = Questionnaire entièrement non rempli

RAISON DE NON-REPONSE (Si non réponse) |__|

1=Refus 2=Absence 3=Incapacité

APPRECIATION DE LA QUALITE DE L'ENQUETE |__|

1=Très bonne 2=Bonne 3=Moyenne 4=Mauvaise 5=Très Mauvaise

NOVEMBRE 2014

CONSENTEMENT APRÈS INFORMATION

Bonjour ! Je m'appelle _____, je suis infirmier et je travaille au centre de santé de _____ (indiquer le nom du centre de santé/Hôpital dans lequel vous travaillez). Nous effectuons en ce moment, une étude dans ce village concernant divers problèmes liés au paludisme.

Comme vous le savez, le paludisme est un grand problème de santé dans notre pays. Il tue chaque année des milliers de personnes, surtout les enfants de moins de cinq ans et les femmes enceintes. Pour l'instant, nous voulons avoir des informations sur la manière dont le paludisme sévit dans le nord du Cameroun et plus particulièrement dans ce village. Ces informations nous aideront pour comprendre comment mieux lutter contre le paludisme dans le nord Cameroun.

Dans le cadre de cette étude, nous travaillons uniquement avec les enfants âgés de 6 mois à 10 ans. Une fois que vous accepteriez la participation de votre enfant à cette étude, nous vous poserons quelques questions sur les résidents de votre ménage, la possession et l'utilisation des moustiquaires et la santé de l'enfant puis nous examinerons l'enfant. Nous prélèverons également une goutte de sang pour examiner au laboratoire à la recherche de l'infection du paludisme et nous effectuerons un test de diagnostic rapide du paludisme. Si l'enfant est positif au test de diagnostic rapide du paludisme, nous le traiterons immédiatement. L'enfant pourra sentir une légère douleur au moment de le piquer pour effectuer le test rapide ou le prélèvement du sang, il peut également ressentir une fatigue due au médicament que nous lui donnerons. Nous vous conseillons que l'enfant doit bien manger chaque fois avant de prendre son traitement. Toutes les informations que vous nous fournirez resteront strictement confidentielles et ne seront transmises à personne en dehors de l'équipe de l'étude. La participation à cette étude est totalement volontaire. Si vous décidez de ne pas participer cela ne portera aucun préjudice à votre relation avec l'équipe de recherche ou avec le personnel sanitaire de votre localité.

Nous souhaiterions que votre/vos enfant(s) participe(nt) à cette étude qui est particulièrement importante pour la santé dans notre pays.

Avez-vous des questions à me poser sur l'étude ?

Si vous avez des questions à tout moment concernant l'étude, vous pouvez contacter

Dr Djélé Sali (Tél : *77 47 30 28*) ou **M. Moussa Souley (Tél : *77 54 34 72*)** ou Dr **Kouambeng Célestin (Tel : *77 77 12 71*)**

Puis-je commence maintenant l'entretien pour recruter votre /vos enfant(s)?

Je soussigné, _____ certifie avoir été bien informé du but et des procédures de l'étude ci-dessus. Toutes les questions que j'ai posées à l'équipe de recherche ont reçu une réponse appropriée. J'accepte librement la participation de mes enfants à l'étude en question. J'ai été informé du fait que je peux me retirer de l'étude à tout moment sans aucun préjudice.

Nom du parent/tuteur :_____ Signature :

Nom du témoin: _____Signature:

MODULE 0 : IDENTIFICATION DU MENAGE

***Code ménage** (Obligatoire)*	
NUMERO SEQUENTIEL DU MENAGE (ne pas remplir)	

*Q01District de santé1. Garoua Urbain 2. Pitoa 3. Mayo Oulo*_____ |__|__|
Q02Aire de santé(CF Code) _____ |__|__|__|
Q03Cluster (quartier/village) (CF Code) _____ |__|__|__|
Q04 NOM DU CHEF DE MENAGE : _____ |
Q05 NOMBRE DE PERSONNES DANS LE MENAGE (Non compris les visiteurs)	

MODULE 1 : CARACTERISTIQUES DU LOGEMENT

Maintenant nous voudrions avoir des informations sur votre logement.

	1 - CARACTERISTIQUES DU LOGEMENT *(Encerclez d'abord le code correspondant à la réponse de l'enquêté, puis reportez-le dans le bac prévu à cet effet)*			
Q06	***A***. *Quel est le nombre total de pièces de votre logement ?*			|__|__|
	B. *Combien de pièces utilisez-vous habituellement pour dormir ?*			|__|__|
Q07	*Quel est votre principal mode d'approvisionnement en eau de boisson ?*	*01=Robinet individuel SNEC/CAMWATER* *02=Autre robinet individuel* *03=Robinet collectif SNEC/CAMWATER (utilisateur principal)* *04=Revendeur d'eau de robinet SNEC/CAMWATER* *05=Borne fontaine publique* *06= Autre robinet collectif*	*07=Forage* *08= Puits à pompe* *09=Puits/Source aménagée* *10= Puits /Rivière/Lac/ Marigot /Source non aménagé(e)* *11= Eau de pluie* *12 =Eau minérale* *13=Autre (à préciser….)*	|__|__|
Q08	*Quel est le principal matériau des murs de votre logement ?*	*1=Béton/Parpaing/Briques cuites* *2=Pierre de taille* *3=Planche* *4=Carabote*	*5=Terre/Brique simple* *6=Pise/terre battue* *7= Nattes/Chaume/Feuille* *8=Autre (à préciser..)*	|__|
Q09	*Quel est le principal matériau du toit de votre logement ?*	*1=Ciment* *2=Tôle/Tuile* *3=Nattes /Chaume/Feuille*	*4=Terre* *5=Autre (à préciser ….)* *……*	|__|
Q10	*Quel est le principal matériau du sol de votre logement ?*	*1=Ciment* *2=Carreaux* *3=Bois*	*4=Terre* *5=Autre (à préciser….)…….*	|__|

MODULE 2 : COMPOSITION DU MENAGE ET UTILISATION DES MOUSTIQUAIRES

Je vais vous demander les noms et prénoms de tous les membres du ménage, en commençant par le chef de ménage et vous poser quelques questions concernant chaque membre.

Q11	*Q12*	*Q13*	*Q14*	*Q15*	*Q16*	*Q17*	*Q18*	*Q19*
N°	*Noms et prénoms des membres du ménage*	*Quel est le lien de parenté de (Nom) avec le Chef de Ménage ?* *CF CODES*	*(Nom) est de quel sexe ?* *1=Masculin* *2=Féminin*	*Quel est l'âge de (Nom) ?* *Inscrivez l'âge en années révolues. 98 pour NSP)*	*Est-ce que (Nom) vit habituellement dans ce village/ quartier?* *1 = Oui 2 = Non*	***Si la réponse est « Oui » à la question précédente (question 15) ,*** *(Nom) a-t-il séjourné plus de six mois dans ce village/quartier* *1 = Oui 2 = Non*	*(Nom) a-t-il dormi sous moustiquai nuit derniè* *1=Oui* → Q19 *2=Non* → MODULE 3	*Si oui, (Nom) a dormi sous quelle moustiquaire ? (indiquer le numéro de la moustiquaire à partir du ODULE 4)*
		└─┴─┘	└─┘	└─┴─┘	└─┘	└─┘	└─┘	└─┴─┘
		└─┴─┘	└─┘	└─┴─┘	└─┘	└─┘	└─┘	└─┴─┘
		└─┴─┘	└─┘	└─┴─┘	└─┘	└─┘	└─┘	└─┴─┘
		└─┴─┘	└─┘	└─┴─┘	└─┘	└─┘	└─┘	└─┴─┘
		└─┴─┘	└─┘	└─┴─┘	└─┘	└─┘	└─┘	└─┴─┘

		|__|__|	|__|	|__|__|	|__|	|__|	|__|	|__|__|
		|__|__|	|__|	|__|__|	|__|	|__|	|__|	|__|__|
		|__|__|	|__|	|__|__|	|__|	|__|	|__|	|__|__|
		|__|__|	|__|	|__|__|	|__|	|__|	|__|	|__|__|
		|__|__|	|__|	|__|__|	|__|	|__|	|__|	|__|__|
		|__|__|	|__|	|__|__|	|__|	|__|	|__|	|__|__|

CODES POUR Q12: LIEN DE PARENTÉ AVEC LE CHEF DE MÉNAGE

CHEF DE MÉNAGE
……**01**

FEMME OU MARI
…… **02**

FILS OU
FILLE…….
……**03**

GENDRE OU BELLE-FILLE
……**04**

PETIT-FILS/FILLE
…… **.05**

PÈRE/MÈRE
……
……**06**

BEAU-PÈRE/BELLE-MERE
……..…**07**

FRÈRE OU SŒUR
……
08

NIÉCE/NEVEU DIRECT
…….. ….**09**

NIÉCE/NEVEU PAR
ALLIANCE……..**1**
0

AUTRES PARENTS
……
.11

ADOPTÉ/EN GARDE/ENFANT DE
LA FEMME/MARI…..**12**

SANS PARENTÉ
……
….**13**

NE SAIT PAS
…….
……

MODULE 3 : POSSESSION ET CONNAISSANCE DU LIEU D'ACHAT DES MOUSTIQUAIRES

Maintenant nous voudrions avoir des informations sur l'achat, la possession et l'utilisation des moustiquaires de votre ménage

No	Questions et Filtres	Reponses	Code
Q20	Avez-vous une moustiquaire dans cette maison installée et utilisée par les membres du ménage? ***(Si oui aller à Q19)***	1. Oui 2. Non	|____|
Q21	Si non, pourquoi n'avez-vous pas de moustiquaire dans votre maison? ***(ALLER AU MODULE 5)***	1. Je n'ai pas les moyens (financiers) 2. Ce n'est pas nécessaire 3. J'utilise autre chose 4. Il n'y a pas beaucoup de moustiques ici 5. Je n'aime pas la moustiquaire 6. Par oubli 7. Autres (à préciser)	|____|
Q22	Si oui, est- ce que les membres de votre ménage dorment sous une moustiquaire de manière continue pendant toute l'année?	1. Oui 2. Non 9. Ne sais pas	|____|
Q23	Combien de moustiquaires y a-t-il dans votre maison ?		|___||___|
Q24	Combien de ces moustiquaires sont imprégnées ***(c'est à dire qui ont été trempées dans un liquide depuis au moins 6 mois)*** (99 si NSP)		|__||__|

