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Titre de la thèse

Le Traitement Intermittent Préventif comme stratégie de lutte contre le paludisme chez les enfants

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RESUME

Le paludisme est l'une des maladies infectieuses la plus fréquente au monde avec 40% de la population mondiale exposée. En dépit des stratégies actuelles de lutte notamment la prise en charge rapide des cas, l'utilisation de matériaux imprégnés et la pulvérisation intra domiciliaire d'insecticide, le paludisme reste une des premières causes de morbidité et de mortalité notamment en Afrique subsaharienne. Cette partie du monde totalise à elle seule plus de 90% des cas de décès par paludisme dont 88% chez les enfants de moins de moins de 5 ans. En absence de vaccin utilisable en santé publique, il y a donc un besoin urgent de trouver une stratégie efficiente et simple de contrôle du paludisme. Le traitement préventif intermittent (TPI) définie comme l'administration d'un antipaludique à dose curative à des intervalles de temps prédéfinis réduit l'incidence du paludisme et apparaît aujourd'hui comme une des stratégies les plus prometteuses. Cette stratégie couplée au Programme Elargi de Vaccination (PEV) chez les enfants de moins de 1 an réduit l'incidence du paludisme de 30%. Des résultats plus importants sont obtenus chez les enfants de 0 à 5 ans voire de 0 à 10 ans lorsque la stratégie est appliquée en ciblant la saison de transmission.

Nos travaux de recherche au Mali ont porté sur :

- l'impact de la mise en œuvre du TPI couplé à la vaccination du PEV (TPin) sur i) la résistance *P. falciparum* à la Sulfadoxine pyrimethamine (SP), ii) la couverture des vaccins du PEV, iii) le taux de mortalité des enfants âgés de 4 à 18 mois.
- l'efficacité du TPI chez les enfants ciblant ciblant la saison de transmission (TPIe) dans un contexte de faible et de forte couverture en des Moustiquaires Imprégnés d'Insecticides (MII).

Nos résultats ont montré qu'après une année de mise en œuvre à l'échelle du district sanitaire, le TPIn a entrainé une augmentation de la couverture des vaccins du PEV. Cette couverture était de 53% en zone de non-intervention contre 69.5% en zone d'intervention ($p<0.01$). Il y a eu une réduction de la mortalité globale de 27% (RR= 0,73, IC95% : 0,55-0,97, p=0,029) chez les enfants âgés de 4 à 18 mois. Les fréquences des marqueurs moléculaires de la résistance de *P. falciparum* à SP en début et en fin la mise en œuvre et entre la zone

d'intervention et la zone de non –intervention après une année de mise en œuvre étaient similaires.

Deux doses de SP données en TPI à 8 semaines d' intervalle durant la saison de transmission réduit le taux d'incidence du paludisme pendant la saison de transmission de 69,4% chez les enfants de moins de 5 ans et de 63,4% chez les enfants de 5-10 ans dans un contexte de très faible utilisation de MII (<5%). Dans une autre étude que nous avons menée, le TPI avec SP + Amodiaquine (AQ) donné en 3 occasions à un mois d' intervalle pendant la saison de transmission a réduit le taux d' incidence du paludisme clinique non compliqué de 82% (IC à 95%: 78%– 85%; P<0.001) et les formes graves de paludisme de 87% (IC à 95% 42% – 99%, P=0.001) chez les enfants âgés de 3 à 59 mois en dépit un taux d'utilisation des MII de plus de 99%.

Nous n'avons pas documenté d'événement indésirable grave lié à l'utilisation de la SP ou de la SP + AQ en TPI durant ces deux études.

Nos résultats étayent la recommandation du TPI, ciblant la saison de transmission ou couplée au PEV, pour la lutte antipaludique chez les enfants.

Mots clés

Paludisme, traitement préventif intermittent, Sulfadoxine-Pyrimethamine, Amodiaquine, enfants, résistance, *Plasmodium falciparum*, Programme Elargi de Vaccination, mortalité, Moustiquaires Imprégnés d'Insecticides, Mali.

