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ACRONYMES ET ABBRÉVIATIONS

AL :	Artheméther-Luméfantrine
AQ :	Amodiaquine
AQ-SP :	Amodiaquine - Sulfadoxine - Pyriméthamine
ASC :	Agent de Santé Communautaire
CMP :	Centre for Medical Parasitology
CPS :	Chimioprévention du Paludisme Saisonnier
CTA :	Combinaison Thérapeutiques à base de dérivés d'Artèmisinine
EDS :	Enquête Démographique et de Santé
G6PD :	Glucose 6 Phosphate Déshydrogénase
HRP 2 :	Histidin Rich Protein 2
HMM :	Home based-Management of Malaria
MILDA :	Moustiquaire Imprégnée d'Insecticide à longue Durée d'Action
MCDC :	Malaria Capacity Development Consortium
NCHS :	National Centre for Health Statistics
OMS :	Organisation Mondiale de la Santé
OPD :	O-Phénylène-Diamine
ORa :	Odds Ratio ajusté
PECADOM :	Prise En Charge à Domicile des cas
PNLP :	Programme National de Lutte contre le Paludisme
PCR :	Polymerase Chain Reaction
SMC :	Seasonal Malaria Chemoprevention
SP :	Sulfadoxine-Piryméthemine
SSOP-ELISA :	Sequence-Specific Oligonucleotide probe Enzyme-Linked Immunosorbent Assay)
SD :	Standard Deviation
TDR :	Test de Diagnostic Rapide
TPIe :	Traitement Préventif Intermittent de l'enfant
TMAC :	Tétra-Méthyl Ammonium Chloride

Résumé en français

Paludisme et anémie sont des affections fréquentes en milieu tropical. Récemment, l'Organisation Mondiale de la Santé a exhorté les pays endémiques à accélérer le processus de mise à échelle des mesures de lutte antipaludique. La mise à échelle de des interventions, en réduisant l'ampleur du paludisme, pourrait contribuer à une réduction significative de la fréquence des anémies tropicales, dans la mesure où paludisme et anémie sont des pathologies fréquemment associées en zone tropicale. Plusieurs stratégies de lutte contre le paludisme sont actuellement disponibles comprenant entre autre : (i) l'accès à un diagnostic précoce au niveau des points de prestation de soins, suivi de la prescription d'antipaludiques efficaces tels que les Combinaisons thérapeutiques à base de dérivés d'Artémisinine (CTA), (ii) le traitement préventif intermittent chez le nourrisson et la femme enceinte, (iii) la prévention du paludisme saisonnier, (iv) la promotion de l'utilisation de la moustiquaire imprégnée d'insecticide à longue durée d'action. Le processus de contrôle du paludisme au niveau de certaines localités, comme le sud-est du Sénégal où la transmission de la maladie reste encore élevée, nécessite l'utilisation combinée de plusieurs mesures de lutte. Toutefois, il existe peu de données concernant la faisabilité d'une telle approche et son impact potentiel sur le paludisme et l'anémie.

Ce projet a été initié dans le but d'étudier la faisabilité, l'efficacité et la tolérance de la mise en œuvre combinée de la prise en charge communautaire des cas et la chimioprévention du paludisme saisonnier (CPS), mais également d'évaluer les principaux facteurs associés à l'anémie chez les enfants de moins de 10 ans. Le projet a été conduit au niveau des 8 villages polarisés par le poste de santé de Bonconto situé au niveau du district de Vélingara, dans la zone du sud-est du Sénégal. Les objectifs du projet ont été couverts à travers : (i) un essai clinique communautaire randomisé qui a permis d'évaluer la faisabilité et l'impact de la combinaison de la prise en charge communautaire des cas et de la prévention du paludisme saisonnier, (ii) une étude cas-témoins investiguant les facteurs associés à l'anémie chez les enfants de moins de 10 ans. Les huit villages situés au niveau de la zone de couverture du poste de santé de Bonconto, ont été randomisés en village d'intervention (prise en charge communautaire des cas combinée à la chimioprévention du paludisme saisonnier) et villages témoins (prise en charge communautaire seule) avec comme critère de jugement principal, l'incidence des épisodes cliniques de paludisme au niveau des deux groupes d'étude. Le rapport d'incidence du paludisme au niveau des villages d'intervention et villages témoins était de

0,15 soit une efficacité protectrice de la combinaison prise en charge des cas + CPS de 85% (IC95% : 39.9%-96.3%, p=0.01). L'accès au traitement par les CTA était de l'ordre 96,4% ; les niveaux de couverture en CPS variaient entre 97,3% (IC95%:91.3–100) en 2010 et 88.8% (IC:84.2–93.6) en 2011. Aucun événement indésirable grave n'a été noté au cours de l'étude. En fin de saison de transmission du paludisme, la prévalence de l'anémie était significativement plus basse au niveau des villages d'intervention (54,1%) comparé aux villages témoins (60,3%) avec un ORa=0,59 soit une efficacité protectrice des interventions contre l'anémie de 41% (IC95% : 18%-58%, p=0,025).

L'étude cas-témoins avait montré que les facteurs significativement associés à l'anémie chez les enfants étaient: le portage de *P. falciparum* (ORa=2,89, IC95%[1,32-6.34]), la présence de traits alpha-thalassémiques (ORa=1,82, IC95% [1.2-3.35]), la malnutrition (ORa=3.37, IC95%[1.93-5.88], âge compris entre 2 et 4 ans (ORa=0.13, IC95%[0.05-0.31]) et l'âge > 5 ans (ORa=0.03, IC95%[0.01-0.08]). La combinaison de la prise en charge communautaire du paludisme et de la chimioprévention du paludisme saisonnier est une approche faisable et bien tolérée pouvant conférer une protection efficace contre le paludisme. Ces deux interventions combinées peuvent induire une réduction significative du paludisme et de l'anémie. Toutefois, pour un contrôle effectif des anémies dont l'étiologie multifactorielle a été bien établie, des approches intégrées prenant en compte les autres causes d'anémie méritent d'être développées.

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Résumé en anglais

Malaria and anaemia are commonly associated in tropical regions. Scaling up antimalarial interventions as advocated by the WHO, will contribute to further reduce malaria burden and may induce a significant reduction in the global anaemia burden. Current antimalarial interventions include : (i) prompt access to diagnostic testing using Rapid Diagnostic Test (RDT) at the point of care and treatment of confirmed cases with effective antimalarial drugs such as Artemisinin Combination Therapy (ACT) for uncomplicated malaria cases and pre-referral rectal artesunate for the initial management of complicated malaria ; (ii) intermittent preventive treatment in infants and pregnant woman; (iii) Seasonal Malaria Chemoprevention (SMC), as well as (iv) Long-Lasting Insecticidal Net (LLIN). Malaria control in many epidemiological situations such as in Senegal will require a multi-intervention strategy with the use of combination of antimalarial interventions. Studies are needed, to document the feasibility as well as the impact on malaria and anaemia of combining several antimalarial interventions. Relevant studies are also needed to assess potential anaemia determinants and risk factors while scaling up antimalarial interventions, for an effective and integrated anaemia control. Because there is limited information about the feasibility of combining several antimalarial interventions at community level, this project aimed to study the feasibility, effectiveness and safety of an integrated malaria control strategy, including RDT, ACTs, Rectal artesunate and SMC delivered by community health workers (CHWs) and to investigate factors associated with anemia among children less than 10 years.

The project was conducted in the South Eastern Part of Senegal and had two components : (i) a cluster randomised trial was designed to assess the feasibility and the impact on malaria burden of combining SMC and HMM, (ii) a case control study was carried to investigate anaemia aetiologies among children less than 10 years of age.

During two malaria transmission seasons, eight communities in eight villages in Senegal were randomised to receive either HMM combined with SMC (intervention arm) or HMM alone (control arm). The overall adjusted rate ratio for incidence of malaria attacks in intervention and control communities was 0.15, indicating a protective effect of HMM+SMC of 85% (95% CI: 39.9%-96.3%, p=0.01). Access to ACT treatment was evaluated at 96.4% while SMC coverage represented 97.3% (95%CI: 91.3–100) in 2010, and 88.8% (95%CI: 84.2–93.6) in 2011. No serious adverse events were recorded. At the end of malaria transmission season, proportion of anaemic children was significantly

lower the intervention arm (54.1%) compare to the control arm (60.3%) with an adjusted odds ratio at 0.59 showing a protective effect of HMM+SMC against anaemia at 41% (95%CI: 18% - 58%, p=0.025). The case control study, demonstrated that the main factors significantly associated with anaemia in these communities were: malaria parasitaemia (aOR=5.23, 95%CI [1.1-28.48]), sickle cell disorders (aOR=2.89, 95% CI[1,32-6.34]), alpha-thalassemia (aOR=1.82, 95% CI[1.2-3.35]), stunting (aOR=3.37, 95% CI[1.93-5.88], age ranged from 2 to 4 years (aOR=0.13, 95% CI[0.05-0.31]) and age > 5 years (aOR=0.03, 95% CI[0.01-0.08]).

It seems feasible and safe to combine SMC with HMM intervention, while achieving high coverage and effectiveness of both SMC and HMM. The combination of these two interventions will result in a significant reduction of malaria and anaemia burden. However, to achieve an effective control of anaemia, integrated approaches taking into account other aetiologies of anaemia need to be developed.

CHAPITRE 1 : REVUE BIBLIOGRAPHIQUE

Chapitre 1: Revue bibliographique

1. Introduction

En dépit des efforts consentis dans la lutte contre le paludisme, cette maladie continue de poser un problème majeur de santé publique. En 2010, l'Organisation Mondiale de la Santé (OMS) estimait le nombre de cas de paludisme entre 154 à 289 millions avec 490 000 à 836 000 décès à travers le monde [1]. Environ 81% des cas de paludisme et 86% des décès surviennent en Afrique où les enfants constituent les catégories les plus vulnérables [1]. Au Sénégal, le paludisme est endémique avec une transmission saisonnière au cours de la saison pluvieuse.

En 2003, le Sénégal a remplacé la chloroquine par les Combinaisons Thérapeutiques à base de dérivés d'Artèmisinine (CTA), en réponse à l'apparition de souches de *Plasmodium falciparum* résistantes à la Chloroquine. La prise en charge du paludisme au Sénégal fait actuellement appel à l'utilisation de la combinaison Artemether-Luméfantrine ou Artésunate-Amodiaquine en schéma thérapeutique de première ligne du paludisme simple et la quinine comme molécule de référence pour la prise en charge du paludisme grave [2]. Afin d'améliorer la qualité du diagnostic du paludisme et optimiser l'utilisation des CTA, les Tests de Diagnostic Rapide (TDR) du paludisme ont été introduits par le Programme National de Lutte contre le Paludisme (PNLP) au niveau des formations sanitaires du pays.

Les autres mesures de lutte antipaludique comprennent, la promotion de l'utilisation des moustiquaires imprégnées d'insecticide, le traitement préventif intermittent chez la femme enceinte, les aspersions intra-domiciliaires qui sont en phase test au niveau de certains districts pilotes. Jusqu'en 2009, la promotion des moustiquaires au Sénégal était limitée à des groupes cibles vulnérables tels les femmes enceintes, les enfants de moins de 5 ans. A partir de 2009, la stratégie de couverture universelle en Moustiquaires Imprégnées à Longue Durée d'Action (MILDA) a été initiée au plan national avec une couverture globale en MILDA évaluée à 63% en 2011 [3]. Le traitement préventif intermittent, est une composante essentielle dans le dispositif de prévention du paludisme chez la femme enceinte et est utilisé au Sénégal avec des taux de couverture de 65% [3].

Au cours des dernières années, une baisse significative du nombre de cas de paludisme a été notée. Ainsi, la morbidité palustre au niveau des points de prestation de soins est passée de 35,7% en 2001 à 3,07% en 2009. Cependant, dans certaines localités telle que la

zone du Sud-Est du Sénégal, la morbidité palustre est restée élevée malgré la mise en œuvre de plusieurs interventions de lutte [2]. Parallèlement à la baisse de la morbidité palustre, la prévalence de l'anémie chez les enfants de moins de 5 ans, a connu une légère baisse entre 2005 (82,6%) et 2010 (76,4%) mais est restée à des niveaux encore élevés *[données des EDS de 2005 et 2010*].

2. La prise en charge communautaire du paludisme

L'accès à un diagnostic précoce, suivi de la prescription d'antipaludiques efficaces constituent des éléments essentiels dans la lute antipaludique. La prise en charge communautaire du paludisme (ou prise en charge à domicile des cas) conçue comme étant la mise à disposition d'un traitement antipaludique aussi proche possible du domicile [4], est une approche visant à contribuer à améliorer l'accès à des molécules antipaludiques efficaces telles que les CTA. Cette approche a été développée dans le but d'améliorer la qualité des pratiques thérapeutiques en milieu communautaire [5]. Elle ciblait initialement la prise en charge du paludisme chez les enfants de moins de 5 ans, en se basant sur l'hypothèse qu'un traitement délivré à domicile dès l'apparition des premiers signes de la maladie, pourrait entrainer une réduction significative de la morbidité et de la mortalité palustre [6]. La mise en œuvre de la stratégie de prise en charge communautaire du paludisme en Ethiopie utilisant la chloroquine avait permis de réduire la mortalité toutes causes confondues de 40%, tandis que la mortalité spécifique liée au paludisme était réduite de 70% [7]. Des études conduites au Burkina Faso avaient démontré qu'une prise charge précoce à domicile des cas de paludisme simple chez les enfants, permettait de réduire de 25 à 50% le risque de survenue de formes sévères [8, 9].

Des études plus récentes conduites en Afrique sub-saharienne ont permis de montrer que l'utilisation des CTA en milieu communautaire pour le traitement des cas de paludisme simple était faisable et bien acceptée, tout en garantissant une bonne efficacité [10-12]. Une étude conduite à Madagascar a montré qu'il est possible d'utiliser les CTA en milieu communautaire de façon correcte avec des niveaux de couverture et d'adhésion élevés [13]. Cette utilisation peut être davantage optimisée par l'introduction des TDR du paludisme en milieu communautaire. Ces tests constituent des moyens simples et fiables pour réduire l'utilisation excessive d'antipaludiques [14].

La prise en charge communautaire du paludisme est une stratégie bien acceptée des communautés et prestataires communautaires (agents de santé communautaire). Elle

pourrait contribuer à accélérer le processus de contrôle du paludisme [15, 16]. Cette stratégie a été recommandée par l'OMS en 2004 et plus de 30 pays dont le Sénégal, l'ont actuellement adoptée comme stratégie nationale de lute antipaludique [17, 18].

Au Sénégal, le PNLP a initié la promotion de l'utilisation des CTA en milieu communautaire sous la désignation de prise en charge des cas à domicile (PECADOM). Cette stratégie est confiée à des agents de santé communautaire ou à des dispensateurs de soins à domicile, et inclut l'utilisation des TDR pour la confirmation des cas, des CTA pour le traitement des formes simples de paludisme. La mise en œuvre de cette intervention a démarré en 2008 au niveau de 20 villages pilotes et a atteint 508 villages en 2009 [19] et 853 villages en 2013.

3. La prévention du paludisme saisonnier

La chimio-prévention du paludisme saisonnier (CPS) initialement désignée sous le terme de traitement préventif intermittent de l'enfant (TPIe), est une nouvelle stratégie visant à réduire la morbidité et la mortalité du paludisme chez les enfants vivant au niveau des zones où la transmission du paludisme est saisonnière. Elle se définit comme étant l'administration de doses curatives d'antipaludiques durant la saison de transmission du paludisme, l'objectif étant de maintenir des concentrations thérapeutiques d'antipaludiques dans le sang, durant toute la période à risque du paludisme, afin de prévenir l'infection palustre [20].

Plusieurs études conduites en Afrique Sub-saharienne ces dernières années, ont permis de démontrer que la CPS est une intervention efficace pouvant contribuer à réduire de façon significative la mortalité et morbidité palustres [21]. Au Sénégal, Cisse et *al* (2006) au cours d'un essai clinique randomisé mené au niveau de la zone rurale de Niakhar, avait démontré que l'administration de Sulfadoxine-Pyriméthamine (SP) et de l'artésunate, 3 fois durant la période de transmission du paludisme, permettait de réduire de 86% l'incidence du paludisme chez les enfants de moins de 5 ans [22]. Au Mali, l'administration de 2 doses de SP à 8 semaines d'intervalle durant la saison de transmission du paludisme, avait révélé une efficacité protectrice de la CPS de 67,5% contre les épisodes cliniques de paludisme [23].
Au Ghana, Kweku et *al* (2008) avaient montré que l'administration mensuelle de la combinaison Artésunate-Amodiaquine pouvait réduire l'incidence du paludisme de 69% [24].

La combinaison de la CPS à l'utilisation des MILDA, avait réduit l'incidence du paludisme de 70 à 82% lors d'études conduites au Mali et au Burkina Faso [25, 26]. Des études menées au Sénégal et en Gambie, ont démontré que la combinaison Amodiaquine (AQ) - Sulfadoxine-Pyriméthamine (SP), constituait le régime thérapeutique le plus efficient dans le cadre d'une telle intervention. En effet, cette combinaison pourrait contribuer à minimiser davantage le risque de résistance au cours d'une stratégie de CPS, permettant ainsi de réserver l'artèmisinine au traitement des épisodes aigus de paludisme [27, 28]. L'alternative à l'AQ-SP pourrait être constituée par la combinaison Pipéraquine (PQ), SP [28, 29].

La CPS est actuellement une stratégie recommandée par l'OMS dans les régions sahéliennes et Sub-sahéliennes d'Afrique de l'Ouest [20, 30]. Toutefois, la meilleure approche pour la mise en œuvre de la CSP n'est toujours pas définie. La combinaison de la CPS à la prise en charge communautaire du paludisme semble être une approche possible, pouvant permettre d'atteindre des niveaux de couverture élevés, dans la mesure où ces deux interventions semblent synergiques [21, 31, 32]

4. Paludisme et autres causes d'anémie

L'infection à *Plasmodium* constitue une cause fréquente d'anémie en zone d'endémie palustre. Cependant, il existe d'autres facteurs pouvant conduire à la survenue d'anémie en milieu tropical. L'anémie peut être induite par une chute du taux d'hémoglobine, du nombre de globules rouges dans le sang, ou de l'hématocrite [33]. Le niveau d'hémoglobine à partir duquel on définit une anémie dépend de certaines conditions physiologiques: âge, sexe, grossesse. Le tableau 1 résume les critères de l'OMS pour la définition de l'anémie selon le taux d'hémoglobine [34].

Table 1: **Définition de l'anémie selon l'OMS** [34].

Statut	Valeur seuil du taux d'hémoglobine (g/dl) permettant de définir l'anémie
Femme non enceinte d'âge supérieur à 15 ans	< 12.0
Femme enceinte	< 11.0
Enfant de moins de 5 ans	< 11.0
Enfant d'âge compris entre 5 – 11.9 ans	11.5
Enfant d'âge compris en 12 – 14,9 ans	< 12.0
Homme de plus de 15 ans	< 13.0

Les mécanismes des anémies peuvent être regroupés en deux catégories :

- ✓ Chute de la production des érythrocytes ;
- ✓ Augmentation de la perte d'érythrocytes à travers une augmentation de leur destruction (hémolyse) ou une perte de sang, ou les deux associés [35].

En milieu tropical, les anémies sont le plus souvent multifactorielles et les maladies infectieuses (surtout le paludisme), les hémoglobinopathies, les enzymopathies et la malnutrition peuvent jouer un rôle majeur dans leur survenue. Les infections parasitaires telles que les parasitoses intestinales peuvent contribuer à la survenue des anémies, du fait de la perturbation de l'absorption et du métabolisme du fer et autres micronutriments, mais aussi du fait de l'augmentation des déperditions de nutriments. Par exemple, les géo-helminthiases habituellement rencontrées en milieu tropical, constituent des causes majeures d'anémie [36, 37]. D'autres infections parasitaires comme les bilharzioses peuvent induire des anémies par perte sanguine en rapport avec l'hématurie causées par *Schistosoma heamatobium* ou la diarrhée sanguinolente due à *Schistosoma mansoni* [38].

Le paludisme demeure une importante cause d'anémie en milieu tropical [39] et l'anémie induite par l'infection à *Plasmodium falciparum*, résulte d'une destruction massive des

globules rouges au niveau du sang périphérique, ou leur séquestration au niveau de la rate [40].

Les anomalies génétiques de l'hémoglobine, conséquence d'une irrégularité structurale ou une réduction de la production de globine peuvent induire des anémies [35]. Plusieurs études ont investigué la distribution ainsi que les conséquences fonctionnelles de ces désordres génétiques ; s'il est bien établi que les porteurs hétérozygotes d'hémoglobinopathie sont partiellement protégés contre le paludisme [41, 42] la part de ces hémoglobinopathies sur la prévalence globale des anémies est moins bien documentée [43]. Des études s'avèrent nécessaires afin de mieux documenter l'effet de ces hémoglobinopathies sur la survenue d'anémie particulièrement dans un contexte de baisse de la prévalence du paludisme dans certaines localités telles que le Sénégal [44, 45].

La drépanocytose est une hémoglobinopathie constamment associée à une anémie hémolytique. Elle est assez fréquente dans le monde avec une incidence de 2,28 pour 1000 naissances vivantes [46]. Cette affection peut se présenter sous une forme homozygote (caractérisée par une présence exclusive de l'hémoglobine S) ou une forme hétérozygote (HbAS). Elle résulte d'une mutation au niveau des chaines β de l'hémoglobine [43].

Les thalassémies constituent des désordres génétiques de l'hémoglobine très prévalent en Afrique sub-saharienne et en Asie [42]. Chaque individu possède normalement une paire de gène alpha au niveau de chaque chromosome 16. Les sujets alpha thalassémiques hétérozygotes (α+ −), présentent une délétion d'un gène alpha ce qui conduit à l'existence de 3 gènes alpha fonctionnels (−α/αα), tandis que les homozygotes ont seulement 2 gènes fonctionnels (−α/-α). Les individus alpha thalassémiques hétérozygotes présentent généralement de légères modifications hématologiques, tandis que les sujets homozygotes présentent fréquemment des anémies microcytaires modérées [47].

La déficience en glucose-6-phosphate déshydrogénase (G6PD) constitue également un désordre génétique relativement fréquent en zone d'endémie palustre et pouvant être à l'origine d'anémie. Il s'agit d'une anomalie enzymatique du globule rouge portée par le chromosome x et résultant de mutations au niveau du gène de la G6PD [48]. En Afrique sub-saharienne, la G6PD peut se présenter sous 3 allèles différents. La G6PD (B) est la forme allélique la plus fréquente ; elle est caractérisée par une activité enzymatique normale. La G6PD (A) est associée à une simple mutation au niveau du codon (c) en

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position 126 où l'Asn est remplacée par Asp (N126D). Dans cette forme, l'activité enzymatique est normale dans 85% des cas. La variété de G6PD (A-) est caractérisée par une substitution au niveau du codon (c) de l'acide aminé en position 68 la Val par la Met (V68M) toujours en conjonction avec la mutation N126D [49]. Pour les sujets présentant l'allèle A- l'activité enzymatique n'est que de 12%; la fréquence de cette forme allélique en Afrique Sub-saharienne, varie entre 5 à 25% [50, 51]. La déficience en G6PD est le plus souvent asymptomatique; toutefois, des anémies hémolytiques aigues peuvent survenir sous l'influence de certains facteurs pouvant créer un stress oxydatif des globules rouges. Ces conditions sont induites par les infections, les anti-inflammatoires, certains antipaludiques tels que la primaquine et la dapsone [48].

La malnutrition est un facteur constamment associé aux anémies en zone tropicale. [52]. En effet, un faible niveau d'accès à certains micronutriments particulièrement au niveau de certains groupes vulnérables tels que les enfants (situation fréquente en milieu sous développé), peut conduire à la malnutrition mais aussi à l'anémie. [35].

5. Justificatif des études

Paludisme et anémie sont des affections fréquentes au niveau des régions tropicales [39, 53]. Selon l'OMS en 2011, le nombre de cas de paludisme dans le monde était estimé entre 154 à 289 million pour un nombre de décès de 490 000 à 836 000 [1]. Plus de 1,6 milliards d'individus à travers le monde présentent une anémie et la prévalence globale des anémies au niveau des pays en voie de développement est estimée à 43% [53]. Paludisme et anémie sont fréquemment associés au niveau des zones où le paludisme sévit à l'état endémique [54, 55]. De ce fait, la mise à échelle des interventions de lutte antipaludique telle que recommandée par l'OMS, devrait contribuer à davantage réduire l'ampleur du paludisme mais elle pourrait aussi induire une réduction du poids de l'anémie liée au paludisme.

Les stratégies actuelles de lutte contre le paludisme comprennent :

- ✓ L'accès à un diagnostic précoce suivi de la prescription d'antipaludiques efficaces tels que les CTA pour les formes simples de paludisme et les rectocaps d'Artèsunate pour le traitement en prè-transfert des formes sévères;
- ✓ Le traitement préventif intermittent du nourrisson et de la femme enceinte ;
- ✓ La prévention du paludisme saisonnier chez l'enfant;

- ✓ La lutte anti-vectorielle (utilisation des moustiquaires imprégnées d'insecticides à longue durée d'action (MILDA), Aspersions intra-domiciliaires…).

Dans certaines situations épidémiologiques comme au niveau des localités du Sud-Est du Sénégal où la transmission du paludisme reste encore élevée, le contrôle du paludisme passera nécessairement par la combinaison de plusieurs mesures de lutte antipaludique [32]. Bien que la combinaison de plusieurs stratégies de lutte antipaludique puisse permettre de réduire de façon considérable l'ampleur du paludisme, il existe peu d'informations concernant, d'une part la faisabilité d'une telle approche, d'autre part l'impact que cela pourrait avoir sur le paludisme et l'anémie. Dés lors, mener des recherches opérationnelles visant à documenter la faisabilité et l'impact de la mise en œuvre combinée de mesures de lutte antipaludique constitue une priorité de santé publique.