MODULE 4 : POSSESSION ET UTLISATIONS DES MOUSTIQUAIRES

		Moustiquaire 1	**Moustiquaire 2**	**Moustiquaire 3**	**Moustiquaire 4**	**Moustiquaire 5**	**Moustiquaire 6**
Q25	Demander à l'enquêté à voir les moustiquaires du ménage 1. VUE 2. Non VUE	|___|	|___|	|___|	|___|	|___|	|___|
Q26	Depuis combien de mois votre ménage a t-il cette moustiquaire? ***(00 si moins d'un mois et 95 si 36 mois ou plus)***	|___||___|	|___||___|	|___||___|	|___||___|	|___||___|	|___||___|
Q27	Comment avez-vous obtenu cette moustiquaire ? 1. Achat 2. MINSANTE 3. don d'une ONG 4. Famille/ami 5. Autre 9. Ne Sait Pas	|___|	|___|	|___|	|___|	|___|	|___|
Q28	Cette moustiquaire est de quelle marque ? 1. Permanet 2. Olyset 3. Autre MILDA 4. Moustiquaire non traitée 9. Ne sait pas	|___|	|___|	|___|	|___|	|___|	|___|
Q29	Est- ce que la nuit dernière, quelqu'un a dormi sous cette moustiquaire? 1. Oui 2. Non 9. NSP	|___|	|___|	|___|	|___|	|___|	|___|

Q30	SI oui, qui a dormi sous cette moustiquaire la nuit dernière ? ***(Noter le numéro (Q11) des personnes à partir du tableau ménage dans le bac et remplir le module utilisation des moustiquaires)***	|___| |___| |___| |___|	|___| |___| |___| |___|	|___| |___| |___| |___|	|___| |___| |___| |___|	|___| |___| |___| |___|	|___| |___| |___| |___|
Q31	Cette moustiquaire a-t-elle des trous ? **1.** Oui → Aller à Q32, Q33 et Q34 **2.** Non **9.** Ne Sait Pas } **Aller au Module 5**	|___|	|___|	|___|	|___|	|___|	|___|
Q32	Nombre de trous pouvant laisser passer un doigt de la main	|___|	|___|	|___|	|___|	|___|	|___|
Q33	Nombre de trous pouvant laisser passer un poing (main fermée)	|___|	|___|	|___|	|___|	|___|	|___|
Q34	Nombre de trous pouvant laisser passer la tête d'une personne	|___|	|___|	|___|	|___|	|___|	|___|

MODULE 5 : EXAMEN PHYSIQUE ET BILAN BIOLOGIQUE DU PALUDISME POUR LES ENFANTS DE MOINS DE 10 ANS DANS LE MENAGE

Situation des enfants éligibles du ménage (enfants de moins de 10ans)

Code ménage	*Code enfant*	*Nom et prénom*	*Age (mois)*		*Température (°C)*	*Conjonctives*	*L'enfant a-t-il eu la fièvre il y a deux semaines (O=Oui ; N=Non)*	*TDR (O=Oui N=Non R=Refus)*	*Résultat*			*ACT (O=Oui N=Non)*	*Parasites*		
													P.falciparum	*P. ovale*	*Charge parasitaire*
										Pf	***pan***				
									Positif						
									Négatif						
									Invalide						
										Pf	***pan***				
									Positif						
									Négatif						
									Invalide						
											P pan				

	f	
Positif		
Négatif		
Invalide		

	P f	***pan***
Positif		
Négatif		
Invalide		

	P f	***pan***
Positif		
Négatif		
Invalide		

	P f	***pan***
Positif		
Négatif		
Invalide		

Appendix 4: Socio-economic and Demographic characteristics of children and their parents in the Pitoa and Mayo-oulo health districts, North Region of Cameroon 2014 (n=362)

Gender of head of family	% (n)	**Family head relationship with child**	% (n)
Male	86.2% (312)	Family head	1.9% (7)
Female	1.8% (50)	Son/daughter	55.5% (201)
Religion	% (n)	Child	42.5% (154)
Christian	41.2% (149)	**Care giver relationship to family head**	
Muslim	56.1% (203)	Husband	8.6% (31)
No religion	2.5% (10)	Wife	91.4% (331)
Marital status of care giver		**Marital status of family head**	
Married	98.3% (356)	Married	100% (362)
Widow	1.7% (6)		
Level of education of care giver		**Level of conventional education of family head**	
None	50.8% (184)	None	26.5% (96)
Primary	47.2% (171)	Primary	71.8% (260)
Secondary	1.9% (7)	Secondary	1.7% (6)

Socio-economic characteristics (continued)

Community membership	% (n)	**Cooperative group**	
Yes	45.3% (164)	Yes	17.1% (62)
No	54.7% (198)	No	82.9% (300)
Religious group		**Njangi group**	
Yes	35.4% (128)	Yes	39.5% (143)
No	64.6% (234)	No	60.5% (219)
Women group (n=50)		**Dance group**	
Yes	4.0% (2)	Yes	8.6% (31)
No	96.0% (46)	No	91.4% (331)
Sports group		**Alumni group**	
Yes	5% (18)	No	100% (362)
No	95% (344)	**Care giver principal activity**	
Cultural group		Skilled	1.1% (4)
Yes	25.7% (93)	Business	1.7% (6)
No	74.3% (269)	Personal land farming	5.2% (19)
Family head principal activity		Rented land farming	0.8% (3)
Skilled	2.5% (9)	Mechanized farming	14.0% (5)
Personal land farming	55.5% (201)	Housewife	77.3% (280)
Rented land farming	14.0% (5)	Petit trader	12.4% (45)
Mechanized farming	3.3% (12)	**Care giver employment status**	
Housewife	1.1% (4)	No work since 12months	11.6% (42)

Trader	2.8% (10)	Working for 12months/5days/week	2.8% (10)
Petit trader	13.5% (49)	Seasonal worker	3.9% (14)
No occupation	19.9% (72)	No response	81.8% (296)
Family head employment status			
Working for 12months/5days/week	1.4% (5)		
Seasonal worker	21.3% (72)		
No response	78.7% (285)		

Socio-economic characteristics (continued)

Type of housing		**House ownership**	
Separate rooms	53.3% (193)	Personal	87% (315)
Apartments	6.9% (25)	Free housing	7.7% (28)
Buckaroo	9.4% (34)	Renting	1.7% (6)
Several same huts	23.2% (84)	Hypothetic	2.8% (10)
Several different huts	7.2% (26)	Others	0.8% (3)
Type of toilet		**Source of cooking energy**	
Modern	3.6% (13)	Electricity	1.7% (6)
Improved pit latrine	1.1% (4)	Charcoal	0.3% (1)
Pit latrine	28.7% (104)	Wood	98.1% (355)
Open pit	14.9% (54)	**Source of water**	
Others	51.7% (187)	Pipe borne water	8.6% (31)
Source of light		Bore hole/wells	51.7% (187)
Gas or kerosene lantern	15.5% (56)	Sachet water	1.4% (5)

Florescent lamp	19.3% (70)	Rain water	38.4% (139)
Electricity	56.9% (206)	**Location of portable water**	
Others	8.3% (30)	Inside the main house	11.6% (42)
Possession of Household Assets **Carpet**		In the compound	6.4% (23)
Yes	9.1% (33)	Else where	82.0% (297)
No	90.9% (329)	**Duration to fetch water**	**Minutes**
Table		Mean	362
Yes	46.1% (167)	Median	14.68
No	53.9% (195)	Min	15.00
Cupboard		Max	0
Yes	21.3% (77)	**Chair**	
No	78.7% (285)	Yes	26.5% (96)
Boat		No	73.5% (266)
No	100% (362)	**Sewing machine**	
Wristwatch		Yes	13.5% (49)
Yes	14.4% (52)	No	86.5% (313)
No	85.6% (310)	**Engine boat**	
Radio		Yes	0.3% (1)
Yes	42.5% (154)	No	99.7% (361)
No	57.5% (208)	**Clock**	
Cable_tv		Yes	18.2% (66)
Yes	17.7% (64)	No	81.8% (296)
No	83.3% (298)	**Television**	

Mobile		Yes	33.1% (120)
Yes	70.2% (254)	No	66.9% (242)
No	29.8% (108)	**Fixed phone**	
Axe		No	100 (362)
Yes	55.2% (200)	**Cutlass**	
No	44.8% (162)	Yes	66.3% (240)
Gas cooker		No	33.7% (122)
No	100% (362)	**Car**	
Freezer		11.9% (43)	
Yes	9.4% (34)	88.1% (319)	
No	90.6% (328)	**Refrigerator**	
Bike		Yes	9.4% (34)
Yes	22.9% (83)	No	90.6% (328)
No	77.1% (279)	**Motorbike**	
Animals		Yes	25.4% (92)
Yes	43.9% (159)	No	74.6% (270)
No	56.1% (203)	**Animal carriage**	
		Yes	0.3% (1)
		No	99.7% (361)

Socio-economic characteristics continued

Range	Cows	Caprins	Sheep	Pig	Chicken	Birds	Rabbits	Horse	Grass cutters and other ruminants
None	71.5% (259)	78.7% (285)	68.8% (249)	100% (362)	75.4% (272)	95.3% (345)	100% (362)	100% (362)	100% (362)
1-5	7.2% (26)	5.5% (20)	9.7% (35)	0 % (0)	9.1% (33)	1.7% (6)	0% (0)	0% (0)	0% (0)
6-10	18.5% (67)	14.6% (53)	11.9% (43)	0 % (0)	6.6% (24)	3.0% (11)	0% (0)	0% (0)	0% (0)
>100	2.8 (10)	1.1% (4)	9.7% (35)	0 % (0)	8.8% (32)	0% (0)	0% (0)	0% (0)	0% (0)

Appendix 5

Conference presentations and articles published

Conference presentations poster and oral

Socio-economic burden and impact of disease in under-ten children, in the North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith Ngondi, Julius Oben. University of Yaounde I, Yaounde. One Health Platform, 6th World One Health Virtual Congress: Advancing science to improve health and security. *Edinburg University, Scotland.* 30th October–03rdNovember2020. **Poster presentation.** See more on www.worldonehealthcongress.org

Host immunity to malaria infection, anaemia and socio-economic impact in under-ten children, for sustainable development in the North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. Organization for Women in Science for the Developing World (OWSD), National Chapter Conference: Official Launching Ceremony and Workshop. Amphi 700, *University of Yaounde I, Yaounde, Cameroon.* 18 December 2019. **Poster presentation**