Intitulé et adresse de l'unité de recherche

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SUMMARY

Malaria is one of the most common infectious diseases in the world and 40% of the world population is exposed to malaria. Despite the current control strategies such as rapid diagnosis and treatment of disease cases, use of insecticide impregnated materials and indoor residuals spraying with insecticides, malaria remained a main cause of morbidity and mortality particularly in sub Saharan Africa. More than 90% of the deaths due to malaria occurred in this region and 88% of these deaths occurred in children aged less than 5 years of age. In absence of vaccine that can be used in public health, there is an urgent need for a simple and efficient control strategy. Malaria intermittent preventive treatment (IPT) defined as the administration of curative dose of anti-malarial drug at predefined time intervals, appears as one of the most promising strategies. Given through the Expanded Program of Immunization (EPI), the strategy reduced the incidence of malaria by 30%. More drastic reductions were obtained in children aged 0-5 years and even 0-10 years when the malaria transmission season was targeted for the administration of the strategy.

Our research work in Mali has assessed the following:

- The impact of implementation of IPT administrated through EPI (IPTi) on: i) the resistance of *P. falciparum* to Sulfadoxine pyrimethamine (SP); ii) EPI vaccine coverage, and iii) mortality of children of 4-18 months of age.
- The efficacy of IPT in children targeting the malaria transmission season (IPTe) in a context of low and high coverage of insecticide impregnated nets (ITN).

We have found that the implementation of IPTi at the district level has resulted in an augmentation of the EPI vaccine coverage. The EPI vaccine coverage was 53% in the non-intervention zone compared to 69.5% in the intervention zone ($p<0,01$). There was a reduction in all cause mortality of 27% (RR= 0.73, 95% CI : 0.55-0.97, p=0.029) in children aged 4-18 months. The frequencies of molecular markers of the resistance of *P. falciparum* to SP were similar at the beginning and the end of the one year implementation period and between the intervention and non-intervention zones.

Two doses of SP given at 8 weeks interval during the transmission season, reduced the incidence of malaria episodes during the transmission season by 69.4% in children aged less

than 5 years and by 63.4% in children aged 5-10 years in a context of very low ITN use (<5%). In another study that we have conducted, IPT with SP + Amodiaquine (AQ) given at three occasions at one month interval during the transmission season reduced the incidence rate of clinical malaria by 82% (95% CI: 78%– 85%; P<0.001), and the incidence of severe and complicated malaria by 87% (95% IC 42% – 99%, P=0.001) in children aged 3 to 59 months of age despite an ITN use of greater than 99%.

There was no serious adverse event related to the use of SP or SP+AQ in IPT during the two studies.

Our results support the recommendation of IPT targeting the transmission season and IPT given through the EPI for malaria control in children.

Key words

Malaria, intermittent preventive treatment, Sulfadoxine-Pyrimethamine, Amodiaquine, children, resistance, *Plasmodium falciparum,* Expanded Program of Immunization, mortality, insecticide impregnated nets, Mali.

LISTE DES ABREVIATIONS

AQ Amodiaquine

CTA Combinaisons Thérapeutiques à base d'Artémisine

DHFR Dihydrofolate reductase

DHPS Dihydropteroate synthetase

IC Intervalle de Confiance

MII Moustiquaires Imprégnés d'Insecticides

MRTC Malaria Research and Training Center de l'Université de Bamako

OMS Organisation Mondiale de la Santé

PEV Programme Elargi de Vaccination

SP Sulfadoxine-Pyrimethamine

TPI Traitement Préventif Intermittent

TPIe Traitement Préventif Intermittent chez les enfants pendant la saison de transmission

TPIn Traitement Préventif Intermittent couplé à la vaccination chez les enfants de moins de 1 an

TPIp Traitement Préventif Intermittent chez les femmes enceintes

UNICEF United Nations of International Children's Emergency Fund

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SOMMAIRE

INTRODUCTION

Le paludisme est une affection causée par des protozoaires parasites du genre *Plasmodium* transmis d'une personne à une autre par la piqûre d'insectes vecteurs, les femelles du genre *Anopheles*. Il existe quatre espèces de *Plasmodium* infectant habituellement l'homme, *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale.* Des cas paludisme humain à *Plasmodium knowlesi* – un paludisme de singe ont été rapportés ces dernières années, dans certaines zones d'Asie du Sud-Est [1,2].