Le renforcement de la lutte antipaludique pourrait contribuer à réduire le fardeau de l'anémie du fait de la relation étroite entre paludisme et anémie. Toutefois, il est à noter qu'en milieu tropical, les causes d'anémie sont le plus souvent multifactorielles [35] et des facteurs tels que les hémoglobinopathies, les enzymopathies érythrocytaires, les facteurs nutritionnels ainsi que les parasitoses intestinales peuvent jouer un rôle important dans la survenue des anémies.

Ainsi, dans le but de générer des informations pouvant permettre d'améliorer la lutte contre les anémies en milieu tropical, il est utile d'analyser les potentiels déterminants des anémies dans des localités où l'intensification des mesures de lutte antipaludique est effective.

Ce projet a été initié dans le but d'évaluer la faisabilité et l'impact de l'introduction combinée de la prise en charge communautaire des cas (utilisant les TDR, CTA, Artésunate rectale) et la CPS par les agents de santé communautaire du Sénégal, mais aussi d'analyser les facteurs associés à l'anémie dans des localités bénéficiant d'une intensification de la lutte antipaludique.

6. Objectifs

6.1 Objectif général

Evaluer l'apport de la mise en œuvre combinée de la prise en charge communautaire des cas et de la chimioprévention du paludisme saisonnier au niveau de la zone du Sud-Est du Sénégal.

6.2 Objectifs spécifiques

- Evaluer la faisabilité de l'utilisation combinée des tests de diagnostic rapide, des ACT et de la chimioprévention du paludisme saisonnier par les agents de santé communautaire du Sénégal.
- Evaluer l'impact de la mise en œuvre combinée de la prise en charge communautaire des cas et de la chimioprévention du paludisme saisonnier sur le paludisme et l'anémie chez les enfants de moins de 10 ans au Sénégal.
- Déterminer les facteurs associés à l'anémie dans des localités à forte couverture en mesures de lutte contre le paludisme au Sénégal.

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CHAPITRE 2: MATERIELS ET METHODES

Chapitre 2 : Matériels et méthodes

1. Cadre d'étude

Ce projet était initialement conçu pour être mené au niveau de l'observatoire démographique de la communauté rurale de Keur Socé, situé dans la zone centre du Sénégal, un des sites sentinelles du Service de Parasitologie de la Faculté de Médecine de Dakar. Les enquêtes de base réalisées au niveau de cette localité, avaient montré une prévalence trés basse du paludisme (1,5%). De ce fait, la mise en œuvre combinée de la CPS et de la prise en charge communautaire des cas, n'était plus justifiée au niveau de cette zone. Cette situation avait conduit à délocaliser le site d'étude au niveau de la zone du sud-est où la prévalence du paludisme était plus élevée. Les résultats de l'enquête de base ont fait l'objet d'un article scientifique soumis à la revue "Journal of Parasitology Research" (http://www.hindawi.com/journals/jpr/).

Au niveau de la zone sud, le poste de santé de Bonconto situé dans le district sanitaire de Vélingara a été choisi comme site d'étude. Le poste de santé de Bonconto est dirigé par un infirmier diplômé d'état et polarise 9 cases de santé dont 8 cases fonctionnelles. Au niveau de chaque case de santé, on retrouve un agent de santé communautaire (responsable de la case), assisté le plus souvent par 2 relais communautaires et 2 matrones. Dans cette localité, la transmission du paludisme est strictement saisonnière durant la saison des pluies (juillet à novembre) avec un pic de transmission entre octobre et novembre. *Plasmodium falciparum* est l'espèce parasitaire prédominante et la transmission est essentiellement assurée par *Anopheles gambiae s.l* [56].

Dans le cadre de la couverture universelle en moustiquaires imprégnées à longue durée d'action (MILDA), les populations de cette localité ont bénéficié d'une distribution généralisée de MILDA. En 2010, la couverture en moustiquaire au niveau de la zone de Bonconto était de 95,6% [45]. Deux campagnes de déparasitage de masse ciblant principalement les enfants âgés de 6 mois à 5 ans sont régulièrement effectués au niveau de la zone de Bonconto dans le cadre de la lutte contre les géohelminthiases. Ces campagnes de masse, font recours à l'administration de masse de mébendazole et ont lieu aux mois de juin et décembre. En décembre 2010, le déparasitage par le mébendazole, avait atteint un niveau de couverture de 95% *(données du poste de santé de Bonconto).*

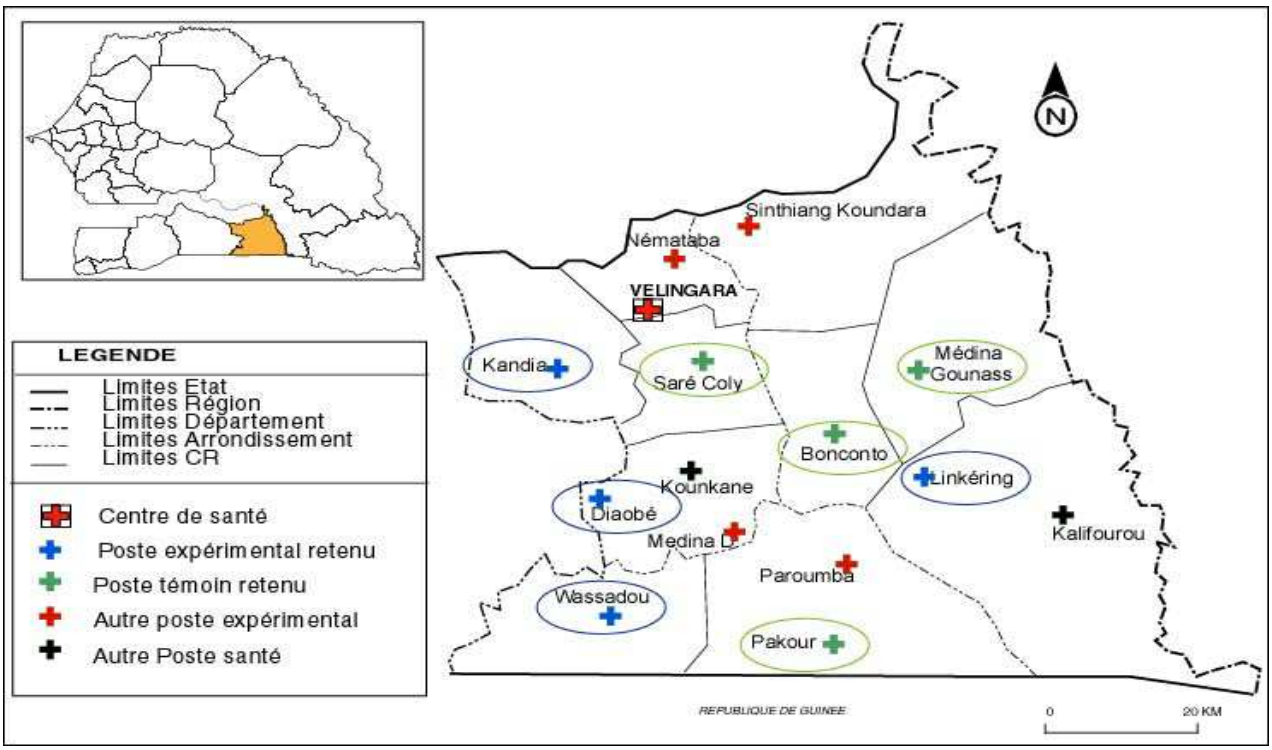

Figure 1: Carte sanitaire du district de Vélingara
(Source: Programme Elargi de Vaccination – Ministère de la santé du Sénégal).

2. Schémas et population d'étude

Les différents objectifs spécifiques du projet, ont été couverts à travers deux études: un essai clinique communautaire randomisé et une étude cas-témoins.

- *La faisabilité et l'impact de la combinaison de la CPS et de la prise en charge communautaire du paludisme ont été évalués en conduisant un essai clinique communautaire randomisé.*

Au niveau des huit villages polarisés par le poste de santé de Bonconto, les agents de santé communautaire (ASC) des cases de santé ont été formés sur l'utilisation des TDR du paludisme, et la prise en charge des cas avec la combinaison artéméther-Luméfantrine (AL) pour le traitement des formes cliniques simples. Les ASC étaient également formés sur l'administration des rectocaps d'artésunate en traitement pré-transfert des formes compliquées de paludisme.

Dans quatre villages tirés au sort les ASC étaient formés pour en plus, effectuer des administrations mensuelles de la combinaison SP-AQ dans le cadre de la CPS avec une dose de SP et 3 doses d'AQ, durant la saison de transmission du paludisme (Septembre -

Octobre – Novembre). Afin d'éviter les phénomènes de contamination pouvant être induits par les partages de comprimés au sein d'une même concession, ou au sein des communautés, l'unité de randomisation était constituée par l'agent de santé communautaire ; chaque agent santé communautaire couvrant un village.

Le critère de jugement principal de l'essai était représenté par l'incidence cumulée du paludisme au cours de la période d'intervention au niveau des 2 groupes d'études (prise en charge communautaire plus CPS versus prise en charge communautaire seule). Un accès palustre était défini par la présence d'une fièvre (température axillaire >37,5°C) associée à un TDR positif.

Les critères secondaires de jugement étaient constitués par :

- ✓ La prévalence du paludisme en fin de saison de transmission au niveau des 2 groupes;
- ✓ La prévalence de l'anémie en fin de saison de transmission au niveau des 2 groupes ;
- ✓ La couverture en CPS définie comme étant la proportion d'enfants ayant reçu les 3 doses de CPS durant la saison de transmission du paludisme ;
- ✓ La tolérance aux médicaments, évaluée par l'incidence des événements indésirables au sein de la population d'étude ;
- ✓ La fiabilité du diagnostic de paludisme, évaluée par comparaison des résultats du TDR et de la microscopie ;
- ✓ L'accès au traitement par les CTA (AL).

    - *L'investigation des facteurs associés à l'anémie chez les enfants était effectuée à partir d'une étude cas-témoins.*

Pour cela, une enquête transversale a été réalisée en décembre 2010 à la fin de la saison de transmission du paludisme, au niveau des 8 villages polarisés par le poste de santé de Bonconto. Cette enquête avait permis de déterminer la prévalence globale de l'anémie (hémoglobine < 11 g/dl) au niveau de la zone d'étude. Les participants à l'étude cas-témoins étaient sélectionnés de façon aléatoire à partir de la liste d'enfants ayant participé à l'enquête de prévalence du mois de décembre. Les enfants âgés de 1 à 10 ans présentant un taux d'hémoglobine < 11 g/dl, étaient considérés comme les cas (sujets présentant une

anémie), tandis que les témoins étaient éligibles s'ils étaient du même groupe d'âge (1 à 10 ans) et leur taux d'hémoglobine supérieur ou égal à 11 g/dl.

3. Interventions

Les deux principales interventions mises en œuvre dans le cadre de cette étude, étaient représentées par:

- la prise en charge communautaire (ou prise en charge à domicile) des cas de paludisme;
- la prévention du paludisme saisonnier chez les enfants (CPS).

Avant le démarrage de l'étude, les tests de diagnostic rapide du paludisme ont été déployés au niveau des cases de santé et les ASC de ces cases formés sur l'utilisation des TDR, le traitement du paludisme par les CTA et l'administration des médicaments de la CPS (AQ-SP). Les TDR utilisés dans le cadre de cette étude, étaient des TDR détectant la protéine *HRP II* de *Plasmodium falciparum* (*P.f Histidin Rich protein II –TDR : Malaria Antigen P.f SD*®).

Les formes simples de paludisme, étaient traitées par la combinaison Artéméther-luméfantrine pour laquelle la posologie journalière variait en fonction de l'âge des enfants. Les enfants présentant des formes sévères de paludisme, recevaient une dose de 10 mg/kg d'artésunate rectale en pré-transfert au poste de santé de Bonconto.

Au niveau des villages où la prise en charge communautaire du paludisme était couplée à la CPS, les enfants âgés de 1 à 10 ans bénéficiaient d'une administration mensuelle de la combinaison AQ-SP. Toutes les doses d'AQ-SP étaient administrées par les ASC en traitement directement observé. Les administrations de CPS étaient organisées au niveau des cases de santé. Aux jours prévus d'administration, il était demandé aux parents d'amener leurs enfants à la case de santé pour recevoir les médicaments de la CPS. Au cas où l'enfant ne se présentait pas à la case de santé, l'ASC devait se rendre au niveau du domicile de ce dernier pour dispenser les médicaments. Pour faciliter l'administration de la combinaison AQ-SP, les posologies des médicaments étaient indiquées sur une fiche qui était distribuée aux ASC pour leur servir de support. Chaque comprimé d'AQ contenait 153 mg d'amodiaquine base, tandis que les comprimés de SP étaient dosés à 500 mg de sulphadoxine et 25 mg de pyriméthamine. Les doses d'AQ-SP étaient fonction du groupe d'âge. Ainsi, les enfants de moins 2 ans recevaient un demi-comprimé de SP ; un comprimé de SP était administré aux enfants dont l'âge était compris entre 2 et 6 ans contre 1 comprimé et demi de SP pour les enfants âgés de 7 à 10 ans. Pour l'amodiaquine,

un demi comprimé était administré aux enfants de moins de 2 ans ; un comprimé pour les enfants d'âge compris entre 2 et 6 ans et un comprimé et demi pour les enfants de 7 à 10 ans. Il a été démontré que ce régime de traitement représente le régime le plus optimal pour minimiser aussi bien les phénomènes de surdosage que les sous dosage à l'amodiaquine [18]. La combinaison Artéméther-luméfantrine (Novartis^{LTD}) ainsi que les TDR étaient fournis par le PNLP, les rectocaps d'artésunate ont été obtenus auprès des laboratoires Mepha^{Ltd} tandis que l'AQ et la SP étaient fournis par Kina Pharm ^{Ltd}.

4. Méthodes de collecte de données

4.1 Méthodes pour évaluer la faisabilité et l'impact de la combinaison de la prise en charge communautaire des cas et prévention du paludisme saisonnier

Les enfants éligibles, vivants au niveau de la zone d'étude, étaient invités à participer à un système de surveillance actif du paludisme. Chaque semaine, tous les enfants inclus dans l'étude étaient examinés par les ASC. A chaque visite de suivi, la température axillaire de l'enfant était mesurée. En cas de fièvre (température supérieure 37,5°C), ou histoire de fièvre dans les 24 heures précédentes, sans causes évidentes de fièvre non palustre, un TDR était effectué par l'ASC. Pour chaque TDR effectué, une goutte épaisse était également préparée par l'ASC afin de comparer ultérieurement les résultats du TDR et de la goutte épaisse. Toutes les informations concernant les conditions cliniques des enfants, ainsi que les résultats des TDR (au cas où un TDR était effectué) étaient consignées sur une fiche-patient. Les enfants fébriles pour qui le TDR du paludisme était négatif, étaient référés au poste de santé en vue d'une meilleure investigation de l'étiologie de la fièvre ; tandis que les enfants présentant un TDR positif, étaient traités et suivi jusqu'à 7 jours après initiation du traitement, afin d'évaluer leurs conditions cliniques. Au cas où les conditions cliniques de l'enfant ne s'amélioraient pas au cours des 3 jours suivants l'instauration du traitement contre le paludisme, il était recommandé aux ASC de référer l'enfant au poste de santé. Il était également demandé aux parents (mère ou adulte responsable) d'amener les enfants à la case de santé si ces derniers présentaient une fièvre en dehors des jours prévus de visite. Les ASC devaient reporter tous les effets secondaires notifiés par les mères d'enfant après prise d'AL (Coartem®). Un questionnaire était élaboré pour recueillir les informations concernant l'administration des médicaments de la CPS (AQ-SP), ainsi que les événements indésirables y afférents. Après chaque période d'administration, les enfants devaient être examinés par un membre de l'équipe de recherche, sa mère (ou adulte responsable) interrogé (e) sur les conditions cliniques de

l'enfant afin d'évaluer la tolérance à l'AQ-SP. Les ASC étaient régulièrement (une fois par semaine) supervisés par un médecin, moniteur local de l'étude. A la fin de chaque saison de transmission, les registres des ASC étaient examinés par les membres de l'équipe de recherche, ce qui permettait de collecter des informations sur les performances des ASC en termes de diagnostic et prise en charge du paludisme.

4.2 Méthodes pour l'étude cas-témoin (anémie)

Dans le cadre de l'étude cas-témoins, chaque enfant recruté avait bénéficié d'un examen clinique puis de prélèvements :

- d'urines pour la recherche de *Schistosoma heamatobium* ;
- de selles pour la recherche de parasites intestinaux ;
- de sang pour le dosage du taux d'hémoglobine, la recherche de *Plasmodium falciparum* et la détection d'hémoglobinopathies.

La mère de l'enfant (ou l'adulte responsable) était interrogée pour déterminer les caractéristiques socio-démographiques de l'enfant, l'existence ou non d'antécédent de fièvre. Les informations concernant les participants à l'étude étaient consignées sur un questionnaire. Le statut nutritionnel des enfants était évalué en mesurant le poids, la taille de l'enfant. Ces éléments, associés au sexe à l'âge de l'enfant, avaient permis de déterminer des Z-scores. Le Z-score poids pour âge était utilisé comme indicateur d'un retard pondéral tandis que le Z-score taille pour âge était utilisé comme indicateur d'un retard de croissance. Les Z-scores étaient calculés en se basant sur les valeurs médianes du *National Centre for Health Statistics* (NCHS).

5. Méthodes biologiques

5.1 Recherche du portage de Plasmodium falciparum

Un prélèvement de sang à la pulpe du doigt était effectué pour la confection d'une goutte épaisse et d'un frottis. Goutte épaisse et frottis étaient colorés au giemsa et lus au niveau du laboratoire de parasitologie de la Faculté de Médecine de Dakar. La goutte épaisse était considérée comme positive devant la présence de formes asexuées de *Plasmodium.* Lorsque la goutte épaisse était positive la densité parasitaire était déterminée en comptant le nombre de formes asexuées pour 200 leucocytes et exprimée en nombre de parasites par µL de sang en utilisant la formule suivante : nombre de parasites x 8000 / 200 ; en assumant que 1 µL de sang contient 8000 leucocytes. Les lames de gouttes épaisses étaient considérées comme négatives lorsque, aucun parasite n'était détecté après avoir

parcouru 200 champs microscopiques. L'examen du frottis permettait de déterminer l'espèce plasmodiale en cause.

5.2 Diagnostic du paludisme par le TDR Malaria Ag SD BiolineTM

Le TDR Malaria Ag SD Bioline ^{TM} est un test immuno-chromatographique capable de détecter l'antigène HRP 2 (Histidin-rich protein 2) de *Plasmodium falciparum*. Le test se présente sous forme de cassette contenant une membrane sensibilisée avec des anticorps monoclonaux de souris, spécifiques de la protéine HRP 2 de *Plasmodium falciparum*. Au niveau de la cassette, on retrouve un puits A destiné à recevoir l'échantillon de sang à tester, un puits S au niveau duquel sera déposé le réactif tampon (fourni par le fabricant) et une colonne de lecture. La colonne de lecture du TDR présente une ligne de contrôle (C) qui doit obligatoirement apparaître pour que le résultat du test soit valide (autocontrôle des procédures). Il existe une deuxième ligne de lecture correspondant à des anticorps spécifiques de la HRP 2 de *Plasmodium falciparum (« P.f »).* L'apparition de cette ligne témoigne d'une infestation à *Plasmodium falciparum*.

Il était recommandé aux ASC d'utiliser les TDR de manière conforme aux instructions du fabricant. Une goutte de sang devait être prélevée par piqure à la pulpe du doigt, puis déposée au niveau du puits A de la casette destiné à recevoir l'échantillon de sang. Dix gouttes de réactif tampon étaient ensuite déposées au niveau du puits S. La lecture devait être effectuée au bout de 15 à 30 minutes. Le résultat du TDR était interprété comme positif en cas d'apparition de deux bandes (ligne contrôle et ligne de positivité). Il était considéré comme négatif lorsque seule la bande contrôle était visible. En l'absence de bande contrôle, le TDR était considéré comme invalide.

5.3 Mesure du taux d'hémoglobine

Une goutte de sang prélevée par piqure à la pulpe du doigt, était déposée au niveau d'une microcuvette pour la détermination du taux d'hémoglobine à l'aide d'un appareil Hemocue (HemoCue® Hb 201).

5.4 Recherche de parasites intestinaux dans les selles et d'oeufs de Schistosoma heamatobium au niveau des urines

Des échantillons de selles étaient prélevés dans des pots en plastique stériles. Ces prélèvements étaient examinés à l'état frais puis après concentration. Pour l'examen direct à l'état frais, des parcelles de selles étaient prélevées à plusieurs endroits de l'échantillon de selle à l'aide d'une baguette fine, puis déposées sur une lame porte objet et mélangées

avec une goutte d'eau physiologique de façon homogène. La suspension était ensuite recouverte d'une lamelle et observée au microscope binoculaire aux grossissements 10 et 40 à la recherche d'œufs et larves d'helminthes, formes végétatives et kystes de protozoaires.

Les selles étaient ensuite examinées après concentration en utilisant la technique de concentration de Ritchie, selon la procédure suivante :

- ✓ Deux grammes de selles étaient mélangés dans 20 ml d'une solution de formol à 10%.
- ✓ Après sédimentation, le mélange était ensuite tamisé à l'aide d'une bande de gaze et émulsionné dans 1 tube à centrifuger avec de l'éther en agitant vigoureusement pendant 30 sec.
- ✓ L'émulsion était ensuite centrifugée à 1500 tour/min pendant 5 minutes dans un tube à fond conique.
- ✓ Le surnageant était rejeté et le culot récupéré en éliminant les couches supérieures.
- ✓ Le culot était examiné entre lame et lamelle au grossissement 10 puis 40.

Un examen de selle était considéré comme positif, en cas de présence d'un ou de plusieurs parasites intestinaux.

Des prélèvements d'urines étaient effectués dans des pots en plastiques stériles entre 10 et 14 heures. La détection d'œufs de *Schistosoma heamatobium* était effectuée par filtration des urines. Un volume de 10 ml d'urines était filtré sur membrane millipore; le filtre permettait de retenir les œufs de *Schistosoma haematobium* dont la taille est plus grande que les mailles du filtre. Ce filtre était ensuite placé sur une lame porte objet et examiné au microscope à l'objectif 10 ou 40 après adjonction d'une goutte de lugol, ce qui permettait de mettre en évidence les œufs de *Schistosoma haematobium* et de les quantifier en comptant le nombre d'œufs par 10 ml d'urines.

5.5 Détection des hémoglobinopathies et enzymopathies

- ***Extraction de l'ADN des échantillons de sang***

Des échantillons de sang étaient prélevés sur papier filtre (Whatman 3M ^{TM}) chez 352 enfants ayant participé à l'enquête de prévalence de décembre 2010. A partir des confettis, une extraction d'ADN était effectuée sur plaque de 96 puits par la méthode du Chelex-100 telle que décrit par Pearce et *al* [57]. Le type d'hémoglobine (A, S, C) ainsi que le polymorphisme de la glucose 6 phosphate déshydrogénase (B, A, A-) étaient déterminés par technique de réaction de polymérisation en chaine (PCR) suivie d'une

technique SSOP-ELISA (*sequence-specific oligonucleotide probe enzyme-linked immunosorbent assay*), tandis que la détection de traits alpha-thalassémiques était faite par PCR [51, 58].

- ***Conditions des réactions de polymérisation en chaine (PCR)***

Les amorces utilisées dans le cadre de la PCR pour la détection du polymorphisme de la G6PD, permettaient d'amplifier un fragment de 352 paires de base, du gène de la G6PD couvrant le siège de la mutation de l'enzyme au niveau du codon (c) en position 68[59]. Le gène de l'hémoglobine était amplifié grâce à des amorces décrites par Modiano et *al* [41] pour produire un fragment de 358 paires de base couvrant les sites de mutation des codons 6 et 26. Les amorces utilisées dans le cadre de ces réactions étaient biotinylées au niveau de l'extrémité 5' par le fournisseur (MWG Biotech, Ebersberg, Germany) [51].
Pour la détection des traits alpha–thalassémiques, les amorces permettaient de produire des bandes de 2200 + 1900 paires de base représentant les variantes africaines de l'alpha globine [58]. Les conditions de la PCR pour la détermination du polymorphisme de la G6PD et des hémoglobinopathies, étaient similaires aux conditions décrites par Enevold et *al* [51]. Toutes les réactions étaient effectuées dans des plaques de PCR à 96 puits. La révélation des produits d'amplification des alpha-thalassémies était faite par électrophorèse sur gel d'agarose à 0,75%.

- ***SSOP-ELISA***

Les procédures de la technologie SSOP-ELISA ont été décrites en détails par Enevold et *al* [51]. Des sondes d'oligonucléotide conjuguées à la digoxigénine au niveau de l'extrémité 3' et comportant de 18 bases couvrant les SNP d'intérêt ont été utilisées. Le produit d'amplification de la PCR a été utilisé pour la sensibilisation des plaques ELISA ; ce produit était dilué dans de l'eau distillée au demi. L'ADN dilué était ensuite dénaturé à 95°C pendant 5 minutes. Deux microlitres du produit dénaturé étaient ajoutés au niveau des puits d'une plaque ELISA préalablement sensibilisée à la streptavidine et contenant une solution de lavage (représentée par du PBS dilué à 0,05% avec du Tween 20) ; le tout était incubé à la température ambiante pendant une heure de temps. Le produit de PCR fixé à la streptavidine, était incubé après adjonction des sondes d'oligonucléotide (concentration de 8 nano moles) en présence d'une solution de tétra-méthyl ammonium chloride (TMAC) à la température de 53°C en utilisant un incubateur à température réglable muni d'un agitateur. Après cette incubation, un lavage était effectué. Les températures de lavage de la solution de TMAC étaient de l'ordre de 62°C pour le typage

de l'hémoglobine et 65°C pour la recherche du polymorphisme de la G6PD. Les plaques étaient secondairement incubées en présence d'anticorps antidigoxigénine conjugués à la peroxydase (Roche Diagnostics) et dilués au 1 : 1000. L'incubation était effectuée à température ambiante pendant une heure. Après cette phase d'incubation, la révélation était effectuée en utilisant une solution d'o-phénylène-diamine (OPD). La réaction était arrêtée par adjonction d'acide sulfurique (H_2SO_4), puis les densités optiques mesurées à 492 nm. Entre chaque étape, les plaques d'ELISA étaient lavées 3 fois en utilisant une solution de lavage tamponnée.