Relationship between nutritional status, malaria, anaemia and socio-economic impact amongst under-ten children in the North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. Federation of African Nutrition Societies Conference: 4th International FANUS Conference: Nutrition in Action for Sustainable Development in Africa. Lemigo Hotel, *Kigali, Rwanda.* 25 – 30 August 2019. Book of abstract page 54, number SY IV.02. Parallel session IV. **Oral presentation**

The relationship between nutritional status, malaria infection, anaemia and socio-economic impact for sustainable development in under-ten children, North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. Alexander Von Humboldt Conference: 1st International AvH Gender Networking Conference: Mindset Change and Gender Empowerment for Sustainable Development. Djeuga Palace Hotel, *Yaounde, Cameroon.* 22 - 25 Jan 2019. Parallel session 3. **Oral presentation**

Host immunity to malaria infection, anaemia and socio-economic impact in under-ten children, North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi,

Julius Oben. University of Yaounde I. Multilateral Initiative on Malaria (MIM) Conference: 7th MIM Pan African Conference: Two Decades of Progress, Challenges and Perspectives in Ending Malaria. Centre International de Conférence Abdou Diouf, *Dakar, Senegal.* 15 -20 April 2018. Book of abstracts page number 79. Parallel session, Immunology 2. Presentation 335. Room 201. **Oral presentation**

Host immunity to malaria infection: Effects on malnutrition and anaemia in under-ten children, North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. Federation of African Immunological Societies Conference: 10th FAIS African Congress of Immunology. Immunology for a Healthy Africa. Hotel Le Royale Conference Hall, *Hammamet, Tunisia*. 03 -- 07 December 2017. Book of abstracts page …..abstract number…. Parallel session. **Oral presentation**

Relationship between Nutritional status, Malaria, Anaemia and Socio-economic Impact amongst under-ten Children in the North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. Cameroon Nutritional Science Society (CNSS) Conference: 3rd National Nutrition Conference: Nutrition for Longevity and Sustainable Development. Amphi 700, *University of Yaounde I, Cameroon.* 10 – 12 October 2017. Book of abstracts page…. Abstract number….. **Oral presentation**

Host immunity to malaria infection, anaemia and socio-economic impact amongst under-ten children in the North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. Keystone Symposia Malaria Conference: Malaria from Innovation to Eradication. Speke Resort Conference Center, *Kampala, Uganda.* 19 -- 23 February 2017. Book of abstracts page…. Poster number…..**Poster presentation**

Cross- sectional assessment: Relationship between malaria, anaemia, nutritional and socio-economic status amongst under-ten children in the North Region of Cameroon. Nobelle Sakwe, Jude Bigoga, Judith-Laure Ngondi, Julius Oben. University of Yaounde I. 23rd Cameroon Bioscience Society Conference: Bioscience and Integrated Management of Living Systems. *Université des Montagnes, Bangangté, Cameroon.* 1 -- 3 December 2016. Parallel session 2. Book of abstracts page …. Abstract number…. **Oral presentation**

Host immunity to malaria infection, anaemia and socio-economic impact among children less than 10 years in Northern Cameroon. Nobelle Sakwe, University of Yaounde I, Yaounde Cameroon. American Society of Tropical Medicine and Hygiene (ASTMH) Conference: 65th Annual Meeting, Atlanta Marriott Marquis and Hilton Atlanta, *Atlanta,*

Georgia, USA. 13 – 17 November 2016. Book of abstracts page 302. Abstract number 962. **Accepted for poster presentation**

See more on www.astmh.org

Nutritional status and malaria infection in children less than 10years old in the North Region of Cameroon. Nobelle Sakwe, Judith-Laure Ngondi, Jude Bigoga, Julius Oben. University of Yaounde I. African Nutrition Leadership Programme: The 14th ANLP Seminar, *Elgro River Lodge, District Potchefstroom, South Africa.* 08 -- 18 March 2016. **Oral presentation**

Article Published

Sakwe N, Bigoga J, Ngondi J, Njeambosay B, Esemu L, Kouambeng C, Nyonglema P, Seumen C, Gouado I, Oben J **(2019). Relationship between malaria, anaemia, nutritional and socio-economic status amongst under-ten children, in the North Region of Cameroon: A cross-sectional assessment.** ***PLoS One*** 2019 Jun 21; **14**(6): e0218442.

Sakwe N, Bigoga J, Ngondi J, Njeambosay B, Esemu L, Kouambeng C, Nyonglema P, Seumen C, Gouado I, Oben J . **Influence of nutritional status on malaria parasitaemia, nutritional status on anaemic status, malaria parasitaemia on anaemia severity amongst under-ten children, in the North Region of Cameroon.** (Paper under review).

Sakwe N, Bigoga J, Ngondi J, Njeambosay B, Esemu L, Kouambeng C, Nyonglema P, Seumen C, Gouado I, Oben J . **Effects of malnutrition on anti-malarial IgG1 and IgG3 antibody responses against crude *Plasmodium falciparum* 3D7 antigen in malaria-infected under-ten children, in the North Region of Cameroon.** (Paper under review).

SUMMARY

ARTICLE PUBLISHED

PLOS ONE

RESEARCH ARTICLE

Relationship between malaria, anaemia, nutritional and socio-economic status amongst under-ten children, in the North Region of Cameroon: A cross-sectional assessment

Nobelle Sakwe[1,2], Jude Bigoga[2,3]*, Judith Ngondi[1], Boris Njeambosay[2], Livo Esemu[2], Célestin Kouambeng[3], Philomena Nyonglema[4], Clovis Seumen[2], Inocent Gouado[5], Julius Oben[1]

1 Laboratory of Nutrition and Nutritional Biochemistry, Department of Biochemistry, University of Yaounde I, Yaounde, Cameroon, **2** Molecular Parasitology and Disease Vector Research Laboratory, the Biotechnology Center, University of Yaounde I, Yaounde, Cameroon, **3** National Malaria Control Programme, Yaounde, Cameroon, **4** Laboratory of Immunology, University of Yaounde I, Yaounde, Cameroon, **5** Laboratory of Nutrition and Food Sciences, Department of Biochemistry, University of Douala, Douala, Cameroon

* jbigoga@gmail.com

Citation: Sakwe N, Bigoga J, Ngondi J, Njeambosay B, Esemu L, Kouambeng C, et al. (2019) Relationship between malaria, anaemia, nutritional and socio-economic status amongst under-ten children, in the North Region of Cameroon: A cross-sectional assessment. PLoS ONE 14(6): e0218442. https://doi.org/10.1371/journal.pone.0218442

Editor: Bishwajit Ghose, University of Dhaka, BANGLADESH

Received: October 24, 2018

Accepted: May 21, 2019

Published: June 21, 2019

Data Availability Statement: All relevant data are within the manuscript.

Funding: The author(s) received no specific funding for this work.

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

Abstract

Background

Despite malaria, malnutrition and anaemia being major public-health challenges in Cameroon, very little has been reported on the interaction between these interconnected health determinants. This study therefore sought to investigate the relationship between malaria, anaemia, nutritional and socio-economic status amongst under—ten children living in six localities within two health districts in the North Region of Cameroon.

Methods

Accordingly, a cross- sectional survey was conducted during the peak malaria season in November 2014, in Pitoa and Mayo-Oulo Health Districts. Three hundred and sixty eight children aged 6months—10 years were enrolled. Structured questionnaires were used to assess socio-economic status. Anthropometric indices were taken using standard methods and nutritional status assessed by calculating Height for Age (HA), Weight for Age (WA) and Weight for Height (WH) z-scores to determine stunting, underweight and wasting respectively. Finger-prick blood samples were used to prepare thin and thick blood smears for microscopy. Whole blood was collected to determine the PCV and blood spots on filter paper were used to extract plasmodium DNA for speciation by PCR.

Results

Overall prevalence rates of malaria, malnutrition and anaemia were 32.9%, 54.1% and 20.6% respectively. Stunting, underweight and wasting were detected in 56.9%, 63.5% and

34.8% of the children respectively. There was a significant association between malaria and malnutrition [OR = 1.89, (95% CI: 1.12–3.19); (p = 0.017)]. Malnutrition was also strongly associated with malaria status [OR = 2.07, (95% CI: 1.22–3.53); (p = 0.007)]. The prevalence rates of mild, moderate and severe anaemia were 8.1%, 9.2% and 3.3% respectively. Both malaria status and anaemia correlated with development index [OR = 0.75, (95% CI: 0.58–0.99); (p = 0.042)] and [OR = 1.45, (95% CI: 1.05–2.00); (p = 0.023)] respectively.

Conclusion

Our findings show a synergistic relationship between malaria and malnutrition. Effective collaboration between malaria control and nutrition intervention programmes is essential for proper case management and improved socio-economic status.

Introduction

Malaria, anaemia, and malnutrition are public-health challenges in paediatric populations in sub-Saharan Africa with malaria and undernutrition being major contributors to childhood mortality [1]. Malaria is a serious vector borne disease affecting mainly impoverished communities. Although children are usually the most vulnerable, the disease can be worsened in those that are malnourished [2, 3]. Despite efforts by control programmes to reduce the disease burden, it still places the lives of many in jeopardy, especially in sub-Saharan Africa, where over 90% of the deaths occur, 78% of which are in children less than 5 years old [4–6]. Recent data show that an estimated 435.000 people died of malaria in 2017, with 219 million cases of malaria reported in 90 countries [7]. In Cameroon, malaria is responsible for 34% of consultations and 47% hospitalization, 21% hospital related deaths of which 38% are in children less than 5years [8]. Previous reports in the same study sight revealed prevalence rates of malaria, malnutrition and anaemia to be 39%, 40.2% and 68.2% respectively [9].

On the other hand, undernutrition is known to cause more than 50% of deaths among under-five children [10]. It is also responsible for most premature mortalities in developing countries that are plagued by poverty and ignorance [11]. Although the World Health Assembly emphasises on the need for all children to be free of malnutrition in all its forms, and stunting rates are dropping, about 159 million around the world are still affected, the number of overweight children is increasing alarmingly and wasting still threatens the lives of 50 million children across the globe [12]. In Cameroon, about 52.000 children reportedly die of malnutrition annually with four of the regions (East, Adamawa, North, Far North) being the most affected [13].

Studies highlighting the relationship between malnutrition and malaria have so far been controversial and complex. While some believe that malaria may cause malnutrition, others observed that malnutrition might enhance susceptibility to malaria [14]. In heavily endemic areas, it is almost inevitable that malaria infection will be associated with anaemia, although it may not be the prime cause of it [15, 16]. Anaemia, an indicator of both poor nutrition and poor health is a common and sometimes serious complication of *P. falciparum* infection [17, 18]. This is frequent in developing countries where its causes are multi-factorial. Anaemia impairs normal development in children and it constitutes a major public health problem in young children in the developing world with wide social and economic implications [19], Cameroon inclusive.