En 2008, selon l'Organisation Mondiale de la Santé [3], on a enregistré 247 millions de cas de paludisme qui ont causé près d'un million de décès dans le monde. L'Afrique subsaharienne reste la région la plus touchée du monde par cette affection, totalisant à elle seule, 89% des cas de la maladie et 85% des décès. L'immense majorité des décès en Afrique (88%), survient chez les jeunes enfants âgés de moins de 5 ans. Près de 20% de l'ensemble des décès dans cette tranche d'âge, sont attribués au paludisme. *P. falciparum* responsable de la pluspart des formes graves et compliquées de paludisme est l'espèce la plus fréquente en Afrique représentant plus de 90% des espèces [3]. Depuis la déclaration de Seattle en 2007 par Bill & Melinda Gates, la communauté internationale fait de plus en plus de plaidoyer pour l'élimination voir l'éradication du paludisme.

Réduire la mortalité des enfants de moins de 5 ans des deux tiers entre 1990 et 2015 est un des objectifs du millénaire pour le développement. Cet objectif ne pourrait être atteint en Afrique sub-saharienne sans une lutte efficace contre le paludisme. Réduire le poids du paludisme est aussi un des objectifs spécifiques du sixième objectif du millénaire pour le développement. Il vise à maitriser le paludisme et à inverser les tendances entre 1990 et

2015 [4]. Pour cela, l'initiative Faire Reculer le Paludisme ou Roll Back Malaria (RBM) née en 1998 de la prise de conscience du poids inacceptable du paludisme a établi un plan [5]. Les chefs d'États africains se sont engagés en avril 2000 à Abuja à intensifier les efforts de lutte a travers la mise en œuvre des interventions, avec comme objectif de diminuer de moitié la mortalité provoquée par le paludisme en 2010 [6].

Au Mali le paludisme reste toujours la première cause de morbidité et la première cause de mortalité selon les statistiques nationales [7, 8]. C'est aussi la première cause (35%) de consultations dans les centres de santé et la première cause d'hospitalisation (45%) dans les services de pédiatrie [9]. L'analyse des statistiques nationales indiquent une augmentation des cas présumés palustres au lieu d'une diminution conformément aux objectifs du RBM [7]. La transmission du paludisme au Mali est saisonnière et survient essentiellement pendant la saison des pluies dont la durée varie de 3 à 6 mois en fonction des zones éco-géographiques. Plus de 80% des cas de paludisme non compliqués ou graves surviennent pendant la courte période de la saison de transmission (Figure 1). Des données récentes montrent que plus de 90% des formes graves et plus de 50% des formes non compliquées surviennent chez les enfants de moins de 5 ans (Figure 2). *P. faclciparum* est l'espèce la plus fréquente au Mali, représentant plus de 95% des espèces.

Figure 1: Distribution des cas de paludisme d'avril 2007 au mois de mars 2008 dans la population générale à Nossoumbougou, cercle de Kolokani, Mali