Il est à noter que toutes les analyses moléculaires effectuées dans le cadre de ce travail, ont été menées au niveau du laboratoire de biologie moléculaire du *Centre for Medical Parasitology* (CMP) de la Faculté de Médecine de Copenhague au Danemark.

6. Gestion and analyse de données

Les données des différentes études ont été saisies sur Excel ™ et analysées en utilisant le logiciel STATA IC 11 ™. Les variables catégorielles étaient décrites en termes d'effectif et de pourcentage de données renseignées. Les données quantitatives étaient décrites en termes de moyenne, d'écart type pour les données ayant une distribution normale ; en l'absence d'une distribution normale, la médiane et l'étendue interquartile étaient utilisées.

Dans le cadre des évaluations d'impact, le critère de jugement principal était représenté par l'incidence des épisodes cliniques de paludisme ; une analyse en intention de traiter était effectuée prenant en compte tous les enfants inclus dans l'étude. Les critères secondaires de jugement comprenaient la prévalence du portage de *Plasmodium falciparum* et de l'anémie en fin de saison de transmission du paludisme. Une analyse en per-protocole a été effectuée en tenant compte du nombre d'enfant ayant participé en l'enquête transversale en fin de saison de transmission.

Toutes les analyses ont été effectuées au plan individuel avec ajustement sur le schéma de l'étude (pour tenir compte de l'effet grappe) par la méthode du « *robust standard error* » [60]. La période à risque pour chaque enfant suivi dans l'étude a été calculée à partir de la date de la première administration de CPS jusqu'à la date de l'enquête transversale de fin de saison de transmission. Après un épisode de paludisme, un enfant n'était plus considéré à risque durant les 28 jours qui suivaient le traitement par la combinaison Artemether-Lumefantrine ; ces enfants étaient censurés de l'analyse pendant une période de 4 semaines. Les délais de survie instantanés au niveau des deux groupes d'étude ont

été comparés par la méthode du Kaplan-Meier avec un test du « *log rank* » stratifié par village (« *cluster* »). Le rapport d'incidence entre le groupe soumis à l'utilisation combinée de la prise en charge communautaire du paludisme + CPS, et le groupe prise en charge communautaire du paludisme seule, a été déterminé après ajustement sur des facteurs confondants tels que l'âge et le sexe, en utilisant un model de régression de Cox ajusté sur le schéma d'étude par la méthode du « *robust standard error* ». L'efficacité protectrice de l'utilisation combinée de la prise en charge communautaire du paludisme et de la CPS a été calculée de la façon suivante : (1- rapport d'incidence) x 100. Les valeurs de p inférieures à 5% ont été considérées comme significatives (en situation bilatérale). Les prévalences du paludisme et de l'anémie en fin de saison de transmission du paludisme, ont été évaluées et comparées au niveau des deux groupes d'étude par méthode de régression logistique avec ajustement sur le schéma d'étude par la méthode du « *robust standard error* ».

Pour évaluer la fiabilité du diagnostic par les TDR, la sensibilité, la spécificité, les valeurs prédictives positive et négative ont été calculées en comparant les résultats du TDR et de la goutte épaisse. Un coefficient de concordance (kappa) entre goutte épaisse et TDR a été calculé ainsi que son intervalle de confiance à 95%. La concordance était considérée comme modérée pour des valeurs de kappa comprises entre 0,21 et 0,60 ; elle était considérée comme bonne lorsque les valeurs de k variaient entre 0,61 et 0,80 et parfaite pour k < 0,80 [61].

Pour évaluer le statut nutritionnel des enfants, les données ont été transférées sur Epi Info (version 3.5 epi 2000). Les Z-scores poids pour âge (retard pondéral), taille pour âge (retard de croissance) ont été dérivés à partir de "*Epinut Anthropometry"* (logiciel Epi Info). Les enfants pour qui les Z-scores étaient inférieurs à moins de 2 déviations standard, par rapport à la médiane de la population de référence qui est celle de la NCHS étaient considéraient comme malnutris. L'analyse des principaux facteurs associés à l'anémie chez les enfants de l'étude était faite par méthode de régression logistique inconditionnelle. Les valeurs de p inférieures à 5% en situation bilatérale ont été considérées comme significatives.

7. Considérations éthiques

Préalablement au démarrage de l'étude, des séances de sensibilisation ont été menées au niveau des villages ciblés par le projet. Un consentement communautaire a été obtenu par l'intermédiaire des leaders communautaires (imams, chefs de village, président de la

communauté rurale de Bonconto). Sur le terrain, un consentement libre et éclairé était requis de la part des mères ou adultes responsables des enfants. Le protocole de recherche a obtenu l'avis favorable du Conseil National de Recherche en Santé; avis éthique N° 027/MSP/DS/CNRS, 18/03/2010. L'étude a par ailleurs était enregistré au niveau de Pan African Clinical Trial Registry (pactr.org) : **PACTR201305000551876.**

CHAPITRE 3 : RESULTATS DE L'ENQUETE DE BASE.

Chapitre 3 : Résultats de l'enquête de base

Ce chapitre est basé sur l'article suivant, soumis à la revue « *Journal of parasitology Research* »

Parasitic infections among children under five years in Senegal: prevalence and effect on anaemia and nutritional status.

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1. Abstract

Although malaria is declining in many countries in Africa, malaria and anaemia remain frequent in children. There are multiple aetiologies to anaemia and intestinal parasites can play a major role in anaemia occurrence. This study was conducted to assess the relationship between malaria parasitaemia, intestinal worms and anaemia, in children <5years in an area with declining of malaria transmission.

A survey was carried out in 30 villages in the central part of Senegal. A two level random cluster sampling technique was used to select study participants. Children less than 5 years were enrolled after parent's inform consent. For each child, blood thick and smear test were performed, haemoglobin concentration measured with HemoCue®, stool samples collected and examined using Ritchie technique.

A total of 736 children were recruited**.** Malaria parasite prevalence was 1.5% [0.7-2.6], anaemia was found in 53.4%[48.2-58.9], while intestinal parasites and stunting represented 26.2% [22.6 – 30.2] and 22% [18.6 – 25.5] respectively. In a Logistic regression analysis, anaemia was significantly associated with malaria parasiteamia (aOR=6.3[1.5-53.5]) and stunting (aOR=2[1.2-3.1] ; no association was found between intestinal parasites and anaemia.

Malaria and anaemia remain closely associated even when malaria is declining. Scaling up antimalarial interventions may contribute to eliminate malaria and reduce the occurrence of anaemia among children.

2. Introduction

Malaria is a major public health problem in African region. According to the World Health Organization (WHO), in 2010 there were an estimated 149 to 274 million cases and 537,000 to 907,000 deaths worldwide [62]. Over 89% of these deaths occur in Africa, mainly in children under 5 years, most of the time outside health facilities [63]. In recent years, significant reductions in the incidence of malaria have been reported in several countries in Africa where malaria was previously moderately endemic [44]. Although the incidence of malaria appears to be declining in a number of African countries, malaria remains an important cause of mortality and morbidity among young children.

Anaemia also remains a major public health problem, not least in malaria endemic areas, where it is primarily seen in young children in areas with stable malaria, but also in adults in malaria unstable areas[64, 65]. Recently, the WHO advocated for the scaling up of malaria control interventions in order to accelerate malaria elimination and consequently, reduce malaria fatal consequence on children health such as anaemia [66, 67]. Several malaria control strategies were developed recently, including (i) early case detection and treatment using rapid diagnostic tests (RDT), treatment with more effective antimalarial drugs : Artemisinin-based Combination Therapy (ACT) for uncomplicated malaria cases and pre-referral rectal Artesunate for the initial management of complicated malaria; (ii)Intermittent Preventive Treatment (IPT) for pregnant women and infants among others. In tropical regions, there are multiple aetiologies to anaemia and intestinal parasites as well as malnutrition can play a major role in anaemia occurrence [37], particularly when malaria prevalence is declining. Thus, exploring the relationship between malaria, intestinal parasites, malnutrition and anaemia can provide useful information in order to guide public health programs aiming at reduction of child health problems in tropical regions.

This study was aiming : (i) to assess the prevalence of malaria parasitaemia, intestinal parasites (IP), anaemia and malnutrition among children <5 years, and (ii) to explore the relationship between malaria, intestinal parasites, malnutrition and anaemia.

The study was conducted prior to the implementation of an interventional study evaluating the impact on malaria burden of a community-based malaria control strategy including effective case management (using RDT and ACT treatment) and IPTc at Lamarame in Senegal, where a demographic surveillance site has recently been established.

3. Population and methods

Study area and population

The study was conducted at the Lamarame health post located in the Keur Socé, a rural community, at 17 Km from the city of Kaolack in central Senegal, and 200 km from Dakar **(figure 1**). The Lamarame health post is in the Ndoffane health district. It covers 34 villages located within a radius of 8 Km from the post, with a total population of about 10,000 inhabitants.

Malaria is endemic in this area with a seasonal pattern of transmission peaking during the rainy season (July-November); entomological inoculation rates range from 9 – 12 infective bites/human/year. Many programs aiming to reduce child health problems are being promoted in this area. These programs included two yearly vitamin A supplementation, and de-worming with Mebendazole, in December and June of each year for children aged 1 to 5 years.

A cross sectional household survey was carried out at the end of the malaria transmission season, in January 2010, one month after the second round of Mebendazole mass administration campaign.

A two level random cluster sampling technique was used with a total of 30 clusters randomly selected based on probability proportional size in the villages. The first household was randomly selected in each cluster, followed by the neighbouring household until a minimum cluster target of 23 children was reached. A minimum sample size of 700 children under 5 years of age was calculated using Epi Info software based on an expected prevalence of anaemia of 35%, with alpha=5% (confidence interval at 95%), power at 90% (beta 10%) and multiplied by 2 for an design effect.

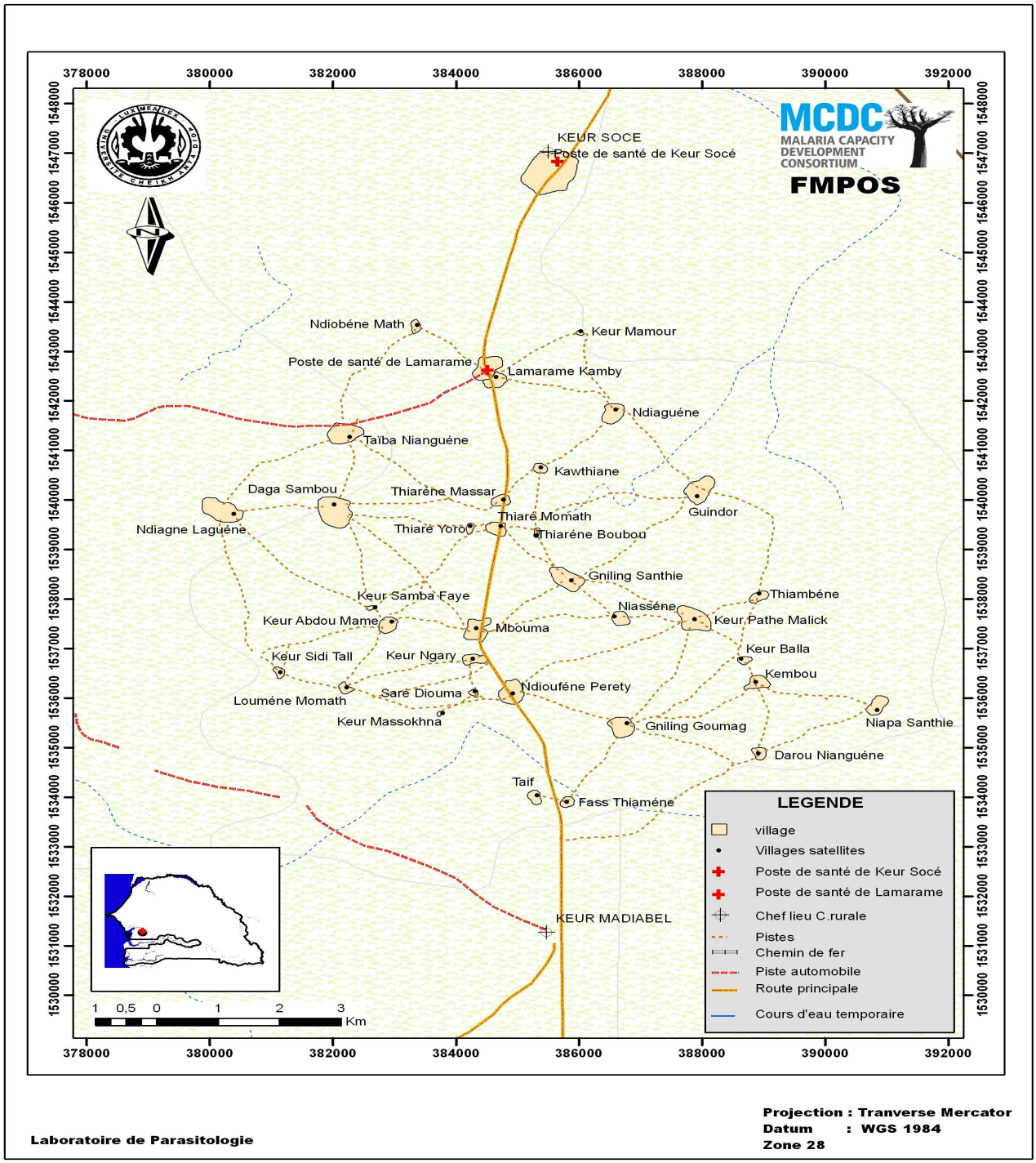

Figure1: Study area location

Data collection

Structured questionnaire

A code was given to every child after parents' informed consent. Each eligible child was examined by a physician prior to a biological assessment which included blood and stool samples. The mother was interviewed directly on the child symptoms as well as socio demographic characteristics using a standard questionnaire. Data obtained from physical examination and parents interview were assigned on a case report form (CRF).

Anthropometry

Children were weighed by a trained assistant, without their shoes using Seca scales; height was measured to the nearest 0.1 cm using a calibrated scale and a sliding head piece. Weight- for-age Z-score was used to denote underweight while height for age Z-score was used as an indicator of stunting. The Z-scores were calculated based on the median values of the National Centre for Health Statistics (NCHS) reference population, United States.

Biological assessment

Blood samples were collected using finger prick blood. The first drop was used to prepare thick and thin smears for the diagnosis of malaria. Thick and thin smears were stained with Giemsa and read by a laboratory technician. Malaria was defined as any asexual parasitemia detected on a thick or thin blood smear. Parasitemia was numbered and expressed by number of trophozoites/µL using the following formula: numbered parasites x 8000 / 200. Absence of malaria parasite in 200 high power ocular fields of the thick film was considered as negative.

The second drop of finger prick blood was drawn into a microcuvette for Hb determination (g/dl) using HemoCue machine (HemoCue® Hb 301). Moderate and severe anaemia were defined as hb concentration below 11 g/dl and 8 g/dl, respectively.

Fresh stools samples were collected into wide mouth screw-cap clean containers. Fecal samples were examined for the detection of intestinal parasite using Ritchie technique. Intestinal parasite was recorded positive by the presence of helminthes and/or protozoans in the faces.

Data analysis

Data were entered in Excel ™ software and analysed using STATA 10™ software. To assess the nutritional status, data were transferred into Epi Info 3.04 d. The Z-scores for weight-for-age (underweight) and height-for-age (stunting) were derived using Epinut Anthropometry. Children who had Z scores below -2 standard deviation (SD) of the NCHS reference population median were considered to be malnourished.

For descriptive data, percentage was used to assess the prevalence of each outcome. Proportions were compared using chi square test or Fisher exact test (univariate analysis); significance level of the different tests was 0.05 two sided. A stepwise logistic regression was done for the determination of risk factors possibly associated with anaemia and under-nutrition. The validity of the final models was tested using Hosmer-Lemeshow goodness of fit test.

Ethical considerations

Prior to the study, a community sensitization was undertaken and community consent was obtained from community leaders (religious guide, village head, etc.…). Informed consent was obtained from parents or children's guardians as well as child assent the day of survey. The study protocol was approved by the Senegalese National Ethical Committee (Conseil National de Recherche en Santé). Patients with malaria parasitaemia, anaemia and/or any intestinal parasite were given treatment by the study physician.

3. Results

Seven hundred and thirty six children (393 male and 343 female) aged 6 months - 5 years with a mean age of 2.5 ±1.3 years participated in this study (**Table 1).**

Table 1: Socio-demographic characteristic of children under 5 years at Lamarame (n=736)

Socio demographic variables	Number	Percentage	CI 95%
Age (months)			
< 12	42	5.7	[4.1 - 7.7]
[12-23]	239	32.5	[28.5 - 36.9]
[24-35]	197	26.8	[23.1 - 30.7]
[36-47]	137	18.6	[15.6 - 22]
[48-60]	121	16.4	[13.6 – 19.6]
Gender			
Male	393	53.4	[48.2 -58.9]
Female	343	46.6	[41.8 - 51.8]
Birth order of children			
[1-3]	393	53.4	[48.2 - 58.9]
[4-5]	340	46.2	[41.4 - 51.4]
Number of children in household			
[1-3]	309	42	[37.4 - 46.9]
[4-5]	216	29.4	[25.6 - 33.5]
> 5	202	27.4	[23.8 - 31.5]
Missing	9	1,2	[0.5 - 2.3]
Residence zone			
Lamarame	116	15.8	[13 - 18.9]
Others villages	620	84.2	[77.7- 91.1]

Prevalence of malaria, anaemia, intestinal parasites and under-nutrition

The overall prevalence of *Plasmodium falciparum* was 1.5%. Moderate anaemia and severe anaemia was found in 53.4% and 12.5% of the children, respectively. A total of 193 (26.2%), children were found with at least one intestinal parasite. Parasites species were represented by *Giardia intestinalis* 15.6%, *Entameaba coli* 10.9, *Hymenolepis nana* 1.9%, *Ascaris lumbricoïdes* 0.4%, *Enterobius vermicularis* 0.4% and T*richuris trichura* 0.1%. Children with 2 two intestinal parasites species represented 3,01% (22 children); one child (0,14%) was coinfected with 3 intestinal parasites species. The overall prevalence of stunting and underweight was 22% and 16.3% respectively. **(Table 2).**

Table 2 : **Prevalence of malaria, anaemia, intestinal parasites and undernutrition among children under 5 years at Lamarame health post. (n=736)**

Variables	Number	Percentage	CI 95%
Malaria parasite			
Yes	11	1.5	[0.7 -2.6]
No	721	98	[90.9 – 100]
Anaemia			
Mean haemoglobin	736	10.1 ± 1.9	
Moderate anaemia (Hb<11)	393	53.4	[48.2 - 58.9]
Severe Anaemia (Hb<8)	92	12.5	[10 – 15.3]
Intestinal parasite prevalence			
Children with at least one intestinal parasite	193	26.2	[22.6 – 30.2]
Parasite species			
Giardia intestinalis	115	15.6	[12.9 – 18.7]
Entameaba coli	80	10.9	[8.6 – 13.5]
Hymenolepis nana	14	1.9	[1 – 3.2]
Ascaris lumbricoïdes	3	0.4	[0.08 – 1.2]
Enterobius vermicularis	3	0.4	[0.08 – 1.2]
Trichuris trichiura	1	0.1	[0.003 – 0.7]
Undernutrition			
Stunting (HAZ < -2SD)	161	22	[18.6 – 25.5]
Underweight (WAZ <-2SD)	120	16,3	[13.5 – 19.5]

Factors associated with anaemia among children under five

Anaemia (Hb <11g/dl) was significantly associated with age range from 12 to 23 months (aOR=3.7 [1.7-7.9], malaria parasite (aOR=6.3 [1.5-53.5]), birth order higher than 3 (aOR=1.6 [1.1-3.5]). Stunting was also significantly associated with anaemia in children under 5 years of age (aOR=2[1.2-3.1]). We did not found any statistically significant association between isolated intestinal parasites such *Giardia intestinalis, Entameaba coli, hymelopis nana, Enterobius vermicularis and Ascaris lumbricoïdes* and anaemia**.** No association was found between anaemia and co-infection with more than one intestinal parasite species (aOR=0,66 [0,28-1,58]. **(Table 3)**

Table 3: **Factors associated with anaemia among children under five at Lamarame health post**

Variables	Anaemia (Hb<11g/dl)		
	Number (%)	aOR* (95% CI)	p value
Age (months)			
< 12	19 (45.2)	1	
12 – 23	199 (83.3)	3.7 [1.7-7.9]	0.001
24 – 35	136 (69)	1.6 [0.7-3.5]	0.20
36 – 47	83 (60.6)	1.1 [0.5-2.4]	0.75
48 – 60	54(44.6)	0.6 [0.3-1.4]	0.26
Gender			
Male	264(67.2)	1	
Female	227(66.2)	0.8 [0.6-1.2]	0.37
Birth order			
[1-3]	254(64.6)	1	
[4-5]	235(69.1)	1.6 [1.1-3.5]	0.04
Number of children within household			
[1-3]	213(68.9)	1	
[4-5]	133(61.6)	0.5 [0.3-0.8]	0.03
>5	137(67.8)	0.6[0.3-1.3]	0.26
Malaria parasite			
No	479(66.4)	1	
Yes	10(90.9)	6.3 [1.5-53.5]	0.03
Stunting			
No	352 (62.4)	1	
Yes	132 (81.9)	2 [1.2-3.1]	0,004
Intestinal parasite			
No	369(68.7)	1	
Giardia intestinalis	73(63.5)	0.9 [0.6-1.4]	0.75
Entameaba coli	49(61.2)	1.1 [0.6-1.9]	0.67
Hymenolepis nana	10(71.4)	1.8 [0.5-6.7]	0.35
Enterobius vermicularis	1(33.3)	0.2 [0.02-2.7]	0.28
Ascaris lumbricoïdes	1(33.3)	0.2 [0.01-2.7]	0.28
Residence zone			
Health post	55(47.4)	1	
Other villages	436(70.3)	2.1[1.3-3.4]	0.001

**Adjusted OR - Hosmer-Lemeshow Goodness of fit test Ch² (8df)=5.49 p=0.70*

Factors associated with under-nutrition among children under five

Multivariate analysis using a logistic regression model, showed that stunting was significantly associated with moderate anaemia (OR 1.7 CI95% [1.1-2.8]) severe anaemia (OR3.5 [1.9-6.4]), residence zone (OR 3.5 CI95% [1.6-8.1]) birth order >3 (OR 2.9 [1.3-6.6]). There was no statistically significant association between malaria and stunting (OR 1.2 [0.3-0.5] as well as isolated intestinal parasites such *Giardia intestinalis, Entameaba coli, hymenolepis nana, Enterobius vermicularis and Ascaris lumbricoïdes.* **(Table 4)**

Table 4 : **Factors associated with stunting among children less than five years at Lamarame health post**

	Stunting (HAZ)		
	Number (%)	**aOR* (95% CI)**	**p value**
Age (months)			
< 12	1 (2.4)	1	
12 – 23	64 (26.8)	7.4 [1 – 57.3]	0.05
24 – 35	49 (24.9)	7.5 [1 – 58.1]	0.05
36 – 47	29 (21.1)	6.6 [0.8 – 52.3]	0.07
48 – 60	18 (14.9)	5 [0.6 – 41]	0.13
Gender			
Male	88 (22.4)	1	
Female	73 (21.3)	0.9 [0.6 – 1.4]	0.82
Birth order			
[1-3]	82 (20.8)	1	
[4-5]	79 (23.2)	2.9 [1.3 – 6.6]	0.01
Number of children within household			
[1-3]	75 (24.2)	1	
[4-5]	43 (19.9)	0.4 [0.2 – 0.8]	0.01
>5	42 (20.8)	0.3 [0.1 – 0.7]	0.01
Malaria parasite			
No	157 (21.8)	1	
Yes	3 (27.3)	1.2 [0.3 – 5]	0.75
Intestinal parasite			
No	123 (22.9)	1	
Giardia intestinalis	21 (18.3)	0.9 [0.5 – 1.6]	0.73
Entameaba coli	17 (21.2)	1.2 [0.7 – 2.3]	0.45
Hymenolepis nana	3 (21.4)	1.3 [0.3 – 5.4]	0.69
Enterobius vermicularis	00	-	
Ascaris lumbricoides	00	-	
Residence zone			
Health post	7 (6)	1	
Other villages	154 (24.8)	3.6 [1.6 – 8.1]	0.002
Anaemia			
No	29 (11.8)	1	
Moderate anaemia (Hb<11g/dl)	93 (23.6)	1.7 [1.1 – 2.8]	0.02
Severe anaemia (Hb<8 g/dl)	39 (39.8)	3.5 [1.9 – 6.4]	0.000

**Adjusted Odds ratio. Goodness of fit test: Hosmer-Lemeshow Chi² (8df) =7.45, p=0.48*

Factors associated with underweight were represented by moderate anaemia (aOR 1.7[1.1-2.8]), severe anaemia (aOR 4.4 [2.3-8.4]) and age range from 24-35 months (aOR 8.9 [1.2-70.6]). **(Table5)**

Table 5 : Factors associated with underweight among children less than five years at Lamarame health post

	Underweight (WAZ)		
Variables	**Number (%)**	**aOR* (95% CI)**	**p value**
Age (months)			
< 12	1(2.4)	1	
12 – 23	38 (15.9)	4.9 [0.6 – 39.5]	0.12
24 – 35	43 (21.8)	8.9 [1.2 – 70.6]	0.03
36 – 47	22 (16)	6.4 [0.8 – 52]	0.08
48 – 60	16 (13.2)	6.2 [0.7 – 50.8]	0.09
Gender			
Male	64 (16.2)	1	
Female	56 (16.3)	1.1[0.7 – 1.6]	0.7
Birth order			
[1-3]	66 (16.8)	1	
[4-5]	54 (15.8)	1.8 [0.8 6 4.2]	0.15
Number of children within household			
[1-3]	58 (18.7)	1	
[4-5]	33 (18.3)	0.5 [0.2 – 1.2]	0.16
>5	28 (13.7)	0.4 [0.1-1.1]	0.09
Malaria parasite			
No	117 (16.2)	1	
Yes	2 (18.2)	0.9 [0.2 – 4.8]	0.9
Intestinal parasite			
No	98 (18.2)	1	
Giardia intestinalis	11 (9.6)	0.6 [0.3 – 1.1]	0.09
Entameaba coli	8 (10)	0.6 [0.3 – 1.3]	0.19
Hymenolepis nana	2 (14.3)	1.3 [0.2 – 6.7]	0.73
Enterobius vermicularis	1 (33.3)	4.4 [0.3 – 61.1]	0.26
Ascaris lumbricoïdes	00	-	-
Residence zone			
Health post	9 (7.7)	1	
Other villages	111 (17.9)	0.6 [0.3 – 1.4]	0.27
Anaemia			
No	24 (9.8)	1	
Moderate anaemia (Hb<11g/dl)	63 (16)	1.7 [1.1 - 2.9]	0.04
Severe anaemia (Hb<8 g/dl)	33 (33.7)	4.4 [2.3 – 8.4]	0.000

**aOR: adjusted odds ratio. Goodness of fit test: Hosmer lemeshow Chi² (8df) 3.35 ;p=0.91*

4. Discussion

Malaria, anaemia, intestinal parasitic infections and malnutrition remain serious public health problems especially in developing countries [36, 68]. We assessed the magnitude of malaria, intestinal parasites carriage, anaemia and under-nutrition.