Several studies [1, 5, 14, 20, 21, 22], have investigated the problem of malaria related to anaemia and nutritional status in children, in different regions of the world, most of whose findings have been inconsistent [23]. In Cameroon, very little information exists on the interaction between these interconnected health determinants especially in the northern parts that bear the highest burden. Thus this study aimed at contributing to a better understanding of the relationship between malaria, malnutrition, anaemia and socio-economic status amongst children (0-10years) in 2 Health districts, (Pitoa and Mayo- Oulo) in the North Region of Cameroon. It will provide baseline information for future studies that will guide better management and control where both malaria and malnutrition prevail.

Materials and methods

Study area and study population

The study was conducted in two health districts, namely, Pitoa Health District, 9.21˚N, 13.31˚E and Mayo-Oulo Health District, 9.46˚N, 13.44˚E, and both peri-urban settings, in the north region of Cameroon. The area is the center of trade of the surrounding agricultural region and also houses several textile processing facilities. Pitoa has a population of about 108.611 inhabitants while Mayo-Oulo is inhabited by about 91.501 individuals. The population depends almost entirely on agriculture (including gardening) for subsistence. Crops grown include; rice, maize, millet, cotton, and sorghum for Pitoa whereas beans peanuts and maize are cultivated in Mayo-oulo. The study area is predominantly populated by individuals of the Hausa ethnic group. Polygamy is a common practice with a number of wives and their children living in separate huts within the family compound. Both health districts have a Sudanese type climate with an annual average rainfall of 700–1000mm, annual average temperature of 26.0–33.0˚C and relative humidity of 15%. Malaria is endemic in the region, with a seasonal mode of transmission, lasting about 6 months and peaking generally during the months of September—November when rainfall is highest. *Plasmodium falciparum* is the predominant (>98%) parasite species in the area.

The study population consisted of children aged 6months to 10 years and of both sexes, after full informed consent. They weighed between 4kg and 30.8 kg. Children with recent (during the last 48hours) or current fever, were considered symptomatic, and were tested using Rapid Diagnostic Tests and immediately treated for free, following the national guidelines for managing uncomplicated malaria, if found positive. Blood smears were taken for all children irrespective of fever status for microscopy.

Study design and sampling procedures

This was a cross-sectional study, carried out in November 2014, during the peak malaria transmission season. It was conducted as part of a larger study to determine the impact of insecticide resistance on Long Lasting Insecticidal Nets (LLIN) and malaria burden in the North of Cameroon.

Initially, a census of all households in the study localities was conducted and all houses given unique survey numbers. Subjects in each household were registered and information including name, sex, age, relationship to the head of the household, demographic and socio-economic data as well as risk factors like, marital status, caregiver level of education, employment status of family head, type of housing, availability and principal source of electricity, drinking water and type of toilets at home, possession of household equipment and participation in social groups, were recorded.

The head of each household was the principal respondent and provided all necessary information for the household. Following completion of the census, the study participants were

recruited from 58 households through systematic random sampling. Visits to households were in the afternoon to include children attending school. The investigation methods included the use of structured questionnaires, clinical evaluation and laboratory investigations.

Ethical considerations

The study was carried out as part of a larger project to assess the Impact of Insecticide Resistance on the effectiveness of LLINS and malaria burden in the North of Cameroon, for which an ethical clearance was obtained from the National Ethics Committee of Cameroon (No. 102/CNE/SE/09; FWA IRB00001954). Participation in the study was strictly voluntary with written informed consent from legal parents/guardian.

Field procedures- data collection

Structured questionnaire. A Structured questionnaire was used for data collection. Certified Nurses, Medical Laboratory Technicians and Interviewers were trained for data and sample collection. The Questionnaires were administered following an informed consent process, to record socio-economic and demographic data such as sex of the child, age of the child, educational status, occupation as well as medical history as concerns malaria, questions on malaria prevention practice like use of LLIN, nutrition /feeding habits and anthropometric measurements were included for each household. Weighing scales were calibrated daily using 5kg weight. All data collection instruments were pretested and validated.

Clinical evaluation and anthropometric measurements. Clinical evaluations were carried out by trained medical personnel (Nurses). Axillary body temperature of the children were taken using digital thermometers and recorded in ˚C (+0.5˚C). The conjunctivas were also examined for paleness (signs of anaemia). Conjunctival pallor was evaluated by everting the lower eyelid and examining the palpebral conjunctiva. This was recorded as either coloured or not coloured. A child was considered febrile if he/she had temperature $\geq$ 37.5˚C or had reported they had fever during the past 48 hours.

With regards to nutritional status, measurements were performed according to standard anthropometric methods and related to age and sex [24]. This was carried out by 2 trained data collectors/interviewers with at least University education, hailing from the region with a good mastery of the local vernacular and French. The children were weighed wearing light clothes only and no shoes by using a portable digital scale accurate to 0.1 kg. In the case of infants ($<$ 2 years), the weight of the mother was recorded and subtracted from the weight of the mother plus the infant, to get the weight of the infant. Length (for children aged $<$ 2 years), and height (for children $>$2 years of age) were measured to the nearest 0.1cm using a non-stretchable tape. Height was measured in a standing position while Length was measured using the same tape, with the child in a horizontal position.

Nutritional status assessment. As indicators of nutritional status, Z-scores for weight-for-height (WH), weight-for-age (WA) and height-for-age (HA) were used. In order to analyse the nutritional status, the Weight and Height of the boys and girls were compared to those of same aged boys and girls measured in the National Centre for Health Statistics (NCHS)/WHO growth reference curves using the nutrition module of the Epi Info 2000 programme. Children were classified as stunted, underweight, or wasted if their HAZ, WAZ, or WHZ was $<$ −2, respectively. While Z-scores $<$ -3.0 was used to classify severely stunted, severely underweight and severely wasted children. The WHO Classification for assessing severity of malnutrition by percentage prevalence ranges of these indicators among children was followed. Children with $<$ −2 and $<$ −3 SD were classified as malnourished and severely malnourished respectively.

Socio-economic status (SES) assessment. To determine the SES of the households, the development index (a composite variable) was computed using socio-economic indicators. Indicators for socio-economic status included; the number of people living in a household, the level of education of caregiver and family head, employment status of family head, type of housing, type of toilet, source of lighting and cooking energy, availability of potable water and possession of household assets.

All the socio-economic indicators were recoded in ranking order of importance and the sum of scores calculated for each household. A straight forward count of the possessions was made where one point was given for each of the possessions to obtain the development index. The index was then divided into quintiles, SES 1 to SES 5 to determine poorest, poor, average, rich and richest SES as seen below (Table 1).

Blood collection and processing. All febrile cases (temperature $\geq$ 37.5 ˚C) or cases with history of fever (during the past 48 hours) were tested with an RDT (SD- Bioline, Standard Diagnostics, INC. Lot No. 05FK60) using finger prick blood samples, and treatment given, if found positive. Still, finger prick blood (one to two drops) was immediately dispensed on properly labelled slides and used to prepare thin and thick blood smears from all participants (irrespective of fever status) for microscopy; blot filter paper (Whatman 3MM) [25] for parasite DNA isolation and species identification by polymerase chain reaction (PCR) and for the determination of the packed cell volume (PCV).

Malaria microscopy. The thick and thin blood smears were air-dried, fixed with methanol (thin smears only) and then stained with Giemsa stain and microscopically examined for the presence of malaria parasite following standard protocol [26]. Each slide was examined by two independent microscopists blinded of each other's results. Parasite density was determined by counting the number of parasites present per 200 white blood cells (WBC) in a thick smear and multiplying by 40 to arrive at an approximate parasite count per microliter of blood. This was based on the assumption that the average WBC count was 8.000/uL blood. The thin smears were used to identify the *Plasmodium* species [26].

PCR identification of *Plasmodium* species. Plasmodium species identification was further confirmed by PCR, using *Plasmodium* DNA extracted by the Chelex Method from a portion of each blood sample collected on filter paper [25, 27].

Determination of the Packed Cell Volume. The PCV was determined by microcentrifugation of the blood filled microcapillary tubes. Anaemia in children was defined as a PCV less than 33% and/or Hb level less than 11g/dl. However, this was further categorized as either mild (Hb between 10.1 and 10.9 g/dL), moderate (Hb between 7.0 and 10.0 g/dL) and severe (Hb $<$ 7.0 g/dL).

Definitions and end-points

Fever was defined as axillary temperature $\geq$ 37.5˚C. Malaria parasitaemia was defined as any asexual parasitaemia detected on a thick or thin blood smear. Confirmed malaria (CM) was

Table 1. Assessment of socio- economic status.

SES	Quintile
Richest (SES 5)	Very high level
Rich (SES 4)	High level
Average (SES 3)	Medium level
Poor (SES 2)	Low level
Poorest (SES 1)	Very low level.

https://doi.org/10.1371/journal.pone.0218442.t001

defined as the presence of any species of *plasmodium*, with an axillary temperature of ≥ 37.5˚C or reported fever in previous 48 hours. The term "parasitaemia" refers to a positive result on expert microscopy. A Haemoglobin level of < 11g/dL was classified as anaemic and further categorized as mild (Hb between 10.1 and 10.9 g/dL), moderate (Hb between 7.0 and 10.0 g/dL) and severe (Hb< 7.0 g/dL). Height–for- age (HAZ), Weight- for-age (WAZ) and Weight-for- height (WHZ) Z-scores were calculated from Center for Disease Control (National Center for Health Statistics)/ World Health Organization (2006) reference values using Epi Info 2000 software (version 2000, Atlanta, GA). Children were classified as stunted, underweight or wasted if the HAZ, WAZ and WHZ was < -2 SD of the NCHS/WHO reference median, respectively. They were classified as having severe stunting, wasting or underweight, if the HAZ, WHZ, or WAZ was <-3, respectively.

Statistical analyses

All data collected were entered in Microsoft Excel 2010 and statistically analyzed using the Statistical Package for Social Sciences (SPSS) Standard version, Release 20.0 (SPSS Inc. 2012). Scale (continuous variables) as parasitaemia and Z-scores were described using measurements of central tendencies (Geometric Mean, Median) and measurements of dispersion (Standard Deviation and range while highlighting the minimum and the maximum values). The scale variables were screened for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests for normality as to decide whether parametric or non-parametric tests should be used for analysis. The variables generally significantly departed from the theoretical normal distribution ($P<0.05$) and non-parametric tests were then used to compare groups for significant differences notably Man-Whitney U test to compare two independent samples.