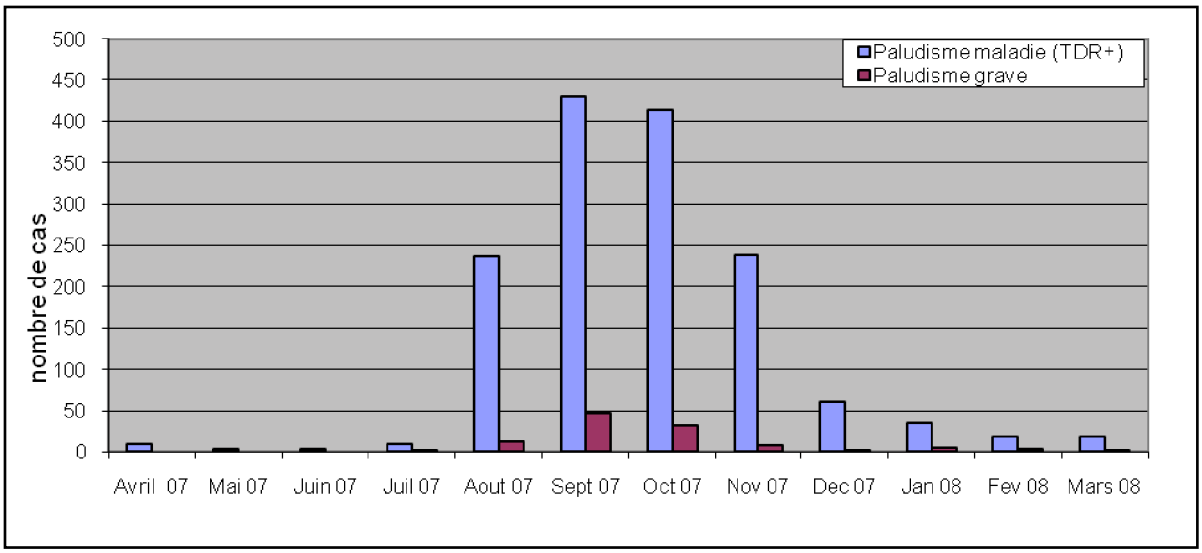
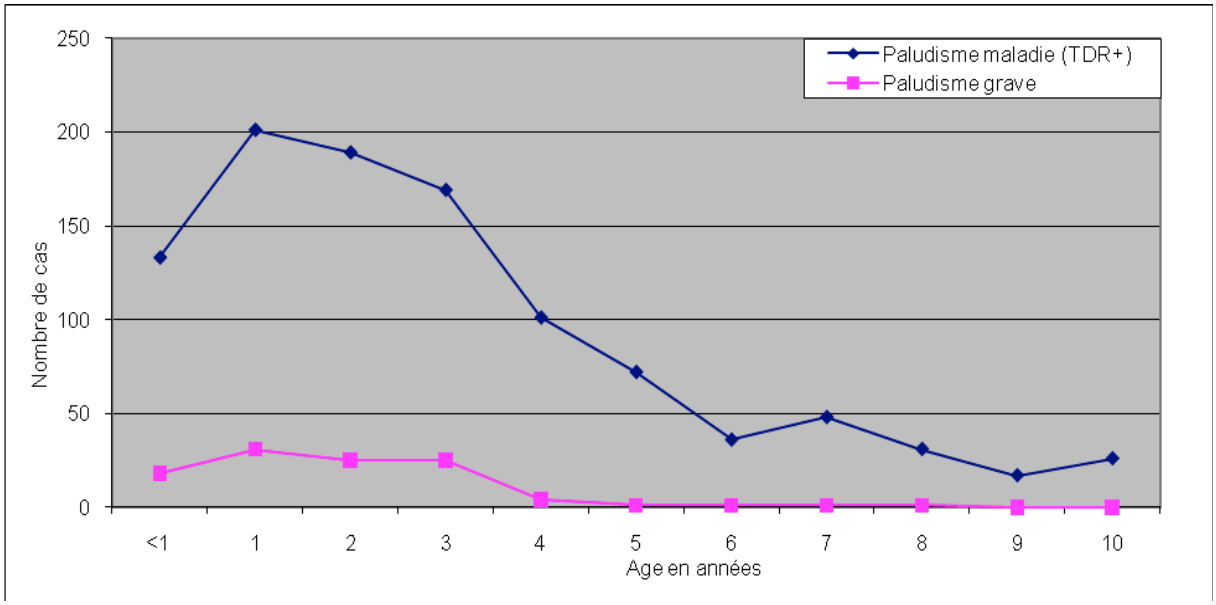