Our study conducted in a malaria endemic area showed an unexpectedly low prevalence of *P. falciparum* infections (1.5%) while a high level of anaemia was observed. Despite the low prevalence of malaria parasiteamia, anaemia was more frequent in children with *P. falciparum* infections (OR 6.3). It thus, appears that malaria is still closely associated with anaemia in this area, even in the context of declining of malaria prevalence. A survey conducted in January 2009 (one year before) in a neighbouring area (Kaolack:17 km from the Lamarame health post), showed a higher prevalence of malaria (7.2%) [69]. These results suggest a decrease of malaria prevalence in this part of Senegal as well as in other areas *(National Malaria Control program annual report 2009*). Scaling up antimalarial strategies such as early case detection and treatment, intermittent preventive treatment as well as long lasting insecticide treated nets, may even contribute to malaria elimination in this area, and significantly reduce anaemia as a public health problem in rural Senegal areas with similar disease patterns. Children with a birth order greater than three had an increased risk of developing anaemia. In other studies, high family size was associated with anaemia and under-nutrition of children [70].

The study showed a high prevalence of intestinal parasite carriage (26.2%); *Giardia intestinalis* was the most frequent parasite (15.6%). The observed prevalence of *Giardia* in this study is consistent with those described in other countries [71, 72]. The deleterious effect of *Giardia intestinalis* on growth and health of children has been shown by several studies [73]. Although we did not find any statistically significant association between *Giardia intestinalis* and anaemia, as well as malnutrition, this parasite is known for its ability to induce diarrhoea [74] and mal-absorption syndrome, and can lead to protein energy malnutrition, vitamin A deficiency and iron deficiency anaemia and vitamin B12 [75]. Mass deworming programme is being promoted at the Lamarame health post to reduce intestinal worms carriage. In December 2009, a large scale of mass administration of mebendazole took place at the Lamarame health post with an estimated coverage of 95% in children aged 1 to 5 years *(Lamarame health post report- unpublished data*). This can explain the low level of prevalence of helminthic infections. While ensuring an effective action against helminthic infections, mass administration of mebendazole does not act against protozoan infections. Albendazole can have an effective action against both protozoan and helminthic infections and can be a suitable molecule in mass deworming programs, particularly in areas were Giardia is frequent. Thus, replacing

mebendazole by albendazole during a mass deworming programme could further reduce the frequency of helminthic infections as well as protozoan infections and, consequently, the occurrence of diarrheal disease and malabsorption syndrome.

Malnutrition remains frequent in children under 5 years with a prevalence of 22% of stunting and 16.3% of underweight. In 2005, the fourth demographic and population health survey, found an average rate of stunting in rural areas of 20.6%, the prevalence of underweight was 21.6% [76]. Our data have confirmed the classical association of anaemia and malnutrition [77]. Indeed, an inadequate intake of macro and micronutrients, or intestinal malabsorption induced or increased by intestinal parasites infections, can play through iron and folate deficiency, a well-documented role in chronic anaemia pathophysiology [37].

Study limitations

This study conducted in a rural senegalese area, has provided useful data for the control of some frequent tropical diseases. However, socio-cultural and behavioural factors which may have a relationship with the occurrence of anaemia and/or malnutrition have not been evaluated in this study, in particular the intake of macro- and micro-nutrients, and the patterns of weaning and supplementary feeding.

Conclusion

Anaemia, intestinal parasites and malnutrition are still frequent in the area of Lamarame, while malaria is declining in this part of Senegal. Despite this decrease, malaria is still playing a major role in anaemia occurrence. The combination of several antimalarial interventions, may contribute to eliminate malaria in this region and reduce the occurrence of anaemia among children.

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Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper

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CHAPITRE 4 : FAISABILITE DE L'UTILISATION DES TDR, ACT, RECTOCAPS D'ARTESUNATE ET CPS PAR LES AGENTS DE SANTE COMMUNAUTAIRE.

Chapitre 4 : Faisabilité de l'utilisation des TDR, ACT, rectocaps d'artèsunate et CPS par les agents de santé communautaire.

Ce chapitre est basé sur l'article suivant, soumis à la revue Transaction of The Royal Society :

Feasibility, safety and effectiveness of combining Home based Malaria Management and Seasonal Malaria Chemoprevention in children less than 10 years in Senegal: A cluster-randomised trial.

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1. Abstract

Current antimalarial strategies recommend (i) prompt access to diagnostic testing and treatment with effective antimalarial drugs such as Artemisinin Combination Therapy (ACT), (ii) intermittent preventive treatment for pregnant woman and infant (iii) Seasonal Malaria Chemoprophylaxis (SMC) and (iv) impregnated bed nets. Combination of antimalarial interventions can significantly reduce malaria burden. However, limited information is available on mechanism of combining antimalarial interventions. This operational research was conducted to assess the feasibility and effectiveness of introducing an integrated malaria control strategy, including HMM (using RDT, ACTs, Rectal artesunate) and SMC delivered by community health workers (CHWs).

A cluster-randomised trial was carried out during 2 transmission seasons (2010-2011) in 8 villages located in the Southeastern part of Senegal. The intervention arm was represented by the combination of HMM+SMC while HMM represented the control arm. Children <10years of age were weekly followed by CHWs. At each visit, axillary temperature was measured and children with malaria suspected fever were RDT tested. Primary end point was the incidence of single or first malaria attack over the follow up period. Secondary end points included : (i) malaria diagnostic accuracy (ii) access to ACT treatment (iii) SMC coverage (iv) safety and drug tolerability.

Among the 1000 enrolled children and followed during two transmission seasons (2010 and 2011), malaria RDT was performed for 190 febrile children and 24% were RDT positive. The adjusted rate ratio comparing incidence of malaria attacks in intervention communities with control communities was 0.15, indicating a protective effect of HMM+SMC at 84.8%, (95% CI[21.2%-97.1%], p=0.005). RDT sensitivity and specificity respectively represented 89.2% (95%CI:70–100) and 86.2% (95% CI:70.2–100). A proportion of 96.4% of RDT confirmed malaria attack received AL. SMC coverage was evaluated at 97.3% (95%CI:91.3–100) in 2010, and 94.1% (95% CI [89.3–99.2] in 2011. No serious adverse event was noted.

This study provided evidence that it is feasible to deliver SMC alongside an effective HMM intervention, while achieving high coverage and effectiveness of both SMC and HMM.

Trial Registration: pactr.org **PACTR201305000551876.**

2. Introduction

Malaria remains a major public health problem in tropical regions. According to the World Health Organization (WHO), in 2010 there were an estimated 149 to 274 million cases and 537,000 to 907,000 deaths worldwide [78]. Approximately 81% of malaria cases and 86% of malaria deaths occur in sub-Saharan Africa, were the most affected individuals are represented by children [78]. In Senegal, it was estimated that of the total mortality among children under 5 years of age, 25% was due to malaria [79].

In view of this situation, there is a need to strengthen malaria case management and malaria prevention at the community level, to reduce the disease. Several malaria control strategies were developed recently, including : (i) prompt access to diagnostic testing using Rapid Diagnostic Test (RDT) at the point of care and treatment of confirmed cases with effective antimalarial drugs such as Artemisinin Combination Therapy (ACT) for uncomplicated malaria cases and pre-referral rectal artesunate for the initial management of complicated malaria[80] ; (ii) intermittent preventive treatment in infants and pregnant woman; (iii) Seasonal Malaria Chemoprevention (SMC)[20] as well as (iv) Long-Lasting Insecticide treated Nets (LLITNs).

To improve access to prompt and effective antimalarial treatment, the strategy of Home based-Management of Malaria (HMM), has been developed [15]. In Senegal, the strategy of HMM is being promoted by the National Malaria Control Programme (NMCP) in some areas were access to health care units is limited. This strategy includes the use of RDT for malaria confirmation and ACT for the treatment of uncomplicated malaria implemented by Community Health Workers (CHWs). The implementation phase started in 2008 in 20 pilot villages and included 508 villages in 2009 [19].

Seasonal Malaria Chemoprevention (SMC), previously referred to, as Intermittent Preventive Treatment in children (IPTc) is a new strategy aiming at reducing malaria morbidity and mortality in children living in areas where malaria transmission is highly seasonal. SMC is defined as the intermittent administration of full treatment courses of an antimalarial medicine, during the malaria transmission season to prevent malarial illness, with the objective of maintaining therapeutic antimalarial drug concentrations in the blood, throughout the period of greatest malarial risk [20]. This strategy is now recommended by the WHO in specific areas of seasonal transmission in the Sahel and sub-Sahel region of West Africa [20, 30].

Recently, the WHO advocated for the scaling up of malaria control interventions in order to further reduce malaria burden in areas where malaria is still a public health problem [18, 81, 82] and efforts are being made by sub-Saharan countries to reduce malaria and reach the pre-elimination phase[78]. Malaria control in some areas will require a multi-intervention strategy with the use of combination of antimalarial interventions [32]. Although combined malaria control strategies at community level are likely to substantially reduce the malaria burden, there is limited information about the feasibility of combining several antimalarial interventions at community level.

Therefore, this study was conducted to assess the feasibility, effectiveness and safety of introducing an integrated malaria control strategy, including HMM (using RDT, ACTs, Rectal artesunate) and SMC delivered by community health workers in Senegal (CHWs).

3. Methods

Study area and population

The study was carried out at the Bonconto health post, located at the Velingara health district in the southeastern part of Senegal, 500 km from the capital city of Dakar. The health post is headed by a nurse and has eight functional health huts staffed with community health workers, serving a total population of 10,016 inhabitants. In this area malaria transmission is seasonal, occurring during the rainy season (July to November) with a peak transmission in October and November. *P. falciparum* is the predominant parasite species, and transmission is mainly due to *Anopheles gambiae sl* [56]. In this area, the National Malaria Control Programme initiated in 2010 a strategy to achieve universal coverage with LLITN. By end 2010, the overall coverage of LLITN in the study area was 95.6% [45].

Study design and interventions

Details on the study design and interventions, and results of the first year of follow-up are described in detail elsewhere [45]. In brief, the study was designed as a cluster randomized trial. Eight CHWs in the eight villages around the Bonconto health post, were trained to diagnose malaria using RDT and provide prompt treatment with artemether-lumefantrine to children < 10 years. Children involved in the trial were assigned to a weekly follow up visit during two transmission seasons (2010 and 2011). During each scheduled visit, axillary temperature was measured for each child. Children who

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presented with fever, without any obvious non-malarial cause of fever received malaria RDT. Patients with uncomplicated malaria were treated by the CHW with artemether-lumefantrine (Coartem®); children presenting with severe malaria received one artesunate suppository (10mg/kg), prior to referral to the Bonconto health post. Both Coartem® and malaria RDTs (*Malaria Ag SD Bioline*^{TM}) were supplied by the National Malaria Control Programme.

Four CHWs were randomized to also administer monthly SMC, comprising a single dose of sulfadoxine-pyrimethamine (SP) plus three doses of amodiaquine (AQ) (taken daily for three days) on three occasions during the transmission season. SP and AQ dosing was according to age [45]. The randomization unit was the CHW in order to avoid contamination (each CHW is covering one village). Due to logistical constraints during the first transmission season, SMC was given in October and November 2010. Given that WHO now recommends 3 to 4 administrations of SMC[20], the study was extended to cover a second transmission season in 2011, when SMC was delivered on 3 occasions to further document the safety and tolerability of the combined interventions.

The primary end point was the incidence rate of malaria attacks in the two groups of communities (HMM+SMC and HMM alone) over the follow up period (8 weeks in 2010, 12 weeks in 2011) throughout an active surveillance system. A malaria attack was defined as presence of fever (temperature > 37.5°C) or episode of fever within the previous 24 hours, without any obvious non-malarial cause of fever, and with a positive RDT. Secondary end points included : (i) SMC coverage defined by the proportion of children who received the 3 doses of SMC during the transmission season, (ii) safety and drug tolerability assessed by the incidence of adverse events during the study period; (iii) malaria diagnostic accuracy assessed by comparing RDT results performed by CHWs and slide microscopy (iv) access to ACT treatment.

Data collection methods

CHWs were trained to perform RDT and blood smears. Information related to children's clinical status as well as RDT results were recorded on a case report form (CRF) by CHW after each visit. Febrile children with negative RDT, were referred to the Bonconto Health post for further investigations while children with malaria confirmed by RDT were treated and followed by the CHWs up to day seven after treatment, to monitor their clinical conditions. In case the child with positive RDT and given Coartem® did not recover by day 3, CHWs were advised to refer the child to the health post. CHWs were advised to

record any adverse event reported by the mothers or caretakers after treatment with Coartem. A questionnaire was designed to collect information on SMC (SP+AQ) drug delivery, as well as drug related adverse events. After each SMC round, the child was seen 24 hours after the last dose (day 4) by a field worker, and the mother/caretaker interviewed on the child clinical conditions, to assess the drug tolerability. CHWs and field workers were weekly supervised by the local clinical monitor. At the end of each transmission season, CHW's registers were examined by the clinical monitor, to assess CHWs performance regarding malaria cases management.

Laboratory methods

Blood samples were collected by CHWs using finger prick blood. The first drop was used to prepare thick and thin smears; the second drop was used for RDT. Prior to the study, all CHWs were trained to perform and interpret results of the Malaria Ag SD Bioline^{TM} RDT according to the manufacturer instructions.[83] Blood slides were sent to the Bonconto health post where the thick and thin smear were stained with Giemsa and stored. At the end of the study, specimens were sent to the Department of Parasitology of the University of Dakar and read by a laboratory technician. A positive slide was defined as any asexual parasitaemia detected on a thick or thin blood smear. Parasite density was determined by counting the number of asexual parasites on the thick smear per 200 white blood cells, and assuming a white blood cell count of 8,000 cells per µL. Absence of malaria parasite in 200 high power ocular fields of the thick film was considered as negative. Laboratory technician were kept blinded to RDT results.

Data management and data analysis

Data were entered in Excel^{TM} software and analysed using STATA 11^{TM} software. For the primary outcome of incidence of malaria attacks, intention-to-treat (ITT) analysis was performed including the total children under observation during each study period. To account for clustering, analysis was based on comparison of the unweighted mean malaria incidence rate at the community level. Two-sided 95% confidence intervals were constructed for the rate ratio as described in Bennett et *al* (2002). Adjustment for covariates (age and gender) was performed by the two-stage method using aggregated residuals from a Poisson regression model fitted to the data for individuals [84].

To assess RDT diagnostic accuracy, sensitivity, specificity, positive predictive and negative predictive values were calculated using microscopy as reference standard. Kappa

(k) value expressed the agreement beyond chance and was calculated with a 95% confidence interval. A k value of 0.21-0.60; 0.61-0.80 and > 0.80 were considered as moderate, good and perfect agreement beyond chance respectively [61].

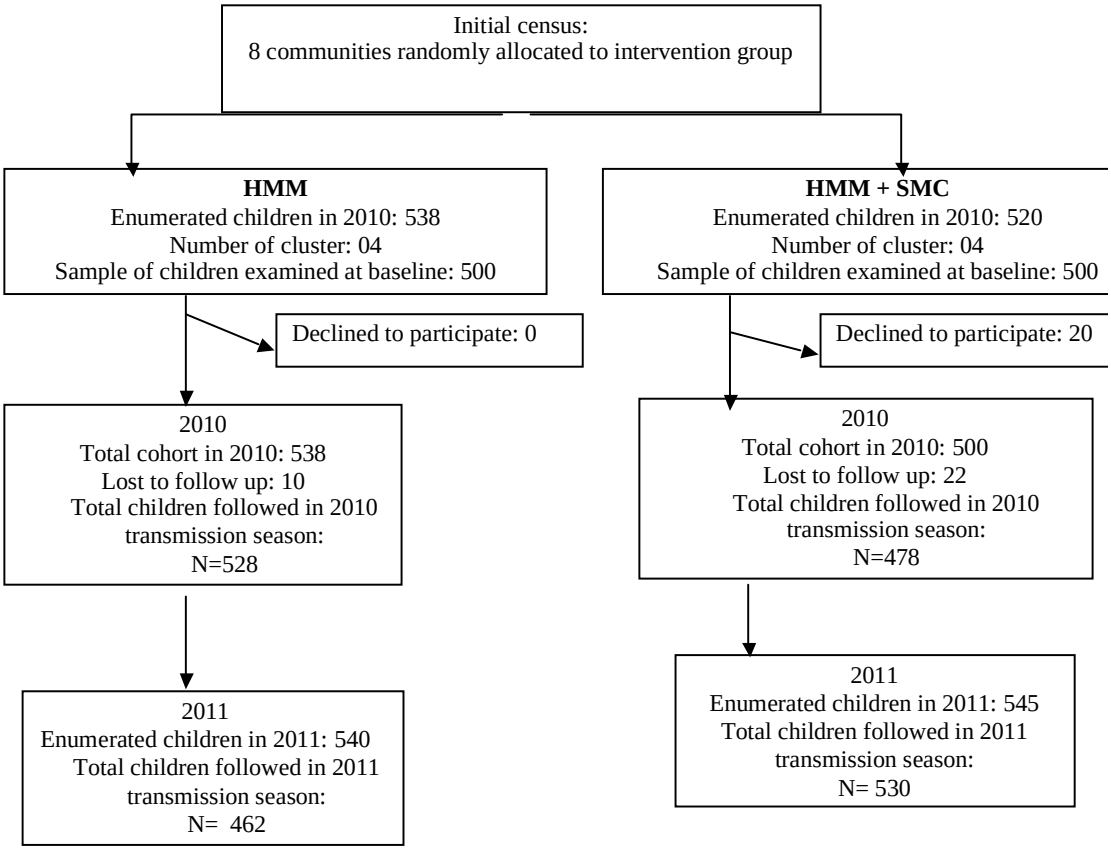
Ethical considerations

Prior to the study, a community sensitization was undertaken and community consent was obtained from community leaders (religious guide, village head). On the field, individual informed consent was obtained from parents or children's guardians prior to each participant enrolment. The study protocol was approved by the Senegalese National Ethical Committee (Conseil National de Recherche en Santé). Approval N 027/MSP/DS/CNRS, 18/03/2010).

4. Results

Study participants characteristics

Eight villages were enrolled and randomized to the intervention group (HMM or HMM+SMC) ; all remained in the study until its completion. In the 2010 transmission season, 1038 children were enrolled; among them, 1000 children (500 in each arm) were sampled for the baseline survey. A total number of 1006 children (528 in the HMM arm and 478 in the HMM+SMC arm) completed the 8 weeks of follow up during the transmission season. In the second transmission season in 2011, 992 children (462 in the HMM arm and 530 in the HMM+SMC arm) represented the total cohort of children followed until the end of the transmission season. (Figure 1).



<u>Figure 1</u>: Study participant's trial profile

At baseline the two study groups were similar in terms of demographics characteristics, bed net usage and *P. falciparum* carriage. Children less than 5 years represented 57.4% in the HMM+SMC and 50% in the HMM arm (p=0.08). The proportion of boys was 45.2% in the HMM+SMC group versus 51.6% in the HMM group (p=0.15). Bed net usage among the study participants was 95.8% in the HMM+SMC arm while that for the HMM arm was at 95.4% (p=0.74). Asymptomatic carriage of *P. falciparum* among study participants prior to the start of the study was 11.2% in the HMM+SMC arm and 9.4% in the HMM arm (p=0.96) (Table 1).

Table 1 : **Study participant's characteristics at baseline survey.**

Parameters	HMM+SMC (n=500)	HMM (n=500)	
	Number (%)	Number (%)	p value
Age group			
<5 years	287 (57.4%)	250 (50%)	0.08
[5 – 10]	213 (42.6%)	250 (50%)	0.11
Gender			
Male	226 (45.2%)	258 (51.6%)	0.15
Female	274 (54.8%)	242 (48.4%)	0.14
***P. falciparum* carriage**	56 (11.2%)	47 (9.4%)	0.96
Bed net usage	479 (95.8%)	477 (95.4%)	0.74

**HMM: Home based malaria management. HMM+SMC: Home based malaria management + Seasonal Malaria Chemoprevention.*

Confirmed malaria cases during the study period

During the two transmission seasons, a total number of 360 fever cases were recorded by the CHWs. A rapid diagnostic test and blood smear were performed for 190 children who presented fever or history of fever within the previous 24 hours Among febrile children who had a malaria test (RDT, microscopy) done, children with a positive malaria RDT was 46.3% (88 children). The frequency of RDT confirmed malaria attacks among febrile patients represented 24% (88/360). Slide microscopy was positive for 83 children (43.7%). Among positive slide microscopy, *Plasmodium falciparum* was identified from 81 samples (97.6%); 2 children (2.4%) were infected both by *P. falciparum* and *P. malariae*. Parasite density among positive children was ranged from 427 to 203,500 trophozoites/µL with a median parasiteamia at 3520 trophozoites/µL. (Table 2).

Table 2 : **Distribution of fever cases and malaria cases among study participants.**

Parameters	Overall distribution	HMM+SMC	HMM
Recorded fever cases	360	68	292
RDT performed among febrile patients (n,%)	190 / 360 (52.7%)	53/68 (77.9%)	137/292 (46.9%)
Positives RDT	88 / 190 (46.3%)	16/53 (30.2%)	72/137 (52.5%)
Negative RDT	102 / 190 (53.7%)	37/53 (69.8%)	65/137 (47.5%)
Positive microscopy	83/190 (43.7%)	14/53 (26.4%)	69/137 (50.4%)
Malaria parasiteamia (median, range)	3520 [427 – 203 500]	4242 [1003 – 46 831]	3449 [427 – 203 500]
Parasite species			
Plasmodium falciparum	81/83 (97.6%)	16/16 (100%)	66/69 (95.7%)
Co-infection (*P. falciparum* + *P. malariae*)	02/83 (2.4%)	00	03/69 (4.3%)

Primary outcome : effectiveness of HMM versus HMM combined with SMC.

The overall incidence rate of RDT confirmed malaria attacks was 34.4 per 1000 person-months at risk in the HMM arm, and 4.91 per 1000 person-months at risk in the HMM+SMC communities. Thus, the adjusted rate ratio comparing incidence of malaria attacks in intervention and control communities was 0.15, indicating a protective effect of HMM+SMC at 85%, (95%CI[39.9%-96.3%], p=0.01). The protective effect of HHM + SMC on malaria incidence in 2010 was 83.5% (95%CI [19.9%-96.6%], p=0.015); in 2011, the combination of HMM and SMC provided a protective effect again malaria attacks at 75.9% (95%CI[23.7%-92.4%], p=0.04) (Table 3).

Table 3 : Effect of combining HMM and IPTc on malaria incidence among children less than 10 years of age over two transmission seasons in 2010 and 2011.

	HMM	HMM + IPTc			
	Incidence rate (1000pers/month)*	Incidence rate (1000pers/month)	aIRR [95%CI] †	Protective Efficacy [95%CI]	p value
Overall study period	34.4	4.91	0.15 [0.037-0.60]	85.1% [39.9%- 96.3%]	0.01
Year 1 (2010)	52.4	8.81	0.17 [0.034-0.80]	83.5% [19.9%-96.6%]	0.01
Year 2 (2011)	21.7	3.25	0.24 [0.076-0.76]	75.9% [23.7% -92.4%]	0.04

**Mean incidence rate across cluster summary, per 1000 person-month at risk.*
aIRRR=adjusted incidence rate ratio;
† *Adjusted for age and sex.*

Secondary outcomes :

Malaria diagnostic accuracy

The RDT performed by CHWs, revealed 88 samples to be positive for malaria and 74 samples had concordant results with microscopy. Thus, 14 RDT results were false positive. Over the 102 negative RDT results, 93 had concordant results with the microscopy and 9 were considered as false negative. No invalid result was noted in the study. Compared to the reference standard, the sensitivity of malaria RDT was 89.2% (95% CI : 70 – 100) while the specificity was evaluated at 86.2% (95%CI : 70.2 – 100). The positive predictive value and negative predictive value were respectively 84.1% (95% CI : 66 – 100) and 91.2% (95%CI : 73.6 – 100). There was a good agreement between the malaria RDT and the reference standard with a Kappa at 0.75 (Standard Error: 0.07, $p < 0.001$) (Table 4).