For the categorical variables such as gender, anaemic status, malaria status etc. they were described using frequencies and proportions. Bivariate association between predictors of malnutrition, anaemia and malaria was computed using the Crammer's V test. Logistic Regression Model (LRM) was used to depict significant predictors or risk factors for malaria, malnutrition and anaemia using the Wald Statistics to generate the significance level and the Odd Ratios (OR) and the 95% CI of ORs. The method used was the SPSS standard regression analysis 'Enter'.

Results

Characteristics of the study population

A total of 368 children residing in the north region of Cameroon, of both sexes, males 175 (48.3%), females 187 (51.7%), aged ≤10 years old with a mean age of 4.7years ± 2.5 (6months to 10years), mean weight of 14.9kg ± 5.5 (4.0 kg to 30.8kg) and mean height of 98.53cm ± 22.08 (27cm to 108cm) were screened for the presence of malaria parasite and anaemia as well as nutritional and socio-economic status assessed. Out of the 368, 362 had complete assessment data. The proportion of children aged <5 years was 182 (50.3%) almost equal to that of those aged 5 years and above 180 (49.7%). The mean ± SD of HAZ, WAZ and WHZ scores were −1.621±2.898, −1.462±1.986 and -0.269±2.547 respectively in the <5 age group while for the ≥5 age group it was −1.346±2.315, −1.503±1.258 and -0.520±2.610 respectively. (Table 2).

Prevalence of malaria. A total of 362 samples were collected from 6 villages (Kirambo, Guizigaré and Boussa in Pitoa Health District; and Mayo- Oulo, Bala and Dourbeye in Mayo-Oulo Health District. The highest malaria prevalence was recorded in Bala (57%) while the lowest was observed in Mayo- Oulo (16.4%), both in the Mayo Oulo health district. Out of the total 362 children with complete data, only 102(28.2%) presented with fever

Table 2. Baseline characteristics of the study population.

	Parameter			All
Age group		**< 5**	**≥ 5**	**Total**
Sex	**Female n (%)**	85(45.5%)	102(54.5%)	187(51.7%)
	Male n (%)	97(55.4%)	78(44.6%)	175(48.3%)
	Mean WAZ	-1.462±1.986	-1.503±1.258	-1.489±1.622
	Mean HAZ	-1.621±2.898	-1.346±2.315	-1.484±2.607
	Mean WHZ	-0.269±2.547	-0.520±2.610	-0.395±2.578
	Children by age group n (%)	182(50.3%)	180(49.7%)	362(100%)
	Parasitaemia n (%)	86(23.8%)	33(9.1%)	119(32.9%)
	Geometric Mean Parasite Density (range)	2224±23008 (40–129600)	1300±10318 (80–69200)	1783 ±19118 (40–129600)
	Confirmed Malaria (RDT) n (%)	52(89.7%)	39(88.6%)	91(89.2%)
	Malnutrition n (%)	101(55.5%)	95(52.8%)	196(54.1%)
	Stunting n (%)	104(57.1%)	102(56.6%)	206(56.9%)
	Underweight n (%)	106(58.2%)	124(68.9%)	230(63.5%)
	Wasting n (%)	57(31.3%)	69(38.3%)	126(34.8%)
	Anaemia	40(22.2%)	34(19.0%)	74(20.6%)

https://doi.org/10.1371/journal.pone.0218442.t002

(temperature ≥ 37,5˚C) at the time of sample collection and were tested on RDT. Amongst these 91(89.2%; 95% CI: 82.0–94.2) were positive for malaria. Microscopic examination of all 362 children revealed 119 malaria positive cases, giving an overall prevalence of 32.9% [95% CI: 28.2–37.8]. *Plasmodium falciparum and P. malariae* were the parasite species found with *P. falciparum* representing 86.6% of single infections. There were only 8.4% single infections of *P. malariae* while mixed infections of *P. falciparum* and *P. malariae* represented 5%. No *Plasmodium ovale* or *Plasmodium vivax* infection was found. There were more infections in males 66(37.7%) compared to the females 53(28.3%) but the difference was not significant (p = 0.058).

Generally, based on the age groups, the prevalence was higher amongst children less than 5 years old compared to the older group. Comparing results from Microscopy, RDT and PCR amongst the age groups, only the prevalence by microscopy showed a significant difference (p = 0.023) between the two age groups. There was no observed significant differences between RDT and PCR (p>0.05) between the <5 and ≥5 age groups.

Parasite densities ranged from 40parasites/μL to 129600parasites/μL of blood with a geometric mean (GMPD) of 1783 ±19118parasites/μL of blood. The difference between the two age groups was not significant (P = 0.096). Of the 185 analysed by PCR, 123(66.5%; 95% CI: 62.4–75.9) were positive. PCR Analysis also confirmed 11 cases that were missed by microscopy (Table 3).

Table 3. Prevalence of malaria and parasite load by age group and by test procedure.

	RDT(Confirmed malaria, T˚C≥37.5)		Microscopy		PCR		Parasitaemia (GM ± SD; Min-Max)	
Age group	**<5**	**≥5**	**<5**	**≥5**	**<5**	**≥5**	**<5**	**≥5**
N	58	44	182	180	99	86	86	33
Positivity	52	39	70	49	67	56	2224±23008 Min = 40 Max = 129600	1300±10318 Min = 80 Max = 69200
Prevalence (%)	89.7	88.6	38.5%	27.2%	67.7	65.1		
Overall	**89.2% (91)**		**32.9% (119)**		**66.5% (123)**		1783 ±19118; Min = 40; Max = 129600	
Cramer's V	V = 0.016; P = 0.870		V = 0.120; P = 0.023		V = 0.027; P = 0.713		U = 1407.000; P = 0.096	

https://doi.org/10.1371/journal.pone.0218442.t003

Prevalence of malnutrition. A total of 196 malnourished children were identified giving an overall prevalence of malnutrition of 54.1% (95% CI: 48.9–59.2). The rate of malnutrition did not differ significantly between the two age groups (P>0.05) with 101 (51.5%) being children aged <5 years and 95 (48.5%) aged ≥5 years.

The overall prevalence rates of stunting, underweight and wasting were 56.9%, 63.5% and 34.8% respectively, showing that most of the children were underweight. Although not significantly different (p = 0.223), the prevalence of malnutrition was higher in the Pitoa Health District 109(55.6%) than Mayo-Oulo 87(44.4%), with the highest prevalence recorded in Dourbeye (60.7%) and lowest in Mayo-Oulo (40.3%).

Malnutrition was significantly higher in males (60.6%) than in females (48.1%). Stunting was also significantly higher in males (66.9%) than females (47.6%). Underweight was more in males (65.7%) than females (61.5%) although the difference was not significant while wasting was more in females (37.9%) than males (31.4%).

The prevalence rates of severe stunting, severe underweight and severe wasting were 29.1%, 20.9% and 9.3% in the <5 age group and 18.3%, 10.0% and 7.8% in the ≥5 age group respectively, with males being generally more severely malnourished than females (p<0.05) as well. Comparisons among age groups in males revealed that the prevalence for severe underweight was significantly higher (p = 0.002) in <5 than the ≥5. Although not significantly different (p = 0.296), severe stunting was higher in the <5 age group than the ≥5 while severe wasting was more in the ≥5's than the <5 age group (p = 0.963). In females, severe stunting and severe underweight were significantly higher (p = 0.028) and (p = 0.003) respectively in the <5 age group compared to the ≥5 while severe wasting was higher in the ≥5 age group than the <5, but not statistically significant (p = 0.185).

Relationship between malaria and nutritional status. Out of the 119 children who had malaria 70(58.8%) were <5years old while 49(41.2%) were ≥5years. For the children infected with malaria parasite, the mean±SD z-scores for HAZ, WAZ and WHZ in the <5 age group were -1.580±2.100, -1.504±2.121 and -0.231±2.729 respectively while for the ≥5 age group, -1.245±1.925, -1.732±1.224 and -1.355±1.566 respectively.

Overall, malaria prevalence in malnourished children was 37.8% (n = 196), of which 67.2% were underweight, 56.3% were stunted and 41.2% were wasted. Among age groups, although not significantly different (p = 0.331), malaria prevalence in malnourished children was higher 33(67.3%) in the ≥5 age group than the <5 age group 41(58.6%).

Relationship between malaria, anaemia and nutritional Status. Anaemia was assessed by Hb levels. Children with haemoglobin levels less than 11 g/dL were considered to be anaemic in accordance with the WHO classification system [28]. Out of the 368 children who were recruited for the project, PCV was successfully obtained for 359 of them. The overall prevalence of anaemia in the study population was 74 (20.6%) at (95% CI: 16.7–25.0) being higher in males 37(21.3%) than in females 37(20.0%), although no significant difference (p>0.05) was observed. Pitoa Health District generally had a significantly higher (p = 0.007) prevalence of anaemia than Mayo-oulo, with the highest in Kirambo (32.8%) and the lowest in Dourbeye (3.7%). Mild, moderate and severe anaemia were prevalent in 29 (8.1%), 33 (9.2%) and 12 (3.3%) children respectively. Children < 5years 40(22.2%) had a higher (p = 0.45) anaemia prevalence when compared to those ≥ 5 years 34(19.0%). (Table 4) In the prevalence of anaemia, no statistically significant differences (p = 0.422) were seen between malaria positive children and malaria negative children, as well as between the malnourished and normal children (p = 0.599).

Relationship between malaria, anaemia, nutritional and socio-economic status. Overall, the categorization of the development index into quintiles revealed that the majority of children in the study population belonged to the poor SES quintile, followed by the very poor.

Table 4. Prevalence of anaemia in the various categories of children by age group.

	Nutritional status				Malaria status				Age group	
	Malnourished		Normal		Malaria positive		Malaria negative			
Age group	≤ 5	>5	≤ 5	>5	≤ 5	>5	≤ 5	>5	≤ 5	>5
N	100	94	80	85	69	48	111	131	180	179
Anaemic	24	18	16	16	15	12	25	22	40	34
Prevalence (%)	24%	19.1%	20.0%	18.8%	21.7%	25.0%	22.5%	16.8%	22.2%	19.0%
Overall	**42 (21.6%)**		**32 (19.4%)**		**27 (23.1%)**		**47 (19.4%)**		**74 (20.6%)**	
Cramer's V	V = 0.059; P = 0.412		V = 0.015; P = 0.849		V = 0.038; P = 0.680		V = 0.072; P = 0.262		V = 0.040 P = 0.450	
Cramer's V	V = 0.028; P = 0.599				V = 0.042; P = 0.422					

https://doi.org/10.1371/journal.pone.0218442.t004

Indicators for socio-economic status included; the number of people living in a household, the level of education of caregiver and family head, employment status of family head, type of housing, type of toilet, source of lighting and cooking energy, availability of potable water and possession of household assets.