Figure 2: Distribution des cas de paludisme par âge chez les enfants de 0-10 ans à Nossoumbougou, cercle de Kolokani, Mali



Depuis l'arrêt de la campagne d'éradication du paludisme, les stratégies de lutte contre le paludisme préconisées par l'OMS visent à réduire la morbidité et la mortalité palustres. Ces stratégies reposent essentiellement sur la prise en charge rapide des cas, l'utilisation des mesures de lutte ou de protection contre les vecteurs et la chimio-prophylaxie (dont les traitements préventifs intermittents).

Le traitement rapide et correct des cas de paludisme maladie permet d'écourter la durée de la maladie et de l'infection plasmodiale, et de limiter la létalité. La mise en œuvre correcte de cette stratégie en Afrique a connu de nombreuses difficultés parmi lesquelles on peut citer les limitations à l'accès aux médicaments antipaludiques et la résistance des parasites. La chloroquine qui était le médicament de choix pour la prise en charge des cas grâce à son coût modique et sa bonne tolérance a été enlevée de la liste des médicaments antipaludiques en Afrique en raison de la résistance élevée des parasites à ce médicament. Les combinaisons thérapeutiques à base d'artémisine (CTA) sont encore efficaces et sont actuellement recommandées pour le traitement de formes non compliquées de paludisme en Afrique. Cependant des cas de résistance de *P. falciparum* aux dérivés de l'artémisine ont été signalés en Asie du Sud-est [10]. Il est craindre que cette résistance ne s'étende à l'Afrique comme cela a été le cas pour la résistance de *P. falciparum* à la chloroquine. Les sels de quinine restent encore le traitement de choix des formes graves et compliquées de paludisme même si des résistances ou des diminutions de sensibilité ont été signalées y compris en Afrique [11]. Le coût des CTA, l'insuffisance et l'inaccessibilité des services de santé dans la plupart des zones d'endémie constituent des handicaps majeurs à la mise en œuvre optimale de cette stratégie pour atteinte l'objectif de l'initiative Faire Reculer le Paludisme (RBM) de faire en sorte qu'au moins 80% des patients aient un diagnostic et un traitement adéquat [5].

L'utilisation de matériaux imprégnés d'insecticide demeure l'une des stratégies phares de la lutte contre le paludisme. L'utilisation des moustiquaires imprégnées d'insecticide (MII) permet de réduire la transmission de plus de 90%, la morbidité d'environ 50% et la mortalité palustres de 17 % [12]. Les taux de couverture de cette stratégie en Afrique sont restés faibles, pendant longtemps et très loin de l'objectif de 60% en 2005 fixé par la conférence d'Abuja et de la couverture universelle recommandée aujourd'hui par l'OMS et RBM. Le prix élevé des MII pour la plupart des ménages en zone d'endémie a été pendant longtemps le principal obstacle. A cela s'ajoute le besoin de changement de comportement des populations en vue de leur utilisation et la durée relativement courte de leur efficacité après imprégnation. Au cours des dernières années de grands efforts ont été faits pour rendre les MII plus accessibles aux populations avec des durées d'action des insecticides plus longues. En conséquence, les niveaux de couverture ont augmenté de façon importante dans la plupart des pays d'endémie en Afrique de 2% en 2000 à 22% en 2008 en moyenne [13] même si elles restent encore largement en dessous des objectifs fixés.