Table 4 : **Malaria diagnostic accuracy using rapid diagnostic test by community health workers at the Bonconto health post in Senegal.**

Parameters	Value	95% CI
RDT sensitivity	74/83 (89.2%)	[70 - 100]
RDT specificity	93/107 (86.2%)	[70.2-100]
Positive predictive value	74/88 (84.1%)	[66 – 100]
Negative predictive value	93/102 (91.2%)	[73.6 – 100]
Interobserver agreement (Kappa, SE*)	0.75 (0.07)	$p < 0.001$

**SE: Standard Error.*

Malaria community case management

Overall, 88 children (55 in the 2010 transmission season and 33 in the 2011 transmission season), presented RDT confirmed malaria attack. Among these, 83 children (94.3%) presented uncomplicated malaria attack while a proportion of 5.6% (5 children) presented severe malaria attack. Pre-referral rectal artesunate was given to the 5 children with severe malaria ; all these 5 children reached the health post after pre-referral rectal artesunate administration. Among patients with uncomplicated RDT confirmed malaria attack, 96.4% were treated with AL by the CHWs ; 3 children with positive RDT did not received AL due to stock out of AL at the health hut. These children were referred to the Bonconto health post for appropriate treatment. Data on malaria community case management as reported by CHW are presented in Table 5.

Table 5 : Malaria case management reported by the CHWs in the area of Bonconto health post, during the study period.

	Overall	2010	2011
RDT confirmed malaria cases	88	55	33
Uncomplicated malaria cases	83/85 (94.3%)	50/55 (90.9%)	33/33 (100%)
Severe malaria cases	05/85 (5.7%)	5/55 (9.1%)	00
Uncomplicated malaria cases treated with AL*	80/83 (96.4%)	55/55 (100%)	30/33 (90.9%)
Severe malaria received pre-referral rectal AS	5/5 (100%)	5/55 (100%)	00

**A number of 3 children in the 2011 transmission season, presented RDT confirmed malaria but did not receive AL. Reasons for non treatment with AL was due AL stock out at the health hut as reported by CHW.*

SMC delivery and coverage

The overall coverage of complete SMC treatment (children who received the full complement of 3 daily doses in each monthly round), was 97.3% in 2010 (2 monthly

rounds), and 88.8% in 2011 (3 monthly rounds). In 2010, 97% of children received complete treatment in October and 97.6% in November. Complete SMC coverage in 2011 was evaluated at 86.6%, 88.7% and 90.6% respectively during September, October and November (Table 6).

Table 6 : IPTc coverage among children under 10 years of age at the Bonconto health post, delivered by CHW.

	Children with 3 doses of IPTc	Dose 1 (AQ-SP)	Dose 2 (AQ)	Dose 3 (AQ)
First transmission season (2010)				
Treatment 1 (October)				
Received	484/500 (97%)	495/500 (99%)	494/500 (98.8%	496/500 (99.2%)
Refused	04/500 (0.8%)	03/500 (0.6%)	01/500 (0.2%)	00
Absent	11/500 (2.2%)	02/500 (0.4%)	05/500 (1%)	04/500 (0.8%)
Treatment 2 (November)				
Received	488/500 (97.6%)	495/500 (99%)	496/500 (99.2%)	497/500 (99.4%)
Refused	00	00	00	00
Absent	12/500 (2.4%)	05/500 (1%)	04/500 (0.8%)	03/500 (0.6%)
Second Transmission season (2011)				
Treatment 1 (September)				
Received	459/530 (86.6%)	487/530 (91.8%)	479/530 (90.3%)	493/530 (93.0%)
Refused	13/530 (2.4%)	02/530 (0.3%)	11/530 (2.1%)	00
Absent	58/530 (10.9%)	41/530 (7.7%)	40/530 (7.5%)	37/530 (6.9%)
Treatment 2 (October)				
Received	470/530 (88.7%)	491/530 (92.6%)	485/530 (91.5%)	494/530 (93.2%)
Refused	08/530 (1.5%)	03/530 (0.6%)	05/530 (0.9%)	00
Absent	52/530 (9.8%)	36/530 (6.7%)	40/530 (7%)	36/530 (6.8%)
Treatment 3 (November)				
Received	480/530 (90.6%)	493/530 (93.0%)	496/530 (93.6%)	491/530 (92.6%)
Refused	02/530 (0.3%)	00	00	02/530 (0.4%)
Absent	48/530 (9.1%)	37 (6.9%)	34/530 (6.4%)	37/530 (6.9%)

Overall IPTc coverage

First transmission season (2010)	Percentage	95%CI
% of children with 2 complete IPTc treatments	97.3%	[91.3 – 100]
Second transmission season (2011)		
% of children with 3 complete IPTc treatment	88.8%	[84.2 – 93.6]

Safety and tolerability

Over the 88 malaria cases treated with AL (Coartem®) or pre-referral rectal artesunate, none of them reported adverse events after treatment.

Overall, 150 children (30%) presented at least one adverse event after SMC administration. The reported adverse events were represented by nausea and/or vomit (9.4%), sleepiness (7%), abdominal pain (6.6%), diarrhoea (6.4%), loss of appetite (3.8%), headache (2.8%), pruritus (2%), cough (0.6%) and dizziness (0.2%). Adverse events intensity, were ranged from minor to moderate. No child presented severe symptoms. The mean duration of the reported adverse events was evaluated at 2 days, with a ranged from 1 to 3 days. No serious adverse event was noted during the study (Table 7).

Table 7 : Adverse events reported during the week after each IPTc treatment among children less than 10 years of age.

Symptoms	Frequency	Average duration (days)	Intensity*	
Headache	14	2 ± 1	Minor Moderate Severe	14 (92.9% 01 (7.1%) 00
Sleepiness	35	2 ± 1	Minor Moderate Severe	16 (45.7%) 19 (54.3%) 00
Dizziness	01	3	Minor Moderate Severe	00 1 00
Abdominal pain	33	2 ± 1	Minor Moderate Severe	20 (60.6%) 13 (39.4%) 00
Nausea - Vomit	47	2 ± 1	Minor Moderate Severe	28 (59.6%) 19 (40.4%) 00
Loss of appetite	19	2 ± 1	Minor Moderate Severe	12 (63.2%) 07 (36.8%) 00
Diarrhoea	32	2 ± 1	Minor Moderate Severe	19 (59.4%) 13 (40.6%) 00
Cough	03	3 ± 2	Minor Moderate Severe	03 00 00
Pruritus	10	3 ± 1	Minor Moderate Severe	05 (50%) 05 (50%) 00
Other symptoms	08	2 ± 1	Minor Moderate Severe	08 00 00

**minor: the symptom does not prevent usual game. Moderate: the symptom prevents the usual game. Severe: the child's clinical condition requires medical consultation or hospitalization.*

5. Discussion

Despite increasing efforts to control malaria, and many African countries' report of a decrease in malaria burden in recent years [44], the disease is still a major public health problem in many sub-Saharan African countries [78].

In order to further reduce malaria burden in endemic areas, the WHO recently advocated for scaling up of antimalarial interventions [18, 82, 85]. Malaria control in some areas may require the use of combination of several antimalarial interventions [32]. This study assessed the feasibility and the effectiveness of combining home based management of malaria and SMC (previously known as IPTc) among children less than 10 years living in a rural area of Senegal.

In the context of an effective HMM programme, SMC had an additional benefit in preventing malaria attacks during the seasonal peak in transmission. Indeed over the study period, malaria incidence was markedly lower in communities who had access to HMM + SMC compared to those who were assigned only to HMM. The data reported in this study are consistent with other findings [31, 45]. The provision of antimalarial treatment through HMM by CHWs, can contribute to improve early access to treatment and thus reduce the progression of the disease to severe illness. Only few children developed severe malaria and these cases occurred only in the first year of the study.

SMC is a new approach aiming at reducing malaria morbidity and mortality. HMM and SMC are two complementary and synergistic strategies. Combining HMM and SMC could thus be a novel and highly effective way of achieving a high level of malaria control in areas where malaria transmission is seasonal [21].

Prompt parasitological confirmation and effective case management are essential tools for malaria control [78]. Malaria RDT are being considered by many endemic countries in sub-Saharan Africa as alternative tools for malaria confirmation in areas where microscopy is not available [86]. This study showed that parasitological confirmation using malaria RDT is an accurate method and can contribute to optimize antimalarial use in areas where microscopy is not available. However, while the method generally has a good level of sensitivity and specificity and there was a good agreement beyond chance when compared to reference microscopy (kappa=0.75), RDTs were false negative and false positive in about 10%. Thus, the accuracy of RDT interpretation under routine practices at community level seems to be lower than under research conditions [87, 88].

Among the study participants who presented an episode of fever only 24% presented RDT confirmed malaria attacks. In this study, CHWs were advised to refer febrile patients with negative RDT to the Bonconto health post. Without any feedback about the diagnostic of the current febrile episode, the study was not able to document the aetiologies of non-malaria febrile illness among study participants. While malaria RDTs can improve malaria diagnostic and targeting of antimalarial drugs, as well as documentation of malaria episodes, there is no comparable test for bacterial diseases[88]. Consequently, other causes of febrile illness remained prevalent among study participants and poorly documented. Febrile illness management in remote areas usually refer to IMCI guidelines [89]. In Senegal, RDT positive patients are being prescribed antimalarial treatment, while RDT-negative patients may be prescribed broad-spectrum antibiotics (trimethoprim-sulfamethoxazole or amoxicillin) and antipyretics [90]. This situation can however lead to miss-use of antibiotics as well as the occurrence and the spread of antibiotic resistance and poor management of febrile patients. Thus, appropriate treatment guidelines for febrile patient management in areas with restricted access to standard diagnostic tools are needed. To achieve this, a better understanding in the epidemiology of non-malaria febrile illnesses is required.

This study showed a high adherence to antimalarial treatment guidelines. Indeed, antimalarial treatment was delivered only to patients with parasitological confirmation. A recent evaluation conducted by the Senegalese National Malaria Control program, showed similar findings in a large scale implementation of HMM [19].

No serious adverse event was recorded among study participants while 30% reported minor to moderate adverse event after SMC treatments. Previous studies, showed that SMC with AQ-SP is safe and well tolerated [27, 29], as well as combining HMM and SMC with Amodiaquine – SP [31, 32]. Parents did not report adverse events after treatment with AL. The fact that notification of adverse events after treatment with AL was based on a self-reporting strategy could have contributed to underestimation of the incidence of adverse event related to AL. However, several studies have demonstrated that AL is a very well tolerated ACT [91-94]. In Senegal, notification of adverse events has been a great challenge for the National Malaria Control Programme although a pharmacovigilence system to monitor ACT drug related adverse events has been established since several years [95]. Strengthening pharmacovigilence systems in African countries in the context of scaling up antimalarial interventions is needed.

Conflict of interest

The authors have no conflicts of interest concerning the work reported in this paper.

Authors' contribution

RCT, CTN, PM, ICB, OG conceived and designed the study. RCT, and KS trained CHWs, supervised the fieldwork and the data collection. RCT analysed the data with the help of MC. RCT wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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CHAPITRE 5 : IMPACT SUR LE PALUDISME ET L'ANEMIE DE L'UTILISATION COMBINEE DE LA PRISE EN CHARGE COMMUNAUTAIRE DU PALUDISME ET DE LA PREVENTION DU PALUDISME SAISONNIER.

Chapitre 5 : Impact sur le paludisme et l'anémie de l'utilisation combinée de la prise en charge communautaire du paludisme et de la prévention du paludisme saisonnier.

Ce chapitre est basé sur l'article suivant:

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MALARIA JOURNAL

RESEARCH Open Access

Impact of combining intermittent preventive treatment with home management of malaria in children less than 10 years in a rural area of Senegal: a cluster randomized trial

Roger CK Tine[1*], Babacar Faye[1], Cheikh T Ndour[2], Jean L Ndiaye[1], Magatte Ndiaye[1], Charlemagne Bassene[1], Pascal Magnussen[3], Ib C Bygbjerg[4], Khadim Sylla[1], Jacques D Ndour[5] and Oumar Gaye[1]

1. Abstract

Current malaria control strategies recommend (i) early case detection using rapid diagnostic tests (RDT) and treatment with artemisinin combination therapy (ACT), (ii) pre-referral rectal artesunate, (iii) intermittent preventive treatment and (iv) impregnated bed nets. However, these individual malaria control interventions provide only partial protection in most epidemiological situations. Therefore, there is a need to investigate the potential benefits of integrating several malaria interventions to reduce malaria prevalence and morbidity.

A randomized controlled trial was carried out to assess the impact of combining seasonal intermittent preventive treatment in children (IPTc) with home-based management of malaria (HMM) by community health workers (CHWs) in Senegal. Eight CHWs in eight villages covered by the Bonconto health post, (South Eastern part of Senegal) were trained to diagnose malaria using RDT, provide prompt treatment with artemether-lumefantrine for uncomplicated malaria cases and pre-referral rectal artesunate for complicated malaria occurring in children under 10 years. Four CHWs were randomized to also administer monthly IPTc as single dose of sulphadoxine-pyrimethamine (SP) plus three doses of amodiaquine (AQ) in the malaria transmission season, October and November 2010. Primary end point was incidence of single episode of malaria attacks over 8 weeks of follow up. Secondary end points included prevalence of malaria parasitaemia, and prevalence of anaemia at the end of the transmission season. Primary analysis was by intention to treat. The study protocol was approved by the Senegalese National Ethical Committee (approval 0027/MSP/DS/CNRS, 18/03/2010).

A total of 1,000 children were enrolled. The incidence of malaria episodes was 7.1/100 child months at risk [95% CI (3.7–13.7)] in communities with IPTc + HMM compared to 35.6/100 child months at risk [95% CI (26.7–47.4)] in communities with only HMM (aOR = 0.20; 95% CI 0.09–0.41; $p = 0.04$). At the end of the transmission season, malaria parasitaemia prevalence was lower in communities with IPTc + HMM (2.05% versus 4.6% $p = 0.03$). Adjusted for age groups, sex, *Plasmodium falciparum* carriage and prevalence of malnutrition, IPTc + HMM showed a significant protective effect against anaemia (aOR = 0.59; 95% CI 0.42–0.82; $p = 0.02$).

Combining IPTc and HMM can provide significant additional benefit in preventing clinical episodes of malaria as well as anaemia among children in Senegal.

2. Manuscript

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Abstract

Background: Current malaria control strategies recommend (i) early case detection using rapid diagnostic tests (RDT) and treatment with artemisinin combination therapy (ACT), (ii) pre-referral rectal artesunate, (iii) intermittent preventive treatment and (iv) impregnated bed nets. However, these individual malaria control interventions provide only partial protection in most epidemiological situations. Therefore, there is a need to investigate the potential benefits of integrating several malaria interventions to reduce malaria prevalence and morbidity.

Methods: A randomized controlled trial was carried out to assess the impact of combining seasonal intermittent preventive treatment in children (IPTc) with home-based management of malaria (HMM) by community health workers (CHWs) in Senegal. Eight CHWs in eight villages covered by the Bonconto health post, (South Eastern part of Senegal) were trained to diagnose malaria using RDT, provide prompt treatment with artemether-lumefantrine for uncomplicated malaria cases and pre-referral rectal artesunate for complicated malaria occurring in children under 10 years. Four CHWs were randomized to also administer monthly IPTc as single dose of sulphadoxine-pyrimethamine (SP) plus three doses of amodiaquine (AQ) in the malaria transmission season, October and November 2010. Primary end point was incidence of single episode of malaria attacks over 8 weeks of follow up. Secondary end points included prevalence of malaria parasitaemia, and prevalence of anaemia at the end of the transmission season. Primary analysis was by intention to treat. The study protocol was approved by the Senegalese National Ethical Committee (approval 0027/MSP/DS/CNRS, 18/03/2010).

Results: A total of 1,000 children were enrolled. The incidence of malaria episodes was 7.1/100 child months at risk [95% CI (3.7-13.7)] in communities with IPTc + HMM compared to 35.6/100 child months at risk [95% CI (26.7-47.4)] in communities with only HMM (aOR = 0.20; 95% CI 0.09-0.41; $p = 0.04$). At the end of the transmission season, malaria parasitaemia prevalence was lower in communities with IPTc + HMM (2.05% versus 4.6% $p = 0.03$). Adjusted for age groups, sex, *Plasmodium falciparum* carriage and prevalence of malnutrition, IPTc + HMM showed a significant protective effect against anaemia (aOR = 0.59; 95% CI 0.42-0.82; $p = 0.02$).

Conclusion: Combining IPTc and HMM can provide significant additional benefit in preventing clinical episodes of malaria as well as anaemia among children in Senegal.

Keywords: Malaria, Intermittent preventive treatment, Home-based management, Anaemia

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Background

Malaria remains a major public health problem in tropical regions. According to the World Health Organization (WHO), in 2009 there were an estimated 169-294 million cases and 628,000-968,000 deaths worldwide. Over 89% of these deaths occur in Africa [1], most of the time outside health facilities [2,3]. In view of this situation, there is need to strengthen malaria case management and malaria prevention at the community level to reduce the burden of disease. Recently, the WHO advocated for scaling up malaria control interventions in order to accelerate malaria elimination [4] Several malaria control strategies were developed recently, including (i) early case detection using rapid diagnostic tests (RDT), prompt treatment with effective anti-malarial drugs, such as artemisinin combination therapy (ACT) for uncomplicated malaria cases, (ii) pre-referral rectal artesunate for severe malaria cases, (iii) intermittent preventive treatment, and (iv) long-lasting insecticide-treated nets (LLIN).

Effective case management is a fundamental element of malaria control. To improve treatment practices at community level, the strategy of home-based management of malaria (HMM) has been developed [5,6]. Home-based management of malaria (HMM) is now considered as an important strategy for reducing severe morbidity and mortality from malaria in resource-poor countries [7,8].

In Senegal, the National Malaria Control Programme (NMCP) has initiated the scaling up of the use of ACT at community level, in the context of HMM strategy, implemented by community health workers (CHWs) in order to strengthen malaria control efforts. This strategy includes the use of RDT for malaria confirmation and ACT for the treatment of uncomplicated malaria.

Intermittent preventive treatment (IPT) is a new approach aiming at reducing malaria morbidity among children or other high-risk individuals. IPT involves administration of anti-malarial drugs at defined time intervals to individuals regardless of whether they are known to be infected with malaria to prevent morbidity and mortality from the infection [9]. IPT was initially recommended for pregnant women involving the administration of at least two doses of sulphadoxine-pyrimethamine (SP) during antenatal visits after the first trimester of pregnancy. More recently the strategy was extended to infants (IPTi) with the administration of three doses of an anti-malarial drug during the expanded programme of immunization (EPI) visits [10]. In children under 5 years of age, several studies have shown IPT to be effective in reducing malaria burden [11,12]. Intermittent preventive treatment of malaria in children less than 5 years of age (IPTc), involves the administration of two to three doses of anti-malarial drug during the high malaria transmission season [13].

Cissé *et al.* during a randomized double-blind controlled trial, conducted in Niakhar in Senegal have shown that administering SP plus artesunate three times during the transmission season can reduce malaria incidence among children under 5 years by 86% [11]. Protective efficacy of IPTc in Mali was estimated at 67.5% with two doses of SP at 8 weeks intervals during the high malaria transmission season [12]. Another study conducted in the rural area of Niakhar (Senegal) demonstrated that the most optimal regimen for IPTc in children is the combination of SP-amodiaquine. To ensure a maximum protective effect, the IPTc should preferably combine two long half-life drugs [14].

In most African countries, anti-malarial interventions are being promoted on an individual basis and many communities are still not getting access to those services [15]. In 2008, it was estimated by WHO that confirmation of malaria cases was done in only 22% on average in most African regions, while less than 15% of patients under 5 years of age suffering from malaria attacks benefitted from treatment with ACT. It thus appears that the use of effective malaria control strategies and their integration into national health systems and services continue to be a challenge in Africa [1]. In addition, individual anti-malarial interventions provide only partial protection in most epidemiological situations [16,17]. Therefore, there is a need to investigate the potential benefits of integrating several malaria interventions, in reducing malaria prevalence and morbidity. This study aimed to assess the impact of combining seasonal intermittent preventive treatment in children (IPTc) with home-based management of malaria (HMM) by community health workers (CHWs) in Senegal.

Methods

Study area and population

The study was carried out at the Bonconto health post, located at the Velingara health district in the south-eastern part of Senegal, 500 km from the capital city of Dakar. The health post is headed by a nurse and has eight functional health huts staffed with community health workers, serving a total population of 10,016 inhabitants. In this area malaria transmission is seasonal, occurring during the rainy season (July to November) with a peak transmission in October and November. *Plasmodium falciparum* is the predominant parasite species and transmission is mainly due to *Anopheles gambiae s.l.* (Konate Lassana, personal communication). In this area, the National Malaria Control Programme initiated the universal coverage of LLIN strategy in 2010.

Study design

The study was designed as a cluster randomized trial. Eight CHWs in the eight villages around the Bonconto health post, were trained to diagnose malaria using RDT and provide prompt treatment with artemether-lumefantrine to children less than 10 years. Four of them were randomized to also administer monthly IPTc with single dose of SP plus three doses of amodiaquine (AQ) in October and November 2010. The randomization unit was the CHW in order to avoid contamination. Each CHW is covering one village. The CHWs were randomized using a random number generator from Excel software.

Primary end point was incidence of single or first malaria attack over 8 weeks of follow up throughout an active surveillance system. Malaria attack was defined as presence of fever (temperature > 37.5°C) with a positive RDT. Secondary end points were prevalence of malaria at the end of the transmission season, and prevalence of anaemia at the end of the transmission season in the two groups.

Interventions

The two main interventions in this study were HMM and IPTc for children aged from one to 10 years. During the study period, RDT were deployed at the level of health huts. The RDT used in this study was based on the detection of the Histidine Rich Protein II (Malaria Antigen P.f SD®), and was provided by the NMCP.

For uncomplicated malaria cases, treatment was done by CHWs using artemether-lumefantrine according to age group; children presenting severe malaria cases received one dose (10 mg/kg) administration of pre-referral artesunate suppositories prior to their transfer to the Bonconto health post. In the four villages with combined HMM and IPTc, all doses of AQ and SP were administered by CHWs under direct observation. IPTc drug delivery was organized at the level of health huts. At scheduled days for IPTc administration, parents were asked to bring their child at the health huts for IPTc delivery. In case a child was not seen at time of administration, the CHW was advised to visit that child at home and give the treatment. To facilitate SP and AQ administration, treatment doses were tabulated on a document and distributed to each CHW to serve as job aid.

Each tablet of AQ contains 153 mg of amodiaquine base while SP tablet contains 500 mg sulphadoxine and 25 mg pyrimethamine. Treatment doses were according to age group. Children under 2 years of age received half a tablet of SP; a whole tablet of SP was given to children age from two to 6 years, while children age from seven to 10 years received one tablet and half of SP. For AQ half a tablet was given to children under 2 years, one tablet and one and a half tablet were given daily for 3 days to children aged 2-7 years and 8-10 years respectively. This drug regimen has been shown to be the most optimal regimen to minimize overdosing as well as under dosing of AQ [18].

Artemether-lumefantrine (Novartis^{LTD}) was provided by the Senegalese NMCP, rectal artesunate was obtained from Mepha^{Ltd}, while AQ and SP were provided by Kina Pharm^{Ltd}.

Data collection

Baseline assessment

Prior to start of the study, meetings were held in the villages to explain the study purpose and answer to the population's questions. Consent was obtained from the community leaders as well as parents or children's guardians. A census of all children aged from one to 10 years leaving in each randomized village was done. A baseline assessment was done prior to the intervention (beginning of October). At baseline, all registered children were examined by a study physician, and their mothers interviewed to assess the use of bed nets, and the presence of any chronic illness which might interfere with the outcome of the trial. Thick and thin blood films were prepared and haemoglobin concentration measured using HemoCue Hb 201®. Children with acute malaria during the baseline study (temperature > 37.5°C and positive RDT) were treated with artemether-lumefantrine and those with anaemia (Hb < 11 g/dl) received oral iron supplementation for 1 month.

Malaria cases detection

An active surveillance system was organized in the eight villages from the date of first IPTc administration, to the end of the transmission season in December. Children were visited at home by CHW once a week during 8 weeks. At each visit children's axillary temperature was measured. If the child had fever (temperature > 37.5°C), or a history of fever within the previous 24 h, a RDT was performed by the CHW. Children with acute malaria (temperature > 37.5°C and positive RDT) received a three-day treatment with artemether-lumefantrine. A follow-up was done by the CHWs up to day seven after treatment, to monitor the patient's clinical conditions. In case the child did not recover on day 3, CHWs were advised to refer the child to the health post. The mothers were encouraged to take their child to the CHWs if the child presented fever in non-scheduled days visits.

Malaria parasitaemia and anaemia prevalence evaluation

A cross-sectional survey was carried out at the end of the malaria transmission season in a subsample of study participants, randomly selected from the list of children less than 10 years living in the eight villages. For each randomly selected child, thick and thin smear test were done, haemoglobin (Hb) concentration measured and anthropometric data collected.

Sample size calculation

With four clusters in each intervention arm and 125 children less than 10 years sampled in each cluster, assuming a current incidence of malaria attacks of 35 per 100 person-month at risk, the study was powered at 80% to detect 20% of reduction of malaria incidence in the HMM + IPTc group at 5% significance level, with a coefficient of variation of 0.3. For the cross sectional survey the total number of children to examine was calculated at 800, based on a prevalence of malaria parasitaemia at 20% in the study area (Senegal MIS 2009) a confidence level at 95% with a precision of 5%, power level at 90% and assuming a percentage of 20% of withdrawal.

Laboratory methods

Blood samples were collected using finger prick blood. The first drop was used for thick and thin smear test for the diagnosis of malaria. Thick and thin smear test were stained with Giemsa and read by a laboratory technician. Malaria parasitaemia was defined as any asexual parasitaemia detected on a thick or thin blood smear. Parasite density was determined by counting the number of asexual parasites per 200 white blood cells, and calculated per μL using the following formula: numbered parasites × 8,000/200 assuming a white blood cell count of 8,000 cells per μL. Absence of malaria parasite in 200 high power ocular fields of the thick film was considered as negative.

The second drop of finger prick blood was drawn into a microcuvette for Hb determination (g/dl) using HemoCue machine (HemoCue® Hb 201). Moderate and severe anaemia were defined as Hb concentration below 11 g/dl and 8 g/dl, respectively.