Children belonging to the average SES quintile were observed to have the highest prevalence of malaria (42.4%) as opposed to those of the rich SES quintile who had the lowest prevalence (18.0%). The overall prevalence of malnutrition was 54.1% with children from the poor SES quintile observed to have the highest prevalence of malnutrition (64.9%) while children in the very rich SES quintile had the lowest prevalence (48.0%). With regards to anaemia, overall prevalence was 20.6%, with children belonging to the poor SES quintile having the highest prevalence of anaemia (26.8%) while those in the rich level had the lowest prevalence (7.1%).

Predictors of malaria, malnutrition, and anaemia. For Malaria, the model demonstrated that location [OR = 1.62, (95% CI: 1.28–2.05); ($p<0.001$)] and nutritional status [OR = 2.07, (95% CI: 1.22–3.53); (p = 0.007)] were significant predictors of malaria, with nutritional status having 2 times greater risk of malaria (Table 5). Other risk factors for malaria were; level of education of care giver ($p<0.001$), development index (p = 0.042) and family head employment status ($p<0.001$).

Table 5. Logistic regression model predicting risk factors of malaria.

Predictors	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Villages	.484	.121	16.001	1	.000	1.622	1.280	2.055
Religion	.378	.312	1.466	1	.226	1.460	.791	2.692
Gender of family head	1.146	.741	2.391	1	.122	3.147	.736	13.457
Family head relationship with child	.195	.121	2.622	1	.105	1.216	.960	1.540
Care giver relationship to family head	-.886	.838	1.118	1	.290	.412	.080	2.130
Marital status of family head	6.247	5424.315	.000	1	.999	516.599	.000	.
Level of education of care giver	-1.153	.318	13.189	1	.000	.316	.169	.588
Level of education of family head	.375	.335	1.253	1	.263	1.455	.754	2.808
Type of housing	-.025	.085	.086	1	.770	.975	.825	1.153
Household size	.298	.199	2.236	1	.135	1.347	.912	1.990
Gender of child	.292	.263	1.229	1	.268	1.339	.799	2.243
Development index	-.282	.138	4.154	1	.042	.755	.576	.989
Nutritional status	.729	.271	7.221	1	.007	2.073	1.218	3.528
Child age	.267	.263	1.032	1	.310	1.306	.780	2.186
Family head employment status	-.393	.084	21.906	1	.000	.675	.573	.796

https://doi.org/10.1371/journal.pone.0218442.t005

Table 6. Logistic regression model predicting risk factors of malnutrition.

Predictors	B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Villages	-.168	.111	2.277	1	.131	.845	.680	1.051
Religion	-.291	.281	1.079	1	.299	.747	.431	1.295
Gender of familyhead	-.241	.609	.156	1	.692	.786	.238	2.594
Family head relationship with child	-.229	.106	4.668	1	.031	.796	.647	.979
Care giver relationship to family head	1.072	.622	2.974	1	.085	2.921	.864	9.877
Marital status of family head	.294	.357	.681	1	.409	1.342	.667	2.702
Level of education of care giver	.879	.278	9.963	1	.002	2.408	1.395	4.156
Level of education of family head	-.478	.289	2.735	1	.098	.620	.352	1.093
Type of housing	-.062	.077	.662	1	.416	.939	.808	1.092
Household size	.087	.178	.237	1	.627	1.090	.769	1.546
Gender of child	.502	.230	4.789	1	.029	1.652	1.054	2.591
Development index	.087	.129	.461	1	.497	1.091	.848	1.404
Anaemic status	.228	.284	.649	1	.421	1.257	.721	2.191
Malaria status	.638	.268	5.680	1	.017	1.892	1.120	3.196
Child age	-.004	.232	.000	1	.985	.996	.632	1.568
Family head employment status	.080	.070	1.293	1	.256	1.083	.944	1.243

https://doi.org/10.1371/journal.pone.0218442.t006

The LRM also demonstrated sex of child [OR = 1.65, (95% CI: 1.05–2.59); (p = 0.029)], level of education of care giver [OR = 2.41, (95% CI: 1.39–4.16); (p = 0.002)], and malaria status [OR = 1.89, (95% CI: 1.12–3.19); (p = 0.017)] as significant predictors of malnutrition as shown above (Table 6). The level of education of the care giver [OR = 2.41, (95% CI: 1.39–4.16); (p = 0.002)] was associated with 2 fold elevated risk of being malnourished. Furthermore, the model also identified other risk factors for malnutrition like family head relationship with child (p = 0.031).

Anaemia had family head relationship with child [OR = 1.41, (95% CI: 1.1–1.8); (p = 0.006)] and socio-economic development index [OR = 1.45, (95% CI: 1.05–2.00); (p = 0.023)] as significant risk factors (Table 7).

Table 7. Logistic regression model predicting risk factors of anaemia.

Predictors	B	S.E.	Wald	Df	Sig.	Exp(B)	95% C.I. for EXP(B)	
							Lower	Upper
Villages	-.043	.136	.099	1	.753	.958	.734	1.251
Religion	.555	.341	2.651	1	.103	1.742	.893	3.399
Gender of familyhead	-.196	.836	.055	1	.815	.822	.160	4.228
Family head relationship with child	.344	.126	7.431	1	.006	1.411	1.102	1.807
Care giver relationship to family head	.486	.742	.430	1	.512	1.626	.380	6.962
Marital status of family head	.050	.464	.012	1	.914	1.051	.424	2.608
Level of education of care giver	-.238	.348	.468	1	.494	.788	.399	1.558
Level of education of family head	-.038	.367	.010	1	.919	.963	.469	1.978
Type of housing	.075	.103	.528	1	.467	1.078	.881	1.319
Household size	-.100	.223	.200	1	.654	.905	.584	1.402
Gender of child	-.052	.283	.034	1	.854	.949	.545	1.652
Development index	.373	.164	5.173	1	.023	1.452	1.053	2.001
Malaria status	.233	.287	.660	1	.416	1.262	.720	2.215
Child age	.363	.324	1.253	1	.263	1.438	.761	2.714
Family head employment status	.344	.280	1.507	1	.220	1.411	.814	2.444

https://doi.org/10.1371/journal.pone.0218442.t007

Discussion

Malaria represents a major public health problem. The overall prevalence of malaria in the study sites was 32.9%. The prevalence of malaria was also observed to be significantly higher ($p<0.01$) in Mayo- Oulo health district (38.5%) than Pitoa health district (27.6%), though both were peri-urban settings, probably because our study covered only 6 villages as opposed to earlier studies which covered a wider range of villages. This result was lower than 44.3% observed by Kimbi et al., in the Mount Cameroon region [29], and could be linked to the recent scale-up of intervention strategies such as the free treatment for malaria in children and the use of LLIN's (Long Lasting Insecticide-treated Nets) by the National Malaria Control Program (NMCP)[30].

Also, our findings revealed that the prevalence of malaria was inversely related to age. Such increased susceptibility of younger children to infections has been attributed to their poorly developed immune systems [31].

The logistic regression analysis demonstrated that location [OR = 1.62, (95% CI: 1.28–2.05); ($p<0.001$)] and nutritional status [OR = 2.073, (95% CI: 1.22–3.53); ($p = 0.007$)] were significant risk factors for malaria. Thus there are 1.62 times more chances that a child will become infected with malaria in Bala than in Mayo-Oulo. The key to effective management of malaria is prompt and accurate diagnosis. The WHO recommends that malaria case management where possible should be based on parasitological diagnosis, except when considering young children in endemic areas where lack of resources or urgency of response temporarily limits its application [32, 33]. Malaria control in Cameroon relies principally on anti-vector intervention using long lasting insecticide nets (LLIN) [2] and mainly through large scale campaign and free distribution of the nets. Therefore the Ministry of public health of Cameroon should continue to implement serious plans to rapidly scale-up control measures against the parasite nation-wide, to further reduce malaria infection to the barest minimum and possibly eradicate it, thereby lessening the socio-economic burden.

With regards to malnutrition, the overall prevalence was 54.1% with an overall prevalence of 56.9% for stunting. Although this is higher than is reported previously in the same region with 40.2% for malnutrition and 37.7% for stunting, [9]. It is similar to those reported by Nkuo-Akenji et al., [34] in the Mount Cameroon area. However, the high malnutrition prevalence observed in our study is in conformity with earlier reports from Africa in general, specifically in sub-Saharan Africa whose prevalence stands at 55.2% for stunting as reported by Kwena et al., 2014. The high stunting prevalence equally underscores the fact that, chronic malnutrition is still a heavy burden in the area, which confirms earlier findings by Ngwa Akonwi et al., 2015. Stunting is generally associated with long term undernutrition whereas wasting is a manifestation of recent and acute undernutrition [35]. The prevalence of malnutrition shows about half of the children in our study (196) to be malnourished which is perfectly in line with the WHO report which states that fully half of the human family, some 3 billion people, suffer from malnutrition of one kind or another. [36]

With regards to malnutrition, the high prevalence is indicative of poor feeding habits, absence of health/ nutritional education and lack of proper knowledge on nutrition and balanced diets as well as the low level of education of the caregivers in the studied area. The observed results on the prevalences of stunting, underweight and wasting in both males and females, was consistent with the observations of Sumbele et al.,[37] in the Mount Cameroon area, Wamani et al., in Tanzania that stunting and underweight were common among males than females in all age groups [38, 39].

A higher prevalence in malnutrition was observed among the <5years (55.5%). This is unlike reports from South Western Ethiopia where older children were more likely to suffer

from undernutrition. This difference is probably because younger children are more vulnerable to infection due to their poorly developed immune systems [31]. Poor feeding habits may also have contributed to the higher prevalence in the <5 age group in the study.

Logistic regression analysis was run and it identified malaria status, sex of child and level of education of caregiver as significant predicting risk factors for malnutrition. As revealed by the odd ratios, the level of education of the care giver was associated with 2 fold elevated risk of being malnourished. This implied that children of illiterate mothers were 2.40 times more likely to become malnourished compared to those that had basic education.