Une autre mesure de protection contre les vecteurs est la pulvérisation d'insecticides à effet rémanent à l'intérieur des habitations. Cette mesure a été préconisée par l'OMS en 2006 après des décennies d'abandon. Elle réduit la transmission du paludisme dans les habitations dans des proportions pouvant atteindre 90 %. Pour être efficace au moins 80% des habitations dans les zones ciblées doivent être traitées. Là aussi les défis majeurs restent la résistance des vecteurs aux insecticides et la brièveté de leur efficacité (3 à 9 mois en fonction des insecticides). La nécessité de traitements itératifs, son coût élevé et les difficultés techniques de sa mise en œuvre (nécessité d'équipes spécialisées) représentent des obstacles majeurs à sa mise en œuvre et à sa diffusion.

La chimio-prévention était réservée aux femmes enceintes vivant en zone d'endémie et aux individus non immuns voyageant dans les zones d'endémie. La chloroquine en a été le médicament de choix. Le développement de la résistance a la chloroquine et les difficultés d'observance du traitement prophylactique ont limité son efficacité. Chez les femmes enceintes vivant en zone d'endémie, elle a à présent été remplacée par le traitement préventif intermittent à la Sulfadoxine pyrimethamine qui s'est révélé plus simple à suivre et plus efficace [14, 15, 16].

Le traitement préventif intermittent (TPI) défini comme l'administration d'un antipaludique à dose curative à des intervalles de temps prédéfinis réduit l'incidence du paludisme et apparaît aujourd'hui comme une des stratégies les plus prometteuses. La stratégie a été évaluée et est aujourd'hui recommandée par l'OMS pour la prévention du paludisme chez les femmes enceintes avec une mise en application dans la plupart des pays d'endémie palustre.

Des études plus récentes, au cours des dix dernières années, ont montré que le TPI couplé au Programme Elargi de Vaccination (PEV) chez les enfants de moins de un an (TPIn) notamment à 2, 3 et 9 mois réduit l'incidence du paludisme maladie de 20 à 60% sans interaction négative avec les vaccins du PEV [17-24]. Cependant si l'absence d'interaction avec les vaccins du PEV et son efficacité sont bien établies dans le cadre d'essais cliniques randomisés et bien suivis, il n'en est pas de même pour l'impact de cette stratégie une fois qu'elle est mise en œuvre dans les politiques de santé, avec d'autres interventions notamment antivectorielles, sur i) la mortalité, ii) le développement de résistances des parasites aux antipaludiques utilisés et iii) la couverture vaccinale du PEV. Il serait en effet important de savoir si l'addition de l'activité TPIn au paquet PEV dans des pays en zone

d'endémie n'entrainerait pas une réduction de la couverture des vaccins du PEV. Cela pourrait être dramatique dans des pays comme le Mali où la couverture des vaccins du PEV reste en dessous 50% selon les dernières enquêtes de démographie et de santé [25, 26].

Une autre approche de l'utilisation de cette stratégie dans les zones de transmission saisonnière intense est de ne donner le TPI que pendant la saison de transmission y compris aux enfants de plus de un an. Ceci a l'avantage de couvrir une population cible plus large par une intervention de durée plus courte. L'analyse de la distribution des cas de paludisme maladie au cours de l'année montre que la large majorité des épisodes cliniques surviennent sur une période relativement courte de 3 à 5 mois. Cela est en faveur de l'utilisation de cette stratégie en ciblant la période de transmission. Une première étude effectuée au Sénégal [27] a montré que le TPI donné à un mois d'intervalle pendant 3 mois de saison de transmission réduit l'incidence du paludisme de 85%. Diverses questions concernant cette approche connue maintenant sous le nom de traitement préventif intermittent chez les enfants (TPIe) ont été ou sont en cours d'évaluation en vue de son adoption et de son intégration dans les politiques de santé. L'efficacité de ces stratégies (dont le TPIn couplé au PEV), en fonction des zones d'endémie, des schémas thérapeutiques et la couverture des autres mesures de lutte restent à établir.