Data analysis and data management

Data were entered in Excel™ software and analysed using STATA 11™ software. For descriptive data, percentage was used to assess the frequency of each outcome. For quantitative data, mean and standard deviation were used to describe normally distributed variables, median and range for other data. Characteristics of all children included in the study were tabulated by study arm.

For the primary end point of incidence of malaria attacks over 8 weeks of follow up, analysis was by intention to treat including all children who attended the baseline survey. For the secondary end points of cross sectional prevalence of malaria and anaemia at the end of the transmission season, analysis was done by per protocol, including all children seen at cross sectional survey at the end of the transmission season.

To assess the impact of combining HMM + IPTc, analysis was conducted at the individual level with adjustment for clustering using robust standard errors [19]. Time at risk was calculated from date of the first IPTc administration to the date of the cross-sectional survey. Children were not considered at risk for 28 days after treatment for malaria attacks, and thus were censored from the analysis for 4 weeks, although no child presented more than one malaria episode. Time at risk to the first malaria episode between the two study arms was compared using Kaplan Meier method with a log rank test stratified by clusters. The incidence rate ratio (IRR) of HMM + IPTC and HMM alone was determined after adjustment by age group and gender using Cox regression model with robust standard errors to account for clustering. The protective efficacy of HMM + IPTc on malaria incidence was calculated as (1-IRR) × 100. *P* values below 5% were considered as significant (two sided). Prevalence of malaria parasitaemia and anaemia at the end of the transmission season were measured in the two groups and compared using a logistic regression analysis with robust standard errors to take into account for the cluster design.

Ethical considerations

Prior to the study, a community sensitization was undertaken and community consent was obtained from community leaders (religious guide, village head). Informed consent was obtained from parents or children's guardians the days of surveys. The study protocol was approved by the Senegalese National Ethical Committee (Conseil National de Recherche en Santé). Approval N 027/MSP/DS/CNRS, 18/03/2010.

Results

Baseline characteristics

One thousand and twenty children aged from 1 to 10 years were registered in the eight villages with functional health huts, covered by the Bonconto health post; 1,000 children (500 in the HMM group and 500 in the HMM + IPTc group) who met the entry criteria were enrolled (Figure 1).

At baseline the two groups were similar in terms of demographic characteristics (age, gender and *P. falciparum* carriage). Prevalence of moderate anaemia and severe anaemia were similar in the two groups, as well as prevalence of under nutrition (stunting, underweight); 95.8% of study subjects in the HMM + IPTc group and 95.4% in the HMM group slept under a LLIN (Table 1).

Impact of the interventions on malaria incidence

Overall, the cumulative incidence of malaria episodes was significantly lower in the HMM + IPTc group. Thus, the Kaplan Meier survival estimates of time to first malaria episode showed a significant difference between the two groups (p = 0.001, log rank test) (Figure 2).

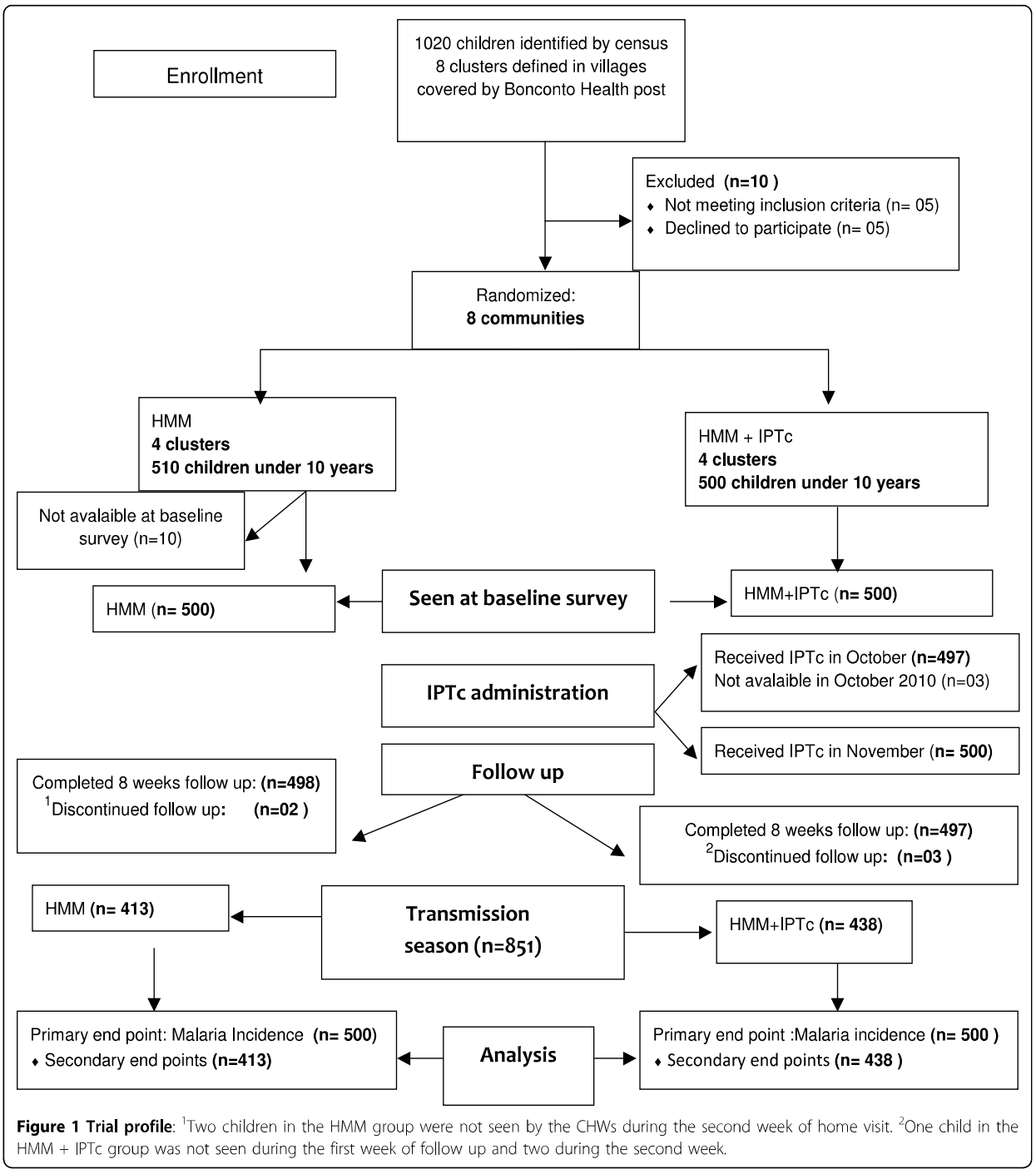

Figure 1 Trial profile: [1]Two children in the HMM group were not seen by the CHWs during the second week of home visit. [2]One child in the HMM + IPTc group was not seen during the first week of follow up and two during the second week.

The incidence of clinical malaria attacks during the study period was 35.6 per 100 children-months at risk in the HMM group while that for children in the HMM + IPTc group was only 7.2 per 100 children-month at risk (p = 0.04). After controlling for age group, and gender, the combination of IPTc + HMM significantly reduced the number of malaria episodes in children: adjusted incidence rate ratio: 0.21 (95% CI [0.10-0.42]); p = 0.04. Thus, the protective efficacy of IPTc + HMM against malaria attacks incidence (all cases) was 79% (95% CI [58%-90%]) (Table 2). During the intervention period, 5/510 children in the HMM group (0.98%) presented severe malaria, while no severe malaria cases were noted in the HMM + IPTc group.

Table 1 Baseline characteristics of children in the two groups

	Interventions	
Parameters	**HMM+IPTc**	**HMM**
Number of children	500	500
Number of clusters	04	04
Mean cluster size (range)	133 (130-145)	120 (102-145)
Age group		
Children under 5 years, %	57.4 (287/500)	50 (250/500)
Children aged from 5 to 10 years, %	42.6 (213/500)	50 (250/500)
Gender, % of male	45.2 (226/500)	51.6(258/500)
P. falciparum carriage, %	11.2 (56/500)	9.4 (47/500)
Moderate anaemia, % (Hb between 11 and 8 g/dl)	58 (290/500)	56.4(282/500)
Severe anaemia (Hb * < 8 g/dl), %	21 (105/500)	24.8(124/500)
Stunting (HAZ < -2SD**), %	46.4 (232/500)	41.2 (206/500)
Bed net usage, %	95.8 (479/500)	95.4 (477/500)

**Hb* haemoglobin; *HAZ* height for age z score

Impact of the interventions on malaria parasitaemia at the end of the transmission season

At the end of the malaria transmission season, 28 children (3.3%) were found with *P. falciparum*. A proportion of 4.6% (95% CI [2.5-6.6]) children in the HMM group had asexual *P. falciparum* (any density) compared with 2.1% (95% CI [0.7-3.3]) in the HMM + IPTc group. The proportion of children with *P. falciparum* parasitaemia at any density, was significantly lower in the HMM + IPTc group (OR = 0.43 (95% CI [0.19-0.95]); p = 0.03), thus IPTc + HMM had a protective efficacy against *P. falciparum* parasitaemia (at any density) of 57% (95% CI [5%-81%]).

Children with parasitaemia at a density > 1,000 parasites/μL at the end of the transmission season, represented 3.39% and 1.37% in the HMM and HMM + IPTc

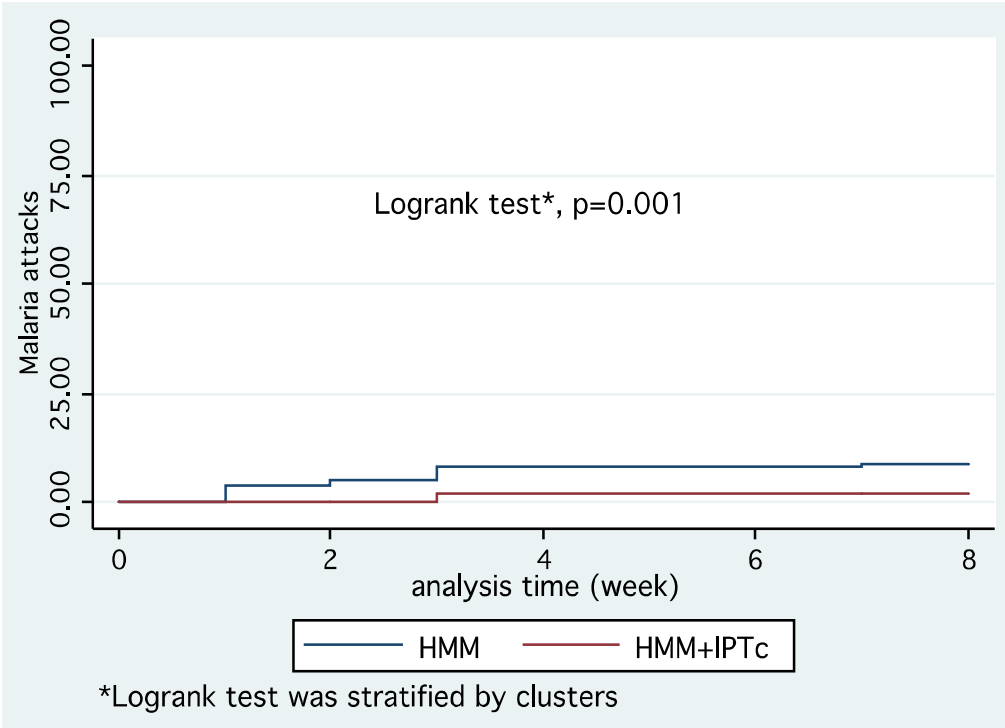

Figure 2 Kaplan-Meier plot comparing time to first episode of malaria attack defined as fever (> 37.5°C) and positive RDT, between the two groups.

Table 2 Impact of IPTc combined to HMM on malaria incidence in the two groups

	Malaria incidence Rate/100 person-month (95% CI)	Unadjusted IRR (95% CI)	Adjusted IRR (95% CI)	Protective efficacy (%) (95% CI)	p value*
Interventions					
HMM	35.5 [25.0-50.3]	1	1		
HMM + IPTc	07.1 [1.1- 9.5]	0.20 [0.04-1.0]	0.21[0.04-0.90]	79 [10; 96]	0.04
Gender					
Female	21.0 [11.1-44.3]	1	1		
Male	22.4 [11.6-48.3]	1.06 [0.62-1.84]	1.18 [0.74-1.86]	-18[-86;26]	0.48
Age groups					
1 years	17.3 [10.1-32.8]	1	1		
[2 - 5 years]	28.5 [13.8-66.3]	1.62 [0.84-3.10]	1.50[0.85-2.65]	-50[-165;15]	0.16
[6- 10 years]	17.4 [09.8-32.8]	1.0[0.74-1.34]	0.98[0.74-1.27]	2 [-27; 26]	0.85

HMM home-based management; *HMM+IPTc* home-based management+intermitent preventive treatment; *IRR* incidence rate ratio. Intraclass correlation coefficients for Gender:0.01, age group:0.007. *All p value are adjusted for clustering by robust standard errors.

groups, respectively (OR = 0.40 (95% CI [0.16-0.96]); p = 0.05) resulting in a protective efficacy at 60% (95% CI [04%-84%]) (Table 3).

Impact of the interventions on anaemia prevalence at the end of the transmission season

Mean Hb concentration among children less than 10 years of age at the end of the malaria transmission season was 10.4 ± 1.98 g/dl in the HMM + IPTc group and 10.2 ± 1.8 g/dl in the HMM group (p = 0.07). Proportion of anaemic children (hb < 11 g/dl) at the end of the transmission season was 54.11% in HMM + IPTc group, while anaemic children represented 60.3% in the HMM group (p = 0.06). In a logistic regression analysis with robust standard errors, HMM + IPTc showed a significant protective effect against anaemia (adjusted Odds Ratio (aOR): 0.59 (95% CI [0.42-0.82]); p = 0.025. The protective efficacy of HMM + IPTc in reducing anaemia among children under 10 years was estimated in this study at 41% (95% CI 95 [18%-58%]).

Anaemia was also significantly associated with *P. falciparum* carriage at the end of the transmission season, (aOR = 2.57; 95% CI [1.1-6.70]; p = 0.026), stunting (aOR = 2.97; 95% CI [2.08-4.23]; p = 0.001), age range from two to 5 years (aOR = 0.14; 95% CI [0.08-0.25]; p = 0.001) and age above 5 years (aOR = 0.04; 95% CI [0.02-0.07]; p = 0.001) (Table 4).

Discussion

Malaria remains a major public health problem in Africa, despite the decline in malaria incidence reported by most African countries in recent years [20]. Early case detection and prompt effective treatment with ACT are essential tools for malaria control. Intermittent Preventive Treatment (IPT) is a new approach aiming at reducing malaria morbidity and mortality. IPT is recommended by the WHO for pregnant women and infants. In children, the strategy is still debated and several studies are in progress [7-9].

This study assessed the potential benefit of combining home based management of malaria with IPTc in an area with high coverage of ITNs. The trial, conducted in a rural area in Senegal, where malaria is highly seasonal, showed that combination of IPTc and HMM can provide substantial benefit in reducing malaria. Indeed, malaria incidence was lower in villages where HMM was combined with IPTc compared to villages with only HMM strategy. *P. falciparum* carriage at the end of the transmission season was significantly lower in communities assigned to IPTc + HMM. No severe malaria cases were noted in the HMM + IPTc arm, while five severe malaria cases were registered in the HMM arm; thus the combination of IPTc and HMM can provide substantial benefit in reducing occurrence of severe malaria cases. The combined interventions also provided

Table 3 Impact of interventions on malaria parasitaemia at the end of the transmission season

	HMM (n = 413)	HMM + IPTc (438)	OR (95% CI)	Protective efficacy (%)	p value
***P. falciparum* parasitaemia**					
> 1,000 parasite/μL	14 (3.39%)	6 (1.37%)	0.40 [0.16 - 0.96]	60% [04 - 84]	0.05
any density	19 (4.60%)	9 (2.05%)	0.43[0.19-0.95]	57% [05 - 81]	0.03

Table 4 Impact of interventions on anaemia prevalence at the end of the transmission season

	Anaemia (Hb inf 11 g/dl)				
	Participants (%)	OR (95% CI)	aOR (95% CI)	Protective efficacy (%) 95% CI	p value
Interventions					
HMM (n = 411)	248 (60.3)	1	1		
HMM+IPTc (n = 438)	237 (54.1)	0.77[0.59 -1.02]	0.59[0.37-0.93]	41%[18;58]	0.025
Gender					
Female (n = 398)	213 (53.5)	1	1		
Male (451)	272(60.3)	1.32[1.01-1.73]	1.19[0.80-1.75]	-19%[-75;20]	0.35
P. falciparum carriage					
No (n = 821)	464 (56.5)	1	1		
Yes (n = 28)	21 (75.0)	2.30[0.83-6.37]	2.56[1.12-5.85]	-156%[-485;-12]	0.026
Stunting (HAZ < -2SD)					
No (n = 546)	265 (48.53)	1	1		
Yes (n = 305)	220 (72.13)	2.72 [1.86-3.98]	2.97[2.41-3.66]	-197[-323;-108]	0.001
Age groups					
Under 2 years n = 179)	162 (90.5)	1	1		
[2-5 years](n = 374)	233 (62.30)	0.17[0.09-0.31]	0.14[0.08-0.24]	86%[76;92]	0.001
[5-10 years] (n = 296)	88 (29.73)	0.04[0.02-0.08]	0.04[0.02-0.07]	96%[93;98]	0.001

Goodness of fit test: Hosmer Lemeshow, Chi 2 (8df) = 7,36, p = 0,49. **aOR* adjusted odds ratio; *OR* odds ratio. Intra-class correlation coefficients for each outcome were as follows: age:0.02, Gender:0.001, stunting:0.08, slide microscopy positive:0.011. All p values are adjusted for clustering by robust standard errors.

an additional benefit in reducing the occurrence of anaemia in children less than 10 years of age.

These results are consistent with data from other trials. Tagbor *et al.*, in a randomized controlled trial conducted in children under 5 years in Ghana, demonstrated that combining IPTc with HMM can significantly reduce the incidence of malaria presumptive fevers [21]. Another trial in the Gambia [17] showed a reduction in the incidence of malaria in children under 5 years of age, when HMM is combined with IPT.

The expansion of malaria control measures at community level has been recommended by the WHO, in order to accelerate malaria elimination [4]. Malaria elimination will require the use of combination of interventions [17], and this study showed that community health workers can play an important role in scaling up anti-malarial interventions and even contribute to the malaria elimination process.

The study showed that combining HMM to IPTc in an area with high coverage of ITN (95%) will provide additional benefit in reducing malaria burden. The high coverage of ITN in the study area means that study participants had access to two or three interventions (HMM and ITNs or HMM, IPTc, ITNs). Thus, a third arm with only ITNs use would be appropriate to better understand the effect on malaria burden of several anti-malarial interventions. In other trials, conducted in Burkina Faso [22] and Mali, [23] IPTc showed a high level of protective efficacy against symptomatic malaria, severe malaria, as well as moderate and severe anaemia in children less than 5 years sleeping under ITNs. It thus appears that IPTc would provide a valuable contribution in reducing malaria by itself, or integrated with other intervention strategies, in areas with highly seasonal malaria [24].

The combination of IPTc and HMM was effective in reducing the magnitude of malaria and anemia in children less than 10 years. Although combined malaria control strategies at community level are likely to reduce malaria burden drastically, there are however, limited information on how the resultant drug pressure (IPTc drugs, ACT) may impact existing drug resistance. Consequently, it is important to monitor drug resistance while scaling up anti-malarial interventions at community level.

Although HMM + IPTc showed a significant protective effect against anaemia, the prevalence of anaemia at the end of the transmission season was still high. Anaemia was closely associated with *P. falciparum* carriage and stunting. It is thus important to implement community-based interventions to reduce anaemia among children in rural areas, to complement interventions against malaria, and malaria related anaemia. These interventions could include, among other things, strengthening and improving children's nutritional status and investigating for other possible causes of anaemia.

Conclusion

Combining IPTc and HMM can provide significant additional benefit in preventing clinical episodes of

malaria as well as anaemia among children in Senegal. IPTc would provide a valuable contribution in reducing malaria, by itself or integrated with other intervention strategies, in areas with highly seasonal malaria.

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Authors' contributions
RCT, CTN, PM, ICB, OG conceived and designed the study. RT, CB and KS trained CHWs, supervised the fieldwork and the data collection. RT analysed the data. RT, CTN, PM, ICB, OG, JLN, BF, MN, JDN wrote the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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CHAPITRE 6: DETERMINATION DES CAUSES D'ANEMIE CHEZ LES ENFANTS DE MOINS DE 10 ANS.

Chapter 6: Investigation des causes d'anémie chez les enfants de moins de 10 ans.

Ce chapitre est basé sur l'article suivant :

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BMC Research Notes

RESEARCH ARTICLE Open Access

The association between malaria parasitaemia, erythrocyte polymorphisms, malnutrition and anaemia in children less than 10 years in Senegal: a case control study

Roger CK Tine[1*], Magatte Ndiaye[1], Helle Holm Hansson[4], Cheikh T Ndour[2], Babacar Faye[1], Michael Alifrangis[4], K Sylla[1], Jean L Ndiaye[1], Pascal Magnussen[3], Ib C Bygbjerg[4] and Oumar Gaye[1]

1. Abstract

Malaria and anaemia (Haemoglobin<11 g/dl) remain frequent in tropical regions and are closely associated. Although anaemia aetiologies are known to be multi-factorial, most studies in malaria endemic areas have been confined to analysis of possible associations between anaemia and individual factors such as malaria.

A case control study involving children aged from 1 to 10 years was conducted to assess some assumed contributors to anaemia in the area of Bonconto Health post in Senegal.

Study participants were randomly selected from a list of children who participated in a survey in December 2010. Children aged from 1 to 10 years with haemoglobin level below 11 g/dl represented cases (anaemic children). Control participants were eligible if of same age group and their haemoglobin level was >= 11 g/dl. For each participant, a physical examination was done and anthropometric data collected prior to a biological assessment which included: malaria parasitaemia infection, intestinal worm carriage, G6PD deficiency, sickle cell disorders, and alpha-talassaemia.

Three hundred and fifty two children < 10 years of age were enrolled (176 case and 176 controls). In a logistic regression analysis, anaemia was significantly associated with malaria parasitaemia (aOR=5.23, 95%CI[1.1-28.48]), sickle cell disorders (aOR=2.89, 95% CI[1,32-6.34]), alpha-thalassemia (aOR=1.82, 95% CI[1.2-3.35]), stunting (aOR=3.37, 95% CI[1.93-5.88], age ranged from 2 to 4 years (aOR=0.13, 95% CI[0.05-0.31]) and age > 5 years (aOR=0.03, 95% CI[0.01-0.08]). Stratified by age group, anaemia was significantly associated with stunting in children less than 5 years (aOR=3.1 95% CI [1.4 – 6.8]), with, sickle cell disorders (aOR=3.5 95% CI [1.4 - 9.0]), alpha-thalassemia (or=2.4 95% CI[1.1-5.3]) and stunting (aOR=3.6 95% CI [1.6-8.2]) for children above 5 years. No association was found between G6PD deficiency, intestinal worm carriage and children's gender.

Malaria parasitaemia, stunting and haemoglobin genetic disorders represented the major causes of anaemia among study participants. Anaemia control in this area could be achieved by developing integrated interventions targeting both malaria and malnutrition.

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Conclusion: Malaria parasitaemia, stunting and haemoglobin genetic disorders represented the major causes of anaemia among study participants. Anaemia control in this area could be achieved by developing integrated interventions targeting both malaria and malnutrition.

Keywords: Anaemia, Malaria, Haemoglobin disorders, Malnutrition, Children

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Background

Anaemia (haemoglobin < 11 g/dl) is a major public health problem affecting 1.62 billion people globally [1]. Africa and Asia are the most affected regions with more than 85% of the absolute anaemia burden [1]. Children and women of reproductive age are most at risk, with global anaemia prevalence estimates of 47% in children younger than 5 years, 42% in pregnant woman and 30% in non-pregnant woman aged 15 – 49 years. [1,2] Causes of anaemia can be broadly classified into decreased erythrocyte production or increased loss of erythrocytes through increased destruction (haemolysis) or blood loss or both [2]. In developing countries, anaemia aetiologies are multi-factorial, and infectious diseases, genetic haemoglobin disorders, as well as malnutrition, can play a major role in anaemia occurrence [3].

Infectious diseases such as intestinal parasite infections contribute to anaemia through impaired absorption and metabolism of iron and other micronutrients or increased nutrient losses. For instance, soil-transmitted helminth infections commonly found in tropical regions are a major cause of anaemia [4,5]. Other parasitic infections such as schistosomiasis are frequent in sub-Saharan countries [6] and can lead to anaemia due to iron deficiency from blood lost from haematuria caused by *Schistosoma haematobium,* or diarrhoea due to *Schistosoma mansoni.* Malaria remains an important cause of morbidity in tropical regions with an estimated 149 to 274 million cases and 539 000 to 906 000 deaths worldwide [7]. *Plasmodium falciparum* is the most pathogenic species and can lead to increased erythrocyte destruction and thus to anaemia [8].

Genetic haemoglobin disorders, which are the consequence from irregular structural or reduced production of the globin chains of haemoglobin, can result in anaemia [2]. Several studies have investigated the distribution and functional consequences of these genetic disorders; while it is clear that heterozygotes (carriers) of the mutations are partially protected against malaria, [9,10] their contribution to the global anaemia burden remains less clear [11] and studies are becoming even more relevant [12] when malaria is declining, as in Senegal, and several other previous high-endemic areas on anaemia [13,14]. Sickle-cell disorders are associated with haemolytic anaemia and an estimated 2.28 individuals per 1000 births worldwide are affected by sickle-cell disorders [15]. Sickle cell disease is the homozygous state for haemoglobin S (HbSS), caused by a mutation in the β-haemoglobin. Sickle cell trait is the heterozygous state of this haemoglobinopathy (mainly HbAS) [12]. Alpha thalassemia is also a genetic haemoglobinopathy highly prevalent in Sub-Saharan Africa, Asia and Melanesia [10]. Normal individuals have duplicate α-genes on each chromosome 16. By contrast, those with heterozygous α+− thalassemia have loss of 1 α-gene, resulting in 3 functional α-genes (−α/αα), whereas homozygotes have only 2 functional α-genes (−α/-α). Heterozygote individuals are characterized by slight haematological changes, whereas homozygote individuals generally have mild microcytic anaemia [16].