Unlike Snow et al., [22] who reported no effect of anthropometric indices on susceptibility to malaria in The Gambia, our results showed an association between malaria and malnutrition. Ehrhardt et al., [1] also identified malnutrition as a major underlying cause of malaria. The relationship between malaria infection and nutritional status was a two-way association. Malnourished children were 2.07 times more likely to become infected by malaria parasite compared to well-nourished children. On the other hand, malaria positive children were 1.89 times more likely to become malnourished than those uninfected. This implies that on one hand, malaria may cause malnutrition, whereas on the other hand, malnutrition may exacerbate the disease. This confirms the controversial synergistic relationship between malaria and malnutrition as previously reported elsewhere [14]. Overall, while children who were stunted and underweight had a higher prevalence of malaria parasite, those wasted had a lower prevalence.

The foremost aim of nutritional assessment studies is to determine the actual magnitude of under nutrition and thereby introduce appropriate nutritional intervention programmes to improve the existing nutrition situation [40–42].

In order to meet up with the World Health Assembly's Global Nutrition Targets 1 and 2 for 2025 on a 40% reduction in the number of children under-5 who are stunted as well as reduce and maintain childhood wasting to less than 5%, then effective strategies need to be sought [43].

Therefore, appropriate measures such as improved nutritional practices could be recommended in areas with high malnutrition [29]. Another way forward would be to expand the health benefits and improve control strategies from the Ministry of Public health for rapid scale-up of already proven nutrition-specific and nutrition-sensitive interventions for the vulnerable malnourished children in the country.

Anaemia is widely distributed and often found in developing countries [44]. Chronic anaemia during childhood is associated with retardation in physical development, cognition and school performance [45], while severe anaemia (haemoglobin < 7g/dL) is responsible for more than half of the deaths attributed to malaria in children under five years of age.

Anaemia is a key public health challenge in Cameroon. Its prevalence as revealed by our study was 20.6%. This is unexpected in a peri-urban community where malaria is hyper endemic. Earlier studies by Jourdan et al., reported prevalence as high as 82% for children attending a clinic in Northern Cameroon [46]. The prevalence of anaemia in our study is lower than 22.6% reported in Brazil in 2011 [47], lower than the 62% recently recorded in under 12 children in Odisha, India in 2016 [48], and the 29% recorded in the Demographic Health Survey (DHS) in Honduras in 2011 amongst 6-59months old [49]. It is also lower than the most recent data (61%) reported by the WHO in 2017 [7].

In our study, the lower prevalence of anaemia observed may probably be due to the low malaria prevalence in the studied area coupled to the fact that ours was a community-based household survey and most of the children examined were healthy children and not necessarily sick children in a hospital. It also revealed mild moderate and severe anaemia to be 8.1%, 9.2% and 3.3% respectively. This indicates that severe anaemia was rare in the area. Meanwhile the WHO prevalence rates for mild, moderate and severe anaemia stood at 25%, 33% and 3% in 2017.

Anthropometry has become a practical tool for evaluating the nutritional status of populations, particularly of children in developing countries and nutritional status is the best indicator of the global well-being of children [41]. Despite the fact that our study on nutritional status was limited to anthropometry, that iron deficiency adversely affects human health is widely recognized. Anaemia could be caused by iron deficiency as a result of; a decrease in haemoglobin concentration; Even though malaria causes chronic anaemia, impaired growth and delayed development in young children [11], in this study, no significant associations were found between anaemia and malaria ($p = 0.422$). Although no significant differences were observed in the prevalence of anaemia between the different sexes, anaemia was higher in males than in females. Similar observations were made in Tanzania [39]. This may be explained by the fact that males were malnourished than females ($p = 0.018$).

The results equally showed that there was no statistically significant association between anaemia and malnutrition ($p = 0.599$). Contrary to our findings, malnutrition was reported in Ghana [1], to be a fundamental factor contributing to malaria-associated morbidity and anaemia, even if the latter exhibits multifactorial patterns. Most findings (in Malaysia and Western Kenya) [21, 50] also reported correlation between education level and anaemia. But our study failed to find such an association.

The LR analysis significantly identified development index and family head relationship with child as significant risk factors for anaemia. Meaning, anaemia correlated with socio economic status ($p = 0.023$). Children with a low SES were 1.45 times more likely to become anaemic [OR = 1.45, (95% CI: 1.05–2.0)] compared to children in the high socio-economic level.

All in all the study highlighted the fact that, malaria and malnutrition may not have been the causes of anaemia in the study population. Our findings show no linear relationship between anaemia and malaria or malnutrition, suggesting the possible involvement of other factors in the development of anaemia in children. Therefore, the results are suggestive of the fact that the anaemia observed in the children may have been due to other presumptive causes like helminthes among others. The prevention as well as timely management of anaemia is essential to attain Sustainable Development Goal- 3 (SDG) on ensuring healthy lives and promoting well-being for all at all ages. Further actions are required to reach the World Health Assembly target of a 50% reduction of anaemia in women of reproductive age by 2025 [36].

Socio-economic status has been defined as a position that an individual or family occupies with reference to prevailing standards of cultural and material possessions, income and education [51]. Our findings revealed that majority of children in the study population belonged to the poor SES quintile, which is not surprising, considering the fact that the community being studied has a low standard of living and confirms previous studies in the same area by Akonwi Ngwa et al., 2015. Socioeconomic factors may influence malaria morbidity, although their respective roles are somewhat controversial [51]. The findings on SES are in support of the fact that, anaemia was significantly associated ($p = 0.023$) to socio-economic status and is in line with earlier findings which state that low economic status, less education, and poor health of mothers due to meagre dietary intake are the main causes of anaemia [48]. Child undernutrition is also influenced by several socio-economic and socio-demographic variables (e.g., sex, age, birth order, education and occupation). This too was seen in our study but we did not find statistically significant association between malnutrition and development index ($p = 0.348$). Socio-economic status did not influence malnutrition.

Conclusion

Control and intervention programmes will have limited effects if we fail to recognize and consequently target the underlying causes of the diseases. Nutritional counselling and health

education interventions of mothers followed by prompt feeding programs have to specifically focus on improving the health of the malnourished and anaemic. These, alongside continuous proper malaria-control measures of proven efficacy, may be apt to reduce the burden of malaria, anaemia, malnutrition and poverty on a large scale. This study therefore elucidates the interplay between the various health determinants and provides preliminary baseline data needed by policy makers, programme planners and implementers, non-governmental organizations, for strategic planning and implementation of the rapid scale-up of nutritional interventions as well as scaled-up malaria parasite control measures in the north region of Cameroon.

Recommendations

Children screened for malaria should also be treated for anaemia and helminthes, especially in the poor communities. Children with pale mucosae should be provided with iron supplementation. There is need for appropriate nutritional and deworming interventions so as to ameliorate the anaemic status of the children. Further research needs to be done to ascertain the exact cause of the anaemia in the children.

Acknowledgments

The authors acknowledge the community and the study participants for their contribution and appreciate the support and cooperation of the parents and guardians of the children in the Pitoa and Mayo—Oulo Health districts, who participated in this study. We are equally grateful to the Biotechnology Center and University of Yaounde I for their support in the accomplishment of this study. The authors are also thankful to officials of the National Malaria Control Programme of the North Region of Cameroon for providing necessary assistance for this study. We would also like to thank the field staff and data collectors for taking their precious time to collect the data. We specially acknowledge the statistical input of Mr. Nana Célestin of the Foundation of Applied Statistics and Data Management (FASTDAM) Buea.

Author Contributions

Conceptualization: Nobelle Sakwe, Jude Bigoga, Judith Ngondi.

Formal analysis: Nobelle Sakwe, Célestin Kouambeng.

Funding acquisition: Nobelle Sakwe.

Investigation: Nobelle Sakwe, Boris Njeambosay, Livo Esemu, Philomena Nyonglema, Clovis Seumen.

Methodology: Nobelle Sakwe.

Resources: Nobelle Sakwe.

Supervision: Jude Bigoga, Judith Ngondi, Inocent Gouado, Julius Oben.

Writing – original draft: Nobelle Sakwe.

Writing – review & editing: Nobelle Sakwe, Jude Bigoga.

References

1. Ehrhardt S, Burchard GD, Mantel C, Cramer JP, Kaiser S, Kuba M, et al. Malaria, anemia, and malnutrition in african children–defining intervention priorities. J Infect Dis. 2006; 194:108–114. https://doi.org/10.1086/504688 PMID: 16741889