Another prevalent genetic disorder in previous and present malarious areas is glucose-6-phosphate-dehydrogenase (G6PD) deficiency. G6PD deficiency is a common chromosome x-linked red blood cell enzymopathy with several polymorphisms arisen from mutations in the G6PD gene [17]. In sub-Saharan Africa, G6PD is essentially a tri-allelic polymorphism. G6PD (B) is the most common allele with normal enzymatic activity; G6PD (A) is associated with a single amino acid substitution at codon (c) 126 where Asn is changed to Asp (N126D), causing around 85% of the normal enzymatic activity. The G6PD (A-) deficiency allele has a single amino acid substitution at c68 from Val to Met (V68M), always in conjunction with the N126D mutation [18]. The G6PD (A-) variant has only around 12% of normal enzymatic activity with frequencies of 5– 25% of the affected population in sub-Saharan Africa [19,20]. Although most individuals with the G6PD (A-) polymorphic variant are asymptomatic, acute haemolytic anaemia can manifest in hetero and homozygote females as well as hemizygote males under oxidative stress of the red blood cells [19]. This condition can be induced by infections, anti-inflammatory agents and chemotherapeutics, including anti-malarials such as primaquine and dapsone [17].

Malnutrition is also known to be a factor associated with anaemia [21]. Restricted access to diverse micronutrients particularly in vulnerable groups living in low-income countries can contribute to malnutrition as well as to anaemia [2].

Overall, in tropical regions anaemia causes present a great diversity. Although anaemia aetiologies are multifactorial, most studies in malaria endemic areas have been confined to the anaemia associated with malaria or with other single factors [22-25]. In recent years, significant reductions in the incidence of malaria have been reported in several African countries where malaria was previously highly to moderately endemic [13]. A study conducted in Senegal (2010), showed a significant reduction of malaria cases in children who had access to several antimalarial interventions. However, despite the significant reduction of malaria, anaemia prevalence remained high in these communities [14]. It remains thus, unclear why anaemia is still high in communities who had access to high coverage of antimalarial interventions. Therefore, this study was undertaken, to document malaria risks for anaemia as well as other possible risks factors and determinants in an area with declining of malaria pattern.

Methods

Study area

The study was conducted in the 8 villages covered by the Bonconto health post, which is located in the Velingara health district in the South-eastern part of Senegal, 500 km from the capital city of Dakar. Mass deworming administration (MDA) program using Mebendazole is being promoted in the area of Bonconto health post with two yearly administrations in children under 5 years. Reports from the health post records showed coverage of mebendazole administration at 95% in December 2010.

Study design and population

A cross sectional survey was carried out in December 2010 at the end of the malaria transmission season, and 3 weeks after the MDA campaign in the 8 villages covered by the Bonconto health post. Study participants were randomly selected from the list of children who participated to the survey in December, using a random number generator from Excel^{TM} software. Children aged from 1 to 10 years with haemoglobin level below 11 g/dl were considered as anaemic and represented the cases while control participants were eligible if of same age group (1 to 10 years) and their haemoglobin level was at least 11 g/dl.

Sample size calculation

Among the investigated factors, malaria is known to be a common risk factor associated to anaemia; thus, malaria prevalence was used as the main outcome for sample size estimation. Based on 80% power, 5% significance level, assuming an overall carriage of malaria parasitaemia among non anaemic children in the study area at 22% (*Senegal Malaria indicator survey 2009*), a minimum odds ratio of 2 and a ratio case/control =1, a number of 354 participants (177 cases and 177 controls) was required in this study.

Data collection

Clinical assessment

Each child was examined by a physician prior to a biological assessment which included blood, stool and urine samples. The mother was interviewed directly to determine socio-demographic characteristics as well as the child history of fever, using a standard questionnaire. Symptoms presented by each child at the day of survey as well as data obtained from physical examination and the parent interview were assigned in a case report form (CRF).

Anthropometric measurements

Children's weight and height were measured by a trained field worker. Weightforage Zscore was used to denote underweight while height-for-age Z-score was used as an indicator of stunting. The Zscores were calculated based on the median values of the National Centre for Health Statistics (NCHS) reference population, United States.

Laboratory methods

Parasites detection Blood samples were collected using finger prick blood. The first drop was used for thick and thin smear tests for the diagnosis of malaria. Thick and thin smear tests were stained with Giemsa. Parasite density was determined by counting the number of asexual parasites per 200 white blood cells, and calculated per μl using the following formula: numbered parasites × 8000 / 200, assuming a white blood cell count of 8,000 cells per μl. Absence of malaria parasites in 200 high power ocular fields of the thick film was considered as negative.

Fresh stools samples were collected into clean containers. Faecal samples were examined for the detection of intestinal parasites using Ritchie technique. Intestinal parasites were recorded positive by the presence of helminths and/or protozoans in the faeces.

Urine samples were collected into clean containers between 10 to 14 h. To determine the presence of *Schistosoma haematobium* eggs in urine, a filtration method using polycarbamate nucleopore filters was used. One aliquot of 10 ml of urine sample was filtered and placed on a slide and examined using light microscopy.

Haemoglobin concentration determination

A drop of finger prick blood was drawn into a microcuvette for Hb determination (g/dl) using a HemoCue machine (HemoCue Hb 201®). Anaemia was defined as Hb concentration below 11 g/dl.

Due to some logistical constraints, samples for haemoglobin concentration, malaria parasite investigation and haemoglobin genetic disorders investigation, were collected in December 2010, while stool and urine samples were collected later in January two weeks after the first samples drawn.

Identification of human genetic polymorphisms in study participants

Samples and DNA extraction

Blood samples were collected on filter paper from 352 children who participated in the survey in December 2010. DNA was extracted from segments of bloodspots on filter paper in 96 well plate formats by chelex-100 methods as described elsewhere [26]. Haemoglobin A, S, C and the G6PD B, A and A-, were determined using PCR followed by a sequence-specific oligonucleotide probe enzyme-linked immunosorbent assay (SSOP-ELISA) while alpha-thalassemia was detected by PCR alone [20-27].

Polymerase chain reaction (PCR) conditions

Primers were used to amplify a 352 base-pair (bp) fragment of the G6PD gene covering the mutation site at codon 68 [27]. The haemoglobin gene was amplified by primers described by Modiano et al. [9] to produce a 358 bp fragment covering the mutation sites at codon 6 and 26. Primers were biotinylated at the 5′end by the supplier (MWG Biotech, Ebersberg, Germany) [20]. For alpha thalassemia, primers were used to produce bands of 2200 + 1900 bp representing the African α-globin variants [28].

The PCR conditions for both the G6PD and HbB gene were similar to the conditions described in [20]. The reactions were performed in 96 well PCR plates on a DNA thermal cycler. Alpha thalassemia PCR products were visualized by electrophoresis on a 0.75% agarose gel.

SSOP-ELISA

The procedures have been described in [20], in brief; oligonucleotide probes of 18 bases covering the SNP of interest were used where the 3′ end was conjugated with digoxigenin. Amplified PCR products were diluted 1:2 in dH_2O, denatured at 95°C for 5 minutes. Two μl of the PCR products were added to streptavidin coated (1μl/ml PBS) ELISA plates containing washing buffer (PBS with 0.05% Tween 20) and incubated for one hour at room temperature. The bound PCR products were incubated with 8 nM of probe in tetra-methyl ammonium chloride (TMAC) solution at 53°C with shaking for an hour. TMAC washing temperatures were set to 62°C for haemoglobin probes and 65°C for the G6PD probes. The plates were incubated with peroxidase conjugated antidigoxigenin antibodies (Roche Diagnostics) 1:1000 in washing buffer at room temperature for one hour, thereafter visualized by o-phenylene-diamine (OPD). The reaction was stopped with H_2SO_4 before measuring the optical density at 492 nm. Between each step, the ELISA plates were washed three times in washing buffer.

Data management and data analysis

Data were entered in Excel ™ software and analysed using STATA 11™ software. To assess the nutritional status, data were transferred into Epi Info. The Zscores for weightforage (underweight) and heightforage (stunting) were derived using Epinut Anthropometry (Epi Info sowfware). Children who had z scores below −2 standard deviations (SD) of the NCHS median reference population were considered to be malnourished. G6PD deficiency (G6PD A-) was defined as hemizygotes males and/or homozygote females. Sickle cell disease was represented by the homozygote state for haemoglobin S (HBSS) while the heterozygote state (HBAS, HBAC), represented sickle cell trait. Alpha-thalassemic children were represented by hetero and homozygote children. For categorical data, percentage was used to assess the frequency of each outcome. For continuous data, mean and standard deviation were used to describe normally distributed variables, median and range for other data. Characteristics of all children included in the study were tabulated by study group. Proportions were compared using chi square test or Fisher exact test where appropriated (univariate analysis); significance level of the different tests was set on 0.05, two sided. A stepwise logistic regression was done for the determination of risk factors possibly associated with anaemia.

Ethical considerations

This study was approved by the Senegalese National Ethical Committee (Conseil National de Recherche en Santé). Informed consent was obtained from parents or children guardian at the time of survey.

Results

Study participants characteristics

A total number of 352 children less than 10 years of age were enrolled in the study (176 cases and 176 controls). Children less than 5 years represented 69% in the anaemic group and 26% in the non-anaemic group (p=0.001). The proportion of boys was 60% in the anaemic group and 53% in the non-anaemic group (p=0.13). A proportion of 50% in both groups had access to seasonal intermittent preventive treatment with SP-AQ. Bed-net ownership in the anaemic group represented 100%, while that for the non-anaemic group represented 98%. (p=0.08) (Table 1).

Among the 176 children in the anaemic group, 122 (69.3%) were less than five years old while the remaining proportion (30.6%) had an age ranged from 5 to 10 years. Overall, mild anaemia was predominant in children above the age of 5 years (63%). Moderate anaemia represented a proportion of 48.4% in children less than 5 years and 33.3% in children with an age from 5 to 10 years (p=0.06). Few children were found with severe anaemia: 6.5% among under 5 years and 3.7% in children above 5 years (p=0.68) (Table 2).

Parasitic infections among study participants

Plasmodium falciparum parasites were identified in 11 children (3.12%). Prevalence of *P. falciparum* was evaluated at 5.11% and 2.14%, respectively in anaemic children and non-anaemic children (p=0.03).

A total number of 97 children (27%) were found with at least one intestinal parasite. The identified intestinal parasites were represented by: *Giardia intestinalis* (12%), *Entameaba coli* (17%), *Strongyloides stercoralis* (0.6%). Hookworms, *Schistosoma haematobium* and *Schistosoma mansoni* were not found (Table 3).

Table 1 Socio demographic characteristics of study participants

	Anaemic group (176)		Non anaemic group (n=176)		
Characteristics	**Number (%)**	**95%CI**	**Number (%)**	**95%CI**	***p value***
Age group					
Under 5	121 (68.75)	[57.04-82.14]	46 (26.14)	[19.13-34.86]	0.001
[5 - 10 years]	54 (30.68)	[23.05-40.03]	130 (73.86)	[61.71-87.70]	0.001
Female	69 (39.20)	[30.50-49.61]	83 (47.16)	[37.56-58.46]	0.13
Male	106 (60.27)	[49.31-72.84]	93 (52.84)	[42.65-64.73]	0.16
Children within household					
[1 – 3]	85 (48.29)	[38.58-59.72]	58 (32.95)	[25.02-42.60]	0.003
[4 -5]	52 (29.54)	[22.06-38.74]	68 (38.64)	[30.00-48.98]	0.07
>5	38 (21.59)	[15.28-21.63]	50 (28.41)	[21.08-37.45]	0.13
Birth order					
[1 -3]	106 (60.22)	[49.31-72.84]	109 (61.93)	[50.85-74.71]	0.74
[4 – 5]	42 (23.86)	[17.19-32.25]	40 (22.73)	[16.23-30.95]	0.80
>5	27 (15.34)	[10.11-22.32]	27 (15.34)	[10.11-22.32]	1
Access to IPTc with SP-AQ	88 (50)	[40.10-61.60]	88 (50)	[41.10-61.60]	1
Bed-net ownership	176 (100)	[85.7-116]	173 (98.30)	[84.19-114]	0.08

A total number of 42 children (24%) in the anaemic group were found with at least one intestinal parasite compared to 55 children (31%) in the non-anaemic group (p=0.12). The prevalence of isolated intestinal parasites was similar between the two study groups. (Table 4).

Erythrocytes polymorphism among study participants

The overall prevalence of sickle cell trait (HbAS and HbAC) was evaluated at 9.94% (35 children) where the majority (33 children) were HbAS while two children were found with HbAC. Children with sickle disease (HbSS) represented 2.8% (10 children).-). Sickle cell disease (HbSS) represented 3.9% (7 children) in the anaemic group and 1.7% (3 children) in the non-anaemic group (p=0.19). A total number of 23 anaemic (13%) and 12 non-anaemic children (6.8%) were found with sickle cell trait (HbAS or HBAC), respectively (p=0.04). Heterozygote alpha-thalassemia (αα/-α) was found in 62 children (17.6%) and homozygote alpha-thalassemia was detected in 16 children (4.5%). Thus, in total the prevalence of alpha-thalassemia (hetero and homozygote) was 22.1% (78 children). Alpha-thalassemia (Hetero and homozygotes) was detected in 48 anaemic children (27.2%) and in 30 non-anaemic children (17%) (p=0.02). Heterozygote alpha-thalassemia was prevalent in 21% of anaemic children and 14% of non-anaemic children (p=0.09). Homozygote alpha-thalassemia represented 6.2% in the anaemic group (11 children) and 2.8% (5 children) in the non-anaemic group (p=0.12) (Table 4).

The G6PD A was detected in 7 children (2%) while 3 children (0.8%) were caring the G6PD A-. Comparison between the two study groups showed a prevalence of G6PD (A) at 1.7% (3 children) in the anaemic group and 2.2% (4 children) in the non-anaemic group (p=0.70). The G6PD (A-) was detected in 3 anaemic children (1.7%) while no child in the non-anaemic group was found with G6PD (A).

Nutritional status

The overall prevalence of stunting among study participants was evaluated at 38.64% (136 children) while underweight and wasting represented 25.85% and 9.38%, respectively (Table 3). Among anaemic children, stunting represented 52% (91 children), whereas only half, 26% (45 children) in the non-anaemic group (p=0.001). Prevalence of underweight was 34% (61 children) in the anaemic group versus 17% (30 children) in the non-

Table 2 Frequency and severity of anaemia among cases stratified by age group

	Under 5years (N=122)		[5 - 10years] (N=54)		p value
	n (%)	**95%CI**	**n (%)**	**95%CI**	
Mild anaemia (Hb<11g/dl)	55 (45.1)	[34.0-58.7]	34 (63.0)	[43.6-87.9]	0.03
Moderate anaemia (Hb <9g/dl)	59 (48.4)	[36.8-62.4]	18 (33.3)	[19.7-52.7]	0.06
Severe anaemia (Hb<7g/dl)	08 (6.5)	[2.8-12.9]	02 (3.7)	[0.4-13.4]	0.68

Table 3 Overall prevalence of parasitic infections, erythrocytes polymorphisms and malnutrition among study participants

Outcome	No / Total No (%)	95%CI
Malaria parasitaemia	11/352 (3.12)	[1.56-5.59]
Intestinal parasite carriage	97/352 (27.56)	[22.35-33.62]
Isolated intestinal parasites		
Giardia intestinalis	43/352 (12.22)	[8.84-16.45]
Entameaba coli	61/352 (17.33)	[13.25-22.26]
Strongyloides stercoralis	02/352 (0.57)	[0.06-2.05]
Genetic disorders		
G6PD A	07/352 (1.99)	[0.79-4.09]
G6PD A-	03/352 (0.85)	[0.17-2.49]
Alpha-thalassemia (Hetero+Homozygote)	78/352 (22.16)	[17.51-27.65]
Heterozygote (αα/-α)	62/352 (17.61)	[13.50-22.58]
Homozygote (–α/-α)	16/352 (4.55)	[2.60-7.38]
Sickle cells disorders (HBSS + HBAS +HbAC)	45 (12.78)	[9.32-17.10]
Sickle cell disease (HBSS)	10/352 (2.84)	[1.36-5.22]
Sickle cell trait (HBAS, C)	33/352 (9.38)	[6.45-13.17]
Malnutrition		
Stunting	136/352 (38.64)	[32.42-45.70]
Underweight	91/352 (25.85)	[20.81-31.74]
Wasting	33/352 (9.38)	[6.45-13.17]

Table 4 Prevalence of parasitic infections, erythrocytes polymorphisms and malnutrition in each study arm

	Anaemic group (n=176)		Non anaemic group (n=176)		
Characteristics	**Number (%)**	**95%CI**	**Number (%)**	**95%CI**	**p value**
Malaria parasitaemia	9 (5.11)	[2.34-9.70]	2 (1.14)	[0.13-4.10]	0.03
Intestinal parasite carriage	42 (23.86)	[17.19-32.26]	55 (31.25)	[23.54-40.67]	0.12
Parasite species					
Giardia intestinalis	21 (11.93)	[7.39-18.23]	22 (12.50)	[7.83-19.92]	0.87
Entameaba coli	26 (14.77)	[9.65-21.64]	35 (18.89)	[13.81-27,65]	0.20
Strongyloides stercoralis	00	–	02 (1.14)	[0.13-4.11]	0.15
Sickle cells					
AS patients	22 (12.57)	[7.83-19.92]	11 (6.25)	[3.11-11.18]	0.04
SS patients	07 (3.97)	[1.59-8.19]	03 (1.70)	[0.35-4.98]	0.19
AC patients	01 (0.57)	[0.01-3.16]	01 (0.57)	[0.01-3.16]	1
Alpha thalassaemia *(Hetero and homozygotes)*	48 (27.27)	[20.11-36.16]	30 (17.05)	[11.50-24.33]	0.02
Heterozygotes Alpha thalassemia	37 (21.02)	[14.80-28.97]	25 (14.20)	[9.19-20.97]	0.09
Homozygotes Alpha thalassemia	11 (6.25)	[3.11-11.18]	05 (2.84)	[0.92-6.23]	0.12
G6PD (B)	148 (84.09)	[71.08-98.78]	134(76.14)	[63.79-90.17]	0.06
G6PD (A)	3 (1.70)	[0.35-4.98]	4 (2.27)	[0.61-5.81]	0.70
G6PD (A-)	3 (1.70)	[0.35-4.98]	00	–	0.08
Stunting	91 (51.70)	[41.62-63.48]	45 (25.57)	[18.64-34.21]	0.001
Underweight	61 (34.66)	[26.51-44.52]	30 (17.05)	[11.50-24.33]	0.001
Wasting	15 (8.52)	[4.77-14.06]	18 (10.23)	[6.06-16.16]	0.58

anaemic group (p=0.001). Wasting was found in a proportion of 8% and 10% respectively in anaemic and non-anaemic children (p=0.58) (Table 4).

Factors associated with anaemia among study participants

In a logistic regression analysis, anaemia was in overall significantly associated with malaria parasitaemia (aOR=5.2, 95%CI [1.1-28.4]), Sickle cell disorders (aOR=2.8, 95%CI [1.3-6.3]), Alpha-thalassemia (aOR=1.8, 95%CI [1.2-3.3]), and stunting (aOR=3.4, 95%CI [1.9-5.9]. Age group was also significantly associated with anaemia, adjusted OR at 0.1 for children aged from 2 to 4 years and 0.03 for children above 5 years of age. (Table 5) Stratified by age group, anaemia was significantly associated with stunting in children less than 5 years (aOR=3.1 95%CI [1.4 – 6.8]). In children aged from 5 to 10 years, the risk factors significantly associated with anaemia were represented by: stunting (aOR=3.6 95%CI [1.6 – 8.2]), sickle cell disorders (aOR=3.5 95%CI [1.4 – 9.0]), alpha-thallasemia (aOR=2.4 95%CI [1.1 – 5.3]) (Table 6). The study did not find any statistically significant association between intestinal parasites and anaemia, nor between anaemia and co-infection with more than one intestinal parasite.

Discussion

Although anaemia aetiologies are often multi-factorial, most studies have been confined to the anaemia associated with malaria or other individual factors [22-25]. This study investigated several factors potentially associated to anaemia among children less than 10 years living in rural area in Senegal. In this area, recently, a randomized trial prior to the present case control study was conducted to assess the impact on malaria of combining home based management and intermittent preventive treatment [14]. Thus, children involved in the case control study, had access to several antimalarial interventions and furthermore, the area has a high coverage of impregnated bed nets.

The high coverage of antimalarial interventions in the study area, and the fact that the study was conducted at the end of the malaria transmission season, could explain the low prevalence of malaria parasitaemia among the study participants (3.1%). Still, malaria prevalence was

Table 5 Distribution of factors associated with anaemia among study participants

	Frequency		Multivariate analysis	
	Anaemic group (n=176)	Non anaemic group (n=176)	aOR (95%CI)	p value
Variables				
Gender				
Female	69 (39.43%)	83 (47.16%)	*Reference*	
Male	106 (60.57)	93 (52.84%)	1.37 [0.82-2.31]	0.22
Age group				
1year	57 (32.76%)	8 (4.55%)	*Reference*	
[2–4 years]	89 (51.15%)	73 (45.48%)	0.13 [0.05-0.31]	0.001
[5 – 10 years]	28 (16.09%)	95 (53.98)	0.03 [0.01-0.08]	0.001
Access to IPTc drug (SP-AQ)				
No	87 (49.71%)	88 (50%)	*Reference*	
Yes	88 (50.29%)	88 (50%)	0.78 [0.45-1.33]	
Stunting				
No	84 (48%)	131 (74%)	*Reference*	
Yes	91 (52%)	45 (25.57%)	3.37 [1.93-5.88]	0.001
Malaria Parasitaemia				
No	166 (94.86%)	174 (98.86%)	*Reference*	
Yes	9 (5.14%)	2 (1.14%)	5.23 [1.1-28.48]	0.04
Sickle cell disorders				
No	145 (82.86%)	161 (91.48%)	*Reference*	
Yes	30 (17.14%)	15 (8.52%)	2.89 [1.32-6.34]	0.008
Alpha Thalassemia				
No	128 (73.14%9	146 (82.95%)	*Reference*	
Yes	47 (28.86%)	30 (17.05%)	1.82 [1.2-3.35]	0.04

aOR: adjusted odds ratio. IPTc: Intermittent Preventive Treatment in children. SP-AQ: Sulfadoxine-Pyrimethamine. Goodness-of-fit-test: Hosmer-Lemeshow, Chi2 (8df)=5.07, p=0.75.

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Table 6 Risk factors significantly associated with anaemia in children less than 5 years and children aged from 5 to 10 years at the Bonconto health post, Senegal

Risk Factors	aOR	95%CI	p value
Children under 5 years (n=122)*			
Stunting	3.1	[1.4 - 6.8]	0.004
Children aged 5 to 10 years (n=54) †			
Stunting	3.6	[1.6 - 8.2]	0.002
Sickle cell disorders	3.5	[1.4 - 9.0]	0.009
Alpha-thalassemia	2.4	[1.1 - 5.3]	0.026

*Analysis of risk factors associated to anaemia among children under 5 years was adjusted on the following variables: P. falciparum infection, G6PD deficiency, Sickle cell disorders, Alpha-thallassemia, Access to IPTc drug (SP-AQ), gender. The number of anaemic children in this age group was = 122. Goodness of fit test: Hosmer-Lemeshow, chi(8df)=0.99, p=0.99. † Analysis of risk factors associated to anaemia among children aged from 5 to 10 years was adjusted on the following variables: P. falciparum infection, G6PD deficiency, Access to IPTc drug (SP-AQ), gender. The number of anaemic children was= 54. Goodness of fit test: Hosmer-Lemeshow, chi(6df)=4.22, p=0.64.

higher in anaemic children compared to non anaemic children and was closely associated to anaemia. Thus, scaling up antimalarial interventions at community level such as early case detection and treatment, intermittent preventive treatment as well as long lasting insecticide treated nets, may be a step towards malaria elimination, but will as well reduce the burden of anaemia. However, other aetiologies need to be considered for an effective and integrated control of anaemia.

Intestinal protozoan infections (*Giardia intestinalis, Entameaba coli*) were the predominant parasites isolated from stool samples among the study participants. No significant association between these parasites and anaemia was observed. Despite that, *G. intestinalis* may induce diarrhoea and mal-absorption syndrome, which can lead to vitamin B12 deficiency as well as iron deficient anaemia [29]. The low prevalence of intestinal helminthic infections could be explained as a result of regular mass deworming administration program in the study area. Indeed, two mass de-worming campaigns using mebendazole (June and December every year) were undertaken in the study period as part of a national policy aiming at improving child survival. As Giardia is still prevalent in the area of Bonconto and it has the potential to induce diarrhoea and mal-absorption syndrome, this parasite could be targeted by public health programs. Thus, shifting from mebendazole to albendazole, which may be used against giardiasis, could contribute to further reduce it in children.

Sickle cell disorders (HbAS, HbAC and HbSS) among study participants were frequent with an overall prevalence at 12.8% which are consistent with other findings; Mbodj et *al.* in 2003, reported a prevalence of HbAS and HbSS in the general population in Senegal at 11.1% [30], in line with Diop et *al.* findings in 2005, of 10% [31]. Alpha-talassaemia (hetero and homozygote) was more prevalent among study participants (22%). In 2006, Nabias et *al.* reported a similar prevalence of alpha-thalassemia (24%) in a cohort of children aged from 2 to 10 years living in a rural area of Senegal [32]. Sickle cell disorders and alpha-thalassemia were strongly associated with anaemia in this study and thus may play a major role in the global burden of anaemia in developing countries [2]. Indeed, as child survival improves, and malaria is decreasing in many African countries, [13] inherited haemoglobin disorders could become an increasingly important disease burden and cause of anaemia in the future [33] *i.e.* beneficial balanced polymorphisms may become unbalanced. However, lack of adequate diagnostic tools in poor African settings can in this context, impact negatively on the investigation of anaemia related to genetic haemoglobin disorders. Therefore, there is a need to strengthen health systems diagnostic capacities in African settings, in order to optimize the investigation of genetic haemoglobin disorders and their consequences such as anaemia.

In this study, a low prevalence of G6PD deficiency was found: G6PD (A-) patients represented a proportion of 0.85%. This is much less than the 12.3% reported in another study from Senegal [31]. G6PD deficiency is usually asymptomatic but can lead to haemolytic anaemia under oxidative stress [19]. Although no significant association was found between the prevalence of G6PD deficiency and anaemia in this study, this genetic disorder was more prevalent in anaemic children.

Malnutrition was frequent in this study with an overall prevalence of 38% for stunting, 25% for underweight and 9% for wasting. Stunting was closely associated to anaemia. Malnutrition is known to be a leading factor to anaemia [34]. An inadequate intake of macro and micronutrients, or intestinal mal-absorption induced or increased by intestinal parasites infections, can play through iron and folate deficiency, a well-documented role in chronic anaemia pathophysiology [5].

Overall, malaria parasitaemia, stunting and some human genetic disorders represented the major causes of anaemia among study participants. Malaria and stunting can be controlled individually or together by developing integrated interventions targeting malaria and malnutrition. A study conducted at the Bonconto health post showed that home based management of malaria can be combined with intermittent preventive treatment, both delivered by community health workers with a significant impact on malaria and anaemia [14]. In addition micronutrient supplementation could be combined to home based management of malaria and IPTc in order to further reduce malnutrition and anaemia prevalence in children less than 10 years. However, studies are needed in such area in order to document the feasibility

as well as the impact of such strategies on child survival. Some studies have shown that vitamin A and iron supplementation (alone), may actually negatively impact child survival and malaria outcome [35,36]. The potential benefit of iron supplementation coupled with effective malaria controls for children living in malaria-endemic regions, need to be investigated.

Study limitations

This study has provided useful data for anaemia prevention and control. The study was designed as a case control study. Although selection of controls was restricted to children aged from 1 to 10 years, children less than 5 years were predominant in the anaemic group. To control for confounding induced by age group, multivariate analysis was done with adjustment by age group among other factors.

Iron deficiency has been shown to be an important factor in the pathophysiology of anaemia. Details on iron deficiency and other micronutrients deficiencies (which have not have not been evaluated in this study), would add to revealing anaemia aetiologies in this part of Senegal. The role of iron deficiency in anaemia occurrence varies according to age groups. Indeed, a study conducted recently in Ivory Coast, found a high prevalence of iron deficiency in infants, without however any significant association with anaemia among infants [37]. Among preschool children, living in areas with high prevalence of soil transmitted helminthic infection (STH) such as hookworm, iron deficiency can play a major role in anaemia occurrence [37]. In this study, no child was found with hookworm infection, neither *Schistosoma* which could contribute to iron deficiency anaemia.

Conclusion

Malaria parasitaemia, stunting and haemoglobin genetic disorders represented the major causes of anaemia among children between 1 and 10 years participating in this study. Anaemia control in this area could be achieved by developing integrated interventions, targeting both malaria and malnutrition.

Competing interests

The authors have no conflicts of interest concerning the work reported in this paper.

Authors' contributions

RCT, CTN, PM, MA, ICB, OG conceived and designed the study. RT, MN and KS collected the data. RT, MA and HHH were responsible for execution and analysis of molecular data on human genetic polymorphisms. RT analysed the data. RT, CTN, PM, ICB, OG, BF, HHH, MA wrote the manuscript. All authors read and approved the final manuscript.

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CHAPITRE 7: DISCUSSION GENERALE, CONCLUSIONS, IMPLICATIONS

Chapitre 7 : Discussion générale, implications et conclusions

1. Discussion générale

Malgré de nombreux efforts consentis dans la lutte contre le paludisme, cette affection continue à poser un problème majeur de santé publique [78], même si certains pays africains ont signalé une baisse de l'incidence du paludisme ces dernières années [44]. L'organisation mondiale de la santé a récemment exhorté les pays africains, à mettre à échelle les interventions à efficacité prouvée, dans le but d'accélérer le processus de contrôle du paludisme [18, 81, 82]. Toutefois, la réduction de l'ampleur du paludisme dans certaines localités où la transmission reste encore élevée telles que la zone du Sud-Est du Sénégal, nécessite la combinaison de plusieurs mesures de lutte antipaludique [32]. Cependant il existe peu d'information permettant de combiner les mesures de lutte antipaludique de la manière la plus optimale et effective possible [21]. Ce projet a été initié dans le but d'étudier la faisabilité et l'impact de l'utilisation combinée de la prise en charge communautaire des cas et de la prévention du paludisme saisonnier chez les enfants de 1 à 10 ans vivant en milieu rural au Sénégal.

La combinaison de la prise en charge communautaire des cas et de la prévention du paludisme saisonnier a permis de réduire de façon significative l'incidence du paludisme chez les enfants. En effet, au cours de la période d'étude, l'incidence du paludisme était significativement plus basse au niveau des communautés où une mise œuvre combinée de la prise en charge des cas et de la prévention du paludisme saisonnier était effectuée. Ces résultats, sont similaires à ceux publiés dans d'autres études [31, 32, 45]. Il est à noter que l'administration d'un traitement antipalustre par les agents de santé communautaire, est un moyen efficace pour améliorer l'accès à une prise en charge précoce, ce qui à terme pourra contribuer à réduire davantage le risque de survenue de formes sévères de paludisme. L'utilisation de la CPS permet quant à elle d'agir en amont de l'infestation plasmodiale et de réduire la survenue d'épisodes cliniques. Il demeure ainsi que CPS et prise en charge communautaire des cas, sont deux stratégies complémentaires et probablement synergiques. La combinaison de ces deux interventions pourrait ainsi constituer une nouvelle approche pouvant permettre d'atteindre des niveaux très importants de réduction du paludisme dans des régions où la transmission du paludisme est saisonnière [21] et les agents santé communautaire constituent des piliers importants sur lesquels les programmes nationaux peuvent s'appuyer pour optimiser davantage la lutte antipaludique.

La confirmation parasitologique des cas et la prescription d'antipaludiques efficaces sont des outils essentiels dans la lutte contre le paludisme [1]. Les tests de diagnostic rapide du paludisme constituent des alternatives intéressantes pour la confirmation biologique des cas de paludisme, spécialement dans des localités où l'accès à la microscopie n'est pas possible. Ces TDR sont largement utilisés en Afrique [86]. Cette étude a montré que les tests de diagnostic rapide du paludisme sont des outils diagnostic fiables pouvant permettre de rationaliser la prescription de médicaments antipaludiques. En effet, cette méthode de diagnostic s'est révélée sensible et spécifique avec un bon niveau de concordance entre les résultats des TDR et la goutte épaisse (méthode de référence ; kappa=0,75). Ces tests sont d'un apport important pour améliorer le niveau de fiabilité et de confiance du diagnostic de paludisme posé par les ASC [16]. Toutefois, il semble que le niveau de sensibilité et spécificité des TDR obtenu au cours de leur utilisation en milieu communautaire (dans les conditions opérationnelles), est plus bas que leur sensibilité dans les conditions habituelles de recherche. [87, 88].

Il est à noter que seul 24% des patients fébriles de l'étude avait présenté un accès palustre confirmé par TDR. Les TDR ont ainsi contribué à mieux documenter les cas cliniques de paludisme et à réduire la fréquence des prescriptions injustifiées d'antipaludiques. S'il est admis que les TDR du paludisme peuvent permettre d'optimiser la prescription d'antipaludique, à l'opposé il n'existe pas de test comparable pour les infections bactériennes [88]. En conséquence, les autres cas de fièvre sont restées prévalent et faiblement documentés au cours de cette étude. La prise en charge des affections fébriles non palustres en milieu communautaire, se base le plus souvent sur les directives de la PECIME (prise en charge intégrée des maladies fébriles de l'enfant) [89]. Au Sénégal, les patients présentant un TDR du paludisme positif, reçoivent généralement un traitement à base de dérivés d'artèmisinine ; en cas de TDR négatif, dans la plupart des cas un traitement antibiotique (Amoxicilline, ou trimethoprime-sulfamethoxazole) ou parfois un traitement antipyrétique est prescrit [90]. Cette situation pourrait conduire à une utilisation excessive d'antibiotique, facteur pouvant contribuer à la survenue de résistance aux antibiotiques mais aussi une prise en charge de mauvaise qualité des affections fébriles. Il apparaît ainsi, que des directives appropriées pour la prise en charge des affections fébriles s'avèrent nécessaire particulièrement dans des localités où l'accès aux outils diagnostiques standard reste limité. Pour y parvenir, une meilleure connaissance de l'épidémiologie des affections fébriles non palustres serait utile. Au cours de cette étude, un bon niveau de respect des directives de prise en charge du paludisme a été noté. En

effet, seuls les patients ayant justifié d'une confirmation biologique du diagnostic de paludisme ont reçu un CTA. Des données similaires ont été rapportées par le PNLP du Sénégal au cours d'une récente évaluation, de la mise à échelle des CTA au niveau communautaire [19].

Aucun événement indésirable grave n'a été répertorié chez les participants à l'étude. Chez une proportion relativement faible d'enfant (30%), des événements indésirables modérés ont été notés après l'administration de la combinaison AQ-SP. Des études précédentes, ont montré que le couplage de la CPS et de la prise en charge communautaire du paludisme utilisant l'association AQ-SP était bien toléré [31, 32].

CPS et prise en charge communautaire du paludisme, sont des interventions efficaces pouvant être d'un apport considérable dans la réduction du paludisme. Bien que la combinaison de ces deux interventions ait montré un effet protecteur contre l'anémie, une fréquence élevée d'enfant de moins de 10 ans avaient un taux d'hémoglobine bas (< 11g/dl) en fin de saison de transmission du paludisme. Il devient dés lors important de mettre en œuvre des interventions à base communautaire visant à réduire davantage le fardeau des anémies en complément des actions menées contre le paludisme. Pour y parvenir, des études s'avèrent nécessaires pour mieux comprendre les facteurs potentiellement associés à l'anémie chez les enfants vivants dans des localités où la transmission du paludisme est saisonnière. En milieu tropical, les causes d'anémie sont le plus souvent multifactorielles et la plupart des études étiologiques sur les anémies en zone d'endémie palustre, se sont limitées à explorer la relation paludisme et anémie [54, 55, 97, 98]. L'étude cas témoins a révélé que plusieurs facteurs peuvent contribuer à la survenue d'anémie chez les enfants de moins de 10 ans après la mise en œuvre combinée de la CPS et de la prise en charge communautaire du paludisme. Malgré la faible prévalence du portage de *Plasmodium falciparum* en fin de saison de transmission, une association étroite était notée entre infestation plasmodiale et anémie. Il apparaît ainsi que la mise échelle et la combinaison d'intervention à efficacité prouvée en milieu communautaire, pourrait constituer une étape vers la prè-élimination du paludisme et pourrait contribuer à réduire davantage la fréquence et les conséquences néfastes des anémies chez les enfants. Cependant, pour une meilleure optimisation de la lutte contre les anémies en milieu rural, les autres facteurs pouvant contribués à la survenue d'anémie méritent d'être pris en considération. Drépanocytose et alpha-thalassémie étaient retrouvés comme facteurs de risque d'anémie chez les participants à l'étude comme démontré par d'autres études [35].

Du fait de l'augmentation constante des efforts de santé publique pour améliorer la survie de l'enfance en Afrique, du fait également de la baisse de l'incidence du paludisme dans certains pays d'Afrique Sub-saharienne [44] les hémoglobinopathies risquent de devenir des causes de plus en plus importantes d'anémie dans les années à venir [99]. Toutefois, le défit posé par l'absence fréquente d'outils diagnostic adaptés permettant une bonne investigation de ces désordres génétiques, particulièrement en milieu rural, pourrait impacter négativement l'investigation correcte des anémies associées à ces désordres génétiques de l'hémoglobine. Dés lors il devient important d'améliorer les capacités diagnostiques des structures sanitaires périphériques, afin d'optimiser l'investigation des anémies ainsi que les facteurs de risque associés.

Au cours de la présente étude, la malnutrition était un facteur étroitement associé à la survenue d'anémie chez les enfants. Ceci a été précédemment décrit dans d'autres études [100]. L'accès limité à une alimentation équilibrée, ou l'existence d'un syndrome de malabsorption induit ou aggravé par certaines parasitoses intestinales, peuvent jouer un rôle prépondérant dans la survenue de déficience en fer et acide folique, et entrainer ainsi des anémies chroniques chez les enfants [37].

Paludisme, malnutrition et hémoglobinopathies (Alpha thalassemia, drépanocytose), sont les causes majeures d'anémie chez les enfants de moins de ans vivant au niveau de la zone de couverture du poste de santé de Bonconto. La lutte contre les anémies au niveau de ces localités, nécessite des approches communautaires intégrées ciblant à la fois le paludisme et la malnutrition. La supplémentation en micronutriment (fer, Vitamine A), pourrait être associée aux stratégies communautaires de lutte contre le paludisme (CPS, PECADOM) dans le but de réduire davantage la prévalence de l'anémie et de la malnutrition chez les enfants en milieu rural; l'administration régulière d'acide folique pourrait également être utile chez les sujets présentant des hémoglobinopathies.

Des études s'avèrent nécessaire dans ce contexte afin de documenter la faisabilité et l'impact (y compris les potentiels effets délétères) de telles stratégies sur la santé et la survie de l'enfance. Certaines études ont montré que la supplémentation en fer et vitamine A pouvait impacter négativement sur la mortalité et morbidité palustre [101, 102]. Le bénéfice potentiel d'une supplémentation en fer couplée à l'utilisation effective d'interventions antipalutres à efficacité prouvée, chez des enfants vivant en zone d'endémie palustre devrait être exploré.

2. Limites des études

Ce projet a contribué à fournir des bases scientifiques pouvant orienter la mise en œuvre de la CPS au Sénégal et renforcer la prise en charge communautaire du paludisme. L'étude a montré que les ASC peuvent utiliser les TDR du paludisme de façon correcte. En conformité avec les directives du PNLP, il était demandé aux ASC participants à l'étude, de référer au poste de santé les patients pour qui le TDR du paludisme était négatif. En l'absence de retour d'information à partir du poste de santé, l 'étude n'était pas en mesure de documenter les principaux tableaux cliniques associés à la survenue de fièvre non palustre chez les enfants inclus. L'examen des registres du poste de santé ou le suivi des patients référés au poste par l'équipe de recherche, aurait permis de mieux documenter les causes de fièvre non palustre au niveau de la zone d'étude. Un tel dispositif, aurait également permis de mieux documenter le niveau de respect du système de référence et contre référence au niveau de la zone d'étude.

L'étude cas témoins a permis une meilleure compréhension des déterminants des anémies au niveau d'une localité avec forte couverture en mesures de lutte contre le paludisme. L'étude n'a toutefois pas exploré la fréquence des déficiences en fer et autres micronutriments chez les enfants. En effet, la carence martiale est connue comme étant un important facteur de risque dans la physiopathologie des anémies et des détails sur la fréquence des carences en fer au niveau de la population d'étude, aurait permis d'apporter plus d'informations concernant les déterminants de l'anémie au niveau de cette localité du Sénégal.

3. Implications et perspectives

Il est faisable de combiner ces deux stratégies au niveau communautaire tout en garantissant des niveaux de couverture élevée pour les deux interventions. La prise en charge communautaire du paludisme est une stratégie recommandée par l'OMS depuis 2003 tandis que la CPS est une stratégie nouvellement recommandée (en 2012) par l'OMS. Le Sénégal a récemment adopté la CPS et envisage de la mettre en œuvre au niveau des foyers éligibles tels que les régions du Sud-Est (Tambacounda, Kolda, Kédougou). Les résultats de la présente étude ont été partagés avec le Ministère de la Santé du Sénégal et ont contribué à fournir des bases scientifiques utiles à l'adoption de la CPS au Sénégal.

La mise en œuvre combinée de la CPS et de la prise en charge communautaire du paludisme pourrait contribuer à réduire sensiblement le fardeau du paludisme au Sénégal. Toutefois, il existe peu d'information sur l'effet que la surpression médicamenteuse additionnelle (CTA, AQ-SP) pourrait avoir sur la survenue ou propagation de la résistance aux antipaludiques. Il devient dès lors, important de surveiller l'efficacité des antipaludiques au niveau des zones où la CPS sera mise en œuvre. Il en résulte que l'introduction de la CPS, va aboutir à une augmentation de la gamme de médicaments antipaludiques disponibles au niveau communautaire. Dans ce contexte, la mise en place d'un système de pharmacovigilance performant pour assurer une surveillance correcte des événements indésirables s'avère nécessaire. Au Sénégal, malgré l'existence d'un programme de pharmacovigilance, la notification d'événement indésirable demeure un défit majeur [103]. Il existe ainsi un besoin d'améliorer la performance des systèmes de pharmacovigilance à l'échelle nationale particulièrement au niveau des zones où la mise en œuvre combinée de la CPS et de la prise en charge communautaire du paludisme sera effectuée.

L'utilisation des tests de diagnostic rapide du paludisme constitue un moyen efficace pour améliorer la qualité du diagnostic et de la prise en charge du paludisme en milieu communautaire. Toutefois, une bonne proportion de cas de fièvre survenant en milieu communautaire, reste encore faiblement documentée du fait de l'inexistence d'outils diagnostiques opérationnels adaptés. Ainsi, des directives de prise en charge des cas de fièvre non palustres adaptés pour le milieu communautaire seraient utiles. Une meilleure connaissance de l'épidémiologie des causes de fièvre non palustre devrait permettre d'optimiser davantage la prise en charge des cas de fièvre non palustre en milieu communautaire.

Les résultats de la présente étude suggèrent un changement significatif au niveau du profil épidémiologique des parasitoses intestinales mais également une modification du spectre des parasites en causes. En effet, le portage parasitaire est devenu plus fréquent chez les enfants d'âge supérieur à 5 ans ; il existe une raréfaction des helminthes contrastant avec la fréquence plus élevée de protozoaires intestinaux (*Giardia intestinalis, Entameaba coli*). Ces modifications pourraient s'expliquer par la conduite de campagnes de déparasitage de masse systématique au mébendazole, effectué au niveau de la zone, deux fois par an et ciblant les enfants de moins de 5 ans. Dans la mesure où *Giardia intestinalis* est très prévalent chez les enfants de la localité et du fait que ce parasite peut induire des effets délétères sur la santé des enfants (diarrhée, syndrome de malabsorption,

malnutrition) ce parasite mériterait d'être ciblé par les actions de santé publique. Ainsi, le remplacement du mébendazole par l'albendazole qui aurait une action à la fois sur les protozoaires et les helminthes, pourrait permettre de rendre la stratégie de déparasitage de masse plus efficiente.

L'élimination du paludisme est un objectif déclaré par l'OMS et beaucoup d'efforts sont entrain d'être menés pour atteindre cet objectif [104]. Diverses approches pour l'élimination du paludisme sont proposées ; ces approches varient en fonction de l'épidémiologie du paludisme [105]. L'adjonction de primaquine au traitement par les CTA au cours du paludisme à *Plasmodium falciparum* est une approche pouvant contribuer à réduire l'intensité de la transmission grâce à l'action antigamétocytaire de la primaquine [106]. Cette stratégie est actuellement recommandée par l'OMS comme stratégie d'élimination du paludisme dans des zones à faible niveau de transmission[107]. Toutefois, il existe encore des inquiétudes sur la tolérance de la primaquine dans la mesure où cette molécule peut induire des anémies hémolytiques chez les sujets présentant une déficience en G6PD[108]. Cette étude a montré une basse prévalence de la déficience en G6PD chez les enfants, ce qui est un argument en faveur de l'utilisation de la primaquine. Toutefois, des études de tolérance et d'efficacité s'avèrent nécessaire afin de mieux documenter la toléra ainsi que le bénéfice potentiel que l'adjonction d'une faible dose de primaquine pourrait avoir sur le paludisme.

4. Conclusions

Ce projet conduit en milieu rural sénégalais, a montré que la mise en œuvre combinée de la prise en charge communautaire du paludisme et de la CPS est une approche faisable par les agents de santé communautaire du Sénégal. Les agents de santé communautaire ont démontré une habileté à utiliser ces deux interventions antipalustres tout en garantissant des niveaux de couverture élevés. Ces deux interventions combinées, ont contribué à réduire l'incidence du paludisme et la fréquence des anémies chez les enfants. Il demeure cependant, que les facteurs nutritionnels, le portage de *Plasmodium falciparum*, les hémoglobinopathies constituent d'importants déterminants des anémies en milieu tropical. Des approches communautaires intégrées ciblant à la fois le paludisme et la malnutrition sont nécessaires pour lutter efficacement contre l'anémie et ses conséquences néfastes sur la santé des enfants vivant en milieu rural au Sénégal.

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VALORISATION SCIENTIFIQUE DES RESULTATS

Valorisation scientifique des résultats

Les résultats de ce travail ont été diffusés à travers des revues scientifiques à comité de lecture et des congrès scientifiques.

- **Articles scientifiques**

1. **TINE R.C**, FAYE B, NDOUR C, NDIAYE J, NDIAYE M, BASSENE C, MAGNUSSEN P, BYGBJERG I.C, SYLLA K, NDOUR J.D, GAYE O. **Impact of combining intermittent preventive treatment with home management of malaria in children less than 10 years in a rural area of Senegal: a cluster randomized trial.** *Malaria Journal* 2011 10:358.

2. **TINE RC**, NDIAYE M, HANSSON HH, NDOUR CT, FAYE B, ALIFRANGIS M, SYLLA K, NDIAYE JL, MAGNUSSEN P, BYGBJERG IC, GAYE O. **The association between malaria parasitaemia, erythrocyte polymorphisms, malnutrition and anaemia in children less than 10 years in Senegal: a case control study.** *BMC Research Notes* 2012 11;5(1):565.

3. TINE R.C, FAYE B, NDOUR C.T, SYLLA K, SOW D, NDIAYE M, NDIAYE J.L, MAGNUSSEN P, ALIFRANGIS M, BYGBJERG I.C, GAYE O**. Parasitic infections among children under five years in Senegal: prevalence and effect on anaemia and nutritional status.** *Article soumis, Journal of Parasitology Resaerch : under review*

4. **TINE R.C,** NDOUR C.T, FAYE B, CAIRNS M, SYLLA K, NDIAYE M, NDIAYE J.L, SOW D, CISSE B, MAGNUSSEN P, BYGBJERG I.C, GAYE O. **Feasibility, safety and effectiveness of combining Home based Malaria Management and Seasonal Malaria Chemoprevention in children less than 10 years in Senegal: A cluster-randomised trial.** *Article soumis, Transaction of the Royal Society: under review.*

5. **TINE R.C**, NDIAYE P, NDOUR C.T, FAYE B, NDIAYE J.L , SYLLA K, NDIAYE M, CISSE B , SOW D, MAGNUSSEN P, BYGBJERG I.C, GAYE O. **Acceptability by community health workers in Senegal of combining home-based malaria management and seasonal malaria chemoprevention.** *In process.*

- **Communication au niveau de congrès scientifiques**

1. **TINE R.C**, FAYE B, NDIAYE J.L, NDOUR C.T, MAGNUSSEN P, BYGBJERB I.C, GAYE O. Prevalence of intestinal parasites, Malaria parasitemia, anaemia and anthropometric status among children under five years of age in Lamarame (Senegal). ASTMH 59th ANNUAL MEETING, Atlanta November 2010. Poster N° 312.

2. **TINE R.C**, NDOUR C.T, FAYE B, NDIAYE J.L, MAGNUSSEN P, BASSENE C, BYGBJERB I.C, GAYE O. Impact of combining intermittent preventive treatment with home management of malaria in children under ten years, in a rural area of Senegal. ASTMH 60th ANNUAL MEETING, Philadelphia December 2011. Poster N° 895.

3. **TINE RC**, HANSSON HH, NDIAYE M, ALIFRANGIS M, FAYE B, NDOUR CT, NDIAYE JL, MAGNUSSEN P, BYGBJERG IC, GAYE O. **The association between malaria parasitaemia, erythrocyte polymorphisms, malnutrition and anaemia in children less than 10 years in Senegal: a case control study.** ASTMH 61th ANNUAL MEETING, Atlanta November 2012. Poster N° 781.

4. **TINE R.C,** FAYE B, NDOUR C.T, NDIAYE J.L, SYLLA K, NDIAYE M, SOW D, NDIAYE D, GAYE O. **Association entre *Plasmodium falciparum*, hémoglobinopathies, malnutrition et anémie chez les enfants de moins de 10 ans au Sénégal: étude cas-témoins.** Communication orale au cours des Journées conjointes des départements de biologie et médecine de la Faculté de Médecine de l'UCAD, Dakar Mai 2012.

5. **TINE R.C,** FAYE B, NDOUR C.T, NDIAYE J.L, SYLLA K, NDIAYE M, SOW D, NDIAYE D, GAYE O. **Association entre *Plasmodium falciparum*, hémoglobinopathies, malnutrition et anémie chez les enfants de moins de 10 ans au Sénégal: étude cas-témoins.** Communication orale au cours des Journées scientifiques de l'hôpital principal de Dakar Juin 2012. A obtenu à cette occasion le prix de la meilleure communication orale

6. **TINE R.C,** FAYE B, NDOUR C.T, MAGNUSSEN P, NDIAYE J.L, SYLLA K, NDIAYE M, SOW D, NDIAYE D, DIENG Y, DIENG T, BYGBJERG I.C, GAYE O. **Prise en charge des cas combinée à la chimioprévention du paludisme saisonnier au Sénégal : faisabilité et impact sur le paludisme.** Communication orale au cours du congrès de la Société Ouest Africaine de Parasitologie (SOAP), Dakar Décembre 2012.