2. Bigoga JD, Nanfack FM, Awono-Ambene PH, Patchoké S, Atangana J, Otia VS, et al. Seasonal prevalence of malaria vectors and entomological inoculation rates in the rubber cultivated area of Niete, South Region of Cameroon. Parasit Vectors. 2012; 5:197. https://doi.org/10.1186/1756-3305-5-197 PMID: 22963986
3. Shankar AH. Nutritional modulation of malaria morbidity and mortality. J Infec Dis. 2000; 182: S37–S53.
4. Benna C. Malaria and Nutrition. Revised edition. Cambridge: University Press; 2001.
5. Mfonkeu JB, Gouado I, Kuate HF, Zambou O, Grau G, Combes V, et al. Clinical Presentation, haematological indices and management of children with severe and uncomplicated malaria in Douala, Cameroon. Pak J Biol Sci. 2008; 11 (20): 2401–2406. PMID: 19137849
6. Breman JG. The ears of the hippopotamus manifestations, determinants and estimates of the malaria burden. Am J Trop Med Hyg. 2001; 64:1–11
7. WHO. World Malaria Report 2018. Geneva, Switzerland, World Health Organization. 2018(http://apps.who.int/iris/bitstream/10665/200019/3/9789241565653_eng.pdf, accessed March 2019).
8. Programme National de Lutte contre le Paludisme-Cameroun. Plan stratégique du Cameroun 2014.
9. Akonwi NF, Ngondi JL, Edoun FL, Dibacto KRE, Oben JE. Multiple Micronutrient Deficiencies are Related to the Nutritional Status of Children Living in North and Far North Regions of Cameroon. Am J Pharm Health Res. 2015; 3: ISSN: 2321–3647.
10. Pelletier DL, Frongillo EA Jr., Schroeder DG, Habicht JP. The effects of malnutrition on child mortality in developing countries. Bull World Health Organ. 1995; 73: 443–448. PMID: 7554015
11. Wierzba TF, El-Yazeed RA, Savarino MS, Rao M, Baddour M, El-Deen AN, et al. The interrelationship of malnutrition and diarrhea in a periurban area outside Alexandria, Egypt. J Pediatr Gastroenterol Nutr.2001; 32:189–196. PMID: 11321391
12. World Health Organization (2015) Global database on Child Growth and Malnutrition. 2015 Joint child malnutrition estimates—levels and trends. UNICEF-WHO-The World Bankproject. Geneva:World-HealthOrganization;2015(http://www.who.int/nutgrowthdb/estimates2015/en/, 2015 edition).
13. Helen Keller International, WFP (2013) What are the current issues in Cameroon? Assessed on the 31st October 2016. https://www.wfp.org/countries/Cameroon.
14. Nyakeriga AM, Troye-Blomberg M, Chemtai AK, Marsh K, Williams TN. Malaria and nutritional status in children living on the coast of Kenya. Am J Clin. Nutr. 2004; 80: 1604–1610. https://doi.org/10.1093/ajcn/80.6.1604 PMID: 15585775
15. Facer CA. Haematological aspects of malaria. In Infection and Haematology, Oxford. Butterworth Heinmann Ltd; 1994: 259–294.
16. Murphy GS, Oldfield EC. Falciparum malaria. Infec Dis Clin North Am. 1996; 10:747–770.
17. Nussenblatt V, Semba RD. Micronutrient malnutrition and the pathogenesis of malarial anaemia. Acta Trop. 2002; 82:321–337. PMID: 12039672
18. Benoist B, McLean E, Egli I, Cogswell M (Eds). Worldwide Prevalence of anaemia 1993–2005. WHO global database on anaemia. Geneva. World Health Organization publication; 2008.
19. Le Cornet M, Hesran JUL, Fievet N, Personne P, Gounoue R, Beyeme M, et al. Prevalence and risk factors for anaemia in young children in southern Cameroon. Am J Trop Med Hyg. 1998; 58:606–611. https://doi.org/10.4269/ajtmh.1998.58.606 PMID: 9598449
20. Deen JL, Walraven GE, von Seidlein L. Increased risk for malaria in chronically malnourished children under 5 years of age in rural Gambia. J Trop Pediatr. 2002; 48: 78–83. https://doi.org/10.1093/tropej/48.2.78 PMID: 12022433
21. Friedman JF, Kwena AM, Mirel LB, Kariuki SK, Terlouw DJ, Philips-Howard PA, et al. Malaria and nutritional status among pre-school children: results from cross-sectional surveys in western Kenya. Am J Trop Med Hyg. 2005; 73: 698. PMID: 16222012
22. Snow RW, Byass P, Shenton FC, Greenwood BM. The relationship between anthropometric measurements and measurements of iron status and susceptibility to malaria in Gambian children. Trans R Soc Trop Med Hyg. 1991; 85:584–589. https://doi.org/10.1016/0035-9203(91)90351-x PMID: 1780980
23. Deribew A, Alemseged F, Tessema F, Sena L, Birhanu Z, Zeynudin A, et al. Malaria and Under-Nutrition: A Community Based Study Among Under-Five Children at Risk of Malaria, South-WestEthiopia. PLoSONE.2010; 5(5):e10775.
24. Cogill B. Anthropometric Indicators Measurement Guide. Food and Nutrition Technical Assistant. 2003.
25. de Lamballerie X, Zandotti C, Vignoli C, Bollet C, de Micco P. A one-step microbial DNA extraction method using "Chelex 100" suitable for gene amplification. Res Microbiol. 1992; 143:785–790. PMID: 1298031

26. Cheesbrough M. District Laboratory Practice in Tropical Countries. Part I. Second edition. Cambridge University Press. Low Price Edition. 2005.

27. Singh B, Bobogare A, Cox-Singh J, Snounou G, Abdullah MS, Rahman HA, et al. A genus- and species-specific nested polymerase chain reaction malaria detection assay for epidemiologic studies. Am J Trop Med Hyg 60. 1999: 687–692. https://doi.org/10.4269/ajtmh.1999.60.687 PMID: 10348249

28. World Health Organization (2001) Iron deficiency anaemia: assessment, prevention and control; a guide for programme managers. Geneva: World Health Organization, 114 p.

29. Kimbi HK, Ajeagah HU, Keka FC, Lum E, Nyabeyeu HN, Tonga CF, et al. Asymptomatic Malaria in School Children and Evaluation of the Performance Characteristics of the PartecCyscope® in the Mount Cameroon Region. J Bacteriol Parasitol. 2012; 3:153.

30. Sumbele IUN, Samje M, Nkuo-Akenji TK. A longitudinal study on anaemia in children with Plasmodium falciparum infection in the Mount Cameroon region: prevalence, risk factors and perceptions by caregivers. BMC Infect Dis. 2013; 13:123. https://doi.org/10.1186/1471-2334-13-123 PMID: 23497273

31. Thorarinsdottir HK, Ludviksson BR, Vikingsdottir T, Leopoldsdottir MO, Ardal B, Jonsson T, et al. Childhood levels of immunoglobulins and mannan-binding lectin in relation to infections and allergy. Scand J Immunol. 2005; 61:466–74. https://doi.org/10.1111/j.1365-3083.2005.01588.x PMID: 15882439

32. Hassan Sel-D, Okoued SI, Mudathir MA, Malik EM. Testing the sensitivity and specificity of the fluorescence microscope (cyscope) for malaria diagnosis. Malar J. 2010; 9:88. https://doi.org/10.1186/1475-2875-9-88 PMID: 20356369

33. Sousa-Figueiredo JC, Oguttu D, Adriko M, Besigye F, Nankasi A, Arinaitwe M, et al. Investigating portable fluorescent microscope (cyscope) as an alternative rapid diagnostic test for malaria in children and women of child–bearing age. Malar J. 2010; 9:245. https://doi.org/10.1186/1475-2875-9-245 PMID: 20799940

34. Nkuo-Akenji TN, Sumbele I, Mankah EN, Njunda AL, Samje M, Kamga L. The burden of malaria and malnutrition among children less than 14 years of age in a rural village of Cameroon. AJFAND. 2008; 8: 252–64.

35. Caulfield LE, de Onis M, Blossner M and Black RE. Undernutrition as an underlying cause of child deaths associated with diarrhoea, pneumonia, malaria and measles. Am J. Trop Med Hyg. 2004; 71: 55–63.

36. World Health Organization (2015) Global database on Child Growth and Malnutrition. 2015 Joint child malnutrition estimates—levels and trends. UNICEF-WHO-The World Bankproject. Geneva:World-HealthOrganization;2015(http://www.who.int/nutgrowthdb/estimates2015/en/, 2015 edition).

37. Sumbele IUN, Bopda OSM, Kimbi HK, Ning TR, Nkuo-Akenji TN. Nutritional status of children in a malaria mesoendemic area: cross sectional study on prevalence, intensity, predictors, influence on malaria parasitaemia and anaemia severity. BMC Public Health. 2015; 15:1099. https://doi.org/10.1186/s12889-015-2462-2 PMID: 26541669

38. Wamani H, Astrom AN, Peterson S, Tommie JK, Tylleskar T. Boys are more stunted than girls in sub-Saharan Africa: a meta-analysis of 16 demographic and health surveys. BMC Pediatr. 2007; 7:17. https://doi.org/10.1186/1471-2431-7-17 PMID: 17425787

39. Kamugisha ML, Crawford N, Savaell Z, Msangeni H, Massaga JJ, Mmbando BP, et al. Malaria and nutritional status in children living in the East Usambara Mountains, north-eastern Tanzania. Tanzania Health Res Bull. 2006; 8:56–61.

40. de Onis M, Yip R, Mei Z. The development of MUAC-for-age reference data recommended by a WHO Expert Committee. Bull World Org. 1997; 75: 11–18.

41. World Health Organization (1995) Physical Status: the Use and Interpretation of Anthropometry: Technical Report Series no. 854. Geneva: World Health Organization, 1995.

42. World Health Organization (2007) WHO child growth standards: head circumference-for-age, arm circumference-forage, triceps skinfold-for-age and subscapular skinfold-for-age: methods and development.

43. Pei Leilei, Ren Lin, and Yan Hong. A survey of undernutrition in children under three years of age in rural Western China. BMC Public Health. 2014; 14:121 https://doi.org/10.1186/1471-2458-14-121 PMID: 24499429

44. WHO (2002) Reducing risks, promoting healthy life. The World Health Report 2002. WHO Regions, Estimates for 2001. Geneva.

45. Grantham-McGregor S, Ani C. A review of studies on the effect of iron deficiency on cognitive development in children. J Nutr. 2001; 131: 649S–666S; discussion 666S–668S. https://doi.org/10.1093/jn/131.2.649S PMID: 11160596

46. Jourdan PM, Laoussou P, Lybie A, Kjetland EF. Indicators of anaemia in under-fives with malaria at a hospital in Northern Cameroon. W Afr J Med. 2008; 27:7–12.

47. Cotta RosângelaMinardiMitre; Oliveira Fabiana de CássiaCarvalho; Magalhães Kelly Alves; Ribeiro AndréiaQueiroz; da Rocha Sant'Ana Luciana Ferreira; Priore Silvia Eloíza, et al. Social and biological determinants of iron deficiency anemia. Cad. SaúdePública. 2011; vol.27: supl.2 Rio de Janeiro.

48. Behera Shuchismita and Bulliyya Gandham. Magnitude of Anaemia and Haematological Predictors among Children under 12 Years in Odisha, India. Anaemia Article ID 1729147. http://dx.doi.org/10.1155/2016/1729147.

49. Mejia Torres RE, Franco Garcia DN, Fontecha Sandoval GA, Hernandez Santana A, Singh P, Mancero Bucheli ST, et al. Prevalence and Intensity of Soil-Transmitted Helminthiasis, Prevalence of Malaria and Nutritional Status of School Going Children in Honduras. PLoS Negl Trop Dis. 2014; 8(10): e3248. https://doi.org/10.1371/journal.pntd.0003248 PMID: 25330010

50. Snarill ZM, Bond UT, Johnson NE. Nutrition and educational achievement of urban primary school children in Malaysia. Asia Pac J Clin Nutr. 2000; 9:264–273. PMID: 24394502

51. Luckner D, Lell B, Greve B, Lehman LG, Schmidt-Ott RJ, Matousek P, et al. No influence of socioeconomic factors on severe malarial anaemia, hyperparasitaemia or reinfection.Trans R Soc Trop Med Hyg. 1998; 92:478–481. https://doi.org/10.1016/s0035-9203(98)90882-3 PMID: 9861356