Au cours de notre thèse nous avons voulu répondre à certaines de ces questions concernant ces deux formes d'utilisation du traitement préventif intermittent chez les enfants. Concernant le TPIe ciblant la saison de transmission, nous avons voulu évaluer son efficacité sur les épisodes d'accès palustres afin d'établir la preuve de son efficacité. Nous avons aussi voulu étudier son impact sur la résistance *in vivo* de *Plasmodium falciparum* à la Sulfadoxine pyrimethamine, et vérifier si l'arrêt de cette stratégie n'entrainait pas un effet rebond en

termes d'augmentation de l'incidence du paludisme. Pour ce qui est du TPIn couplé au PEV, nous avons évalué l'impact de sa mise en œuvre à large échelle sur la mortalité des enfants de la classe d'âge concernée, sur la fréquence des mutations des gènes dhfr et dhps, marqueurs moléculaires de la résistance à la SP et sur la couverture des vaccins du PEV.

Notre travail est divisé en six parties complémentaires.

Dans le premier chapitre, nous avons voulu répondre à la question de l'efficacité du TPIe sur les épisodes d'accès palustres, de son impact sur la résistance *in vivo* de *P. falciparum* à la SP et du risque de rebond d'incidence du paludisme à son arrêt.

Dans la deuxième partie, nous avons mesuré l'impact de la mise en œuvre du TPIn sur la fréquence des mutations des gènes dhfr et dhps, marqueurs moléculaires de la résistance à la SP.

Dans la troisième partie, nous avons évalué l'impact de la mise en œuvre du TPIn sur la couverture des vaccins du PEV et,

dans la quatrième partie, son impact sur la mortalité des enfants de la classe d'âge concernée.

Dans la cinquième partie de notre travail, nous avons évalué l'efficacité du TPIe (en utilisant les combinaisons de médicaments ayant montré la meilleure efficacité) chez les enfants dormant sous moustiquaires imprégnées d'insecticide à longue durée d'action.

Enfin dans la dernière partie nous essayons de tirer les conclusions des différents travaux présentés et proposons quelques perspectives de recherche.

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CHAPITRE I

Efficacité du traitement préventif intermittent ciblant la saison de transmission sur les épisodes de paludisme maladie chez les enfants au Mali, son impact sur la résistance *in vivo* de *P. falciparum* à la Sulfadoxine-Pyriméthamine et sur l'incidence des accès palustres après sa cessation

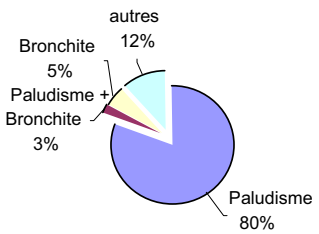
1. Efficacité du traitement préventif intermittent ciblant la saison de transmission sur les épisodes de paludisme maladie chez les enfants au Mali, son impact sur la résistance in vivo de *P. falciparum* à la SP et sur l'incidence des accès palustres après sa cessation

1.1 Position du problème

Au début des années 2000, la prise en charge rapide des cas et l'utilisation de matériaux imprégnés d'insecticide étaient les meilleures stratégies de lutte contre le paludisme mais leur mise en application était très lente et difficile à cause de la pauvreté, de l'analphabétisme des populations, de même que l'insuffisance et l'inaccessibilité des services de santé dans la plus part des zones d'endémie. En même temps, la résistance croissante de *P. falciparum* à la chloroquine qui était le médicament de choix en termes d'accessibilité et de tolérance pour le traitement et la prophylaxie antipaludique, a eu pour conséquence une augmentation de la morbidité et de la mortalité palustre [1, 2]. Il existe un besoin urgent de trouver une stratégie de lutte contre le paludisme plus simple et plus efficace. Le traitement préventif intermittent par la Sulfadoxine Pyrimethamine était déjà proposé comme alternative à la prophylaxie à la chloroquine chez les femmes enceintes avec des résultats très encourageant y compris au Mali. Au cours de la même période, un essai contrôlé randomisé réalisé en Tanzanie [3] a montré que le traitement intermittent avec SP à l'âge de 2, 3 et 9 mois réduit l'incidence du paludisme de 59% et celle de l'anémie grave de 50% chez les enfants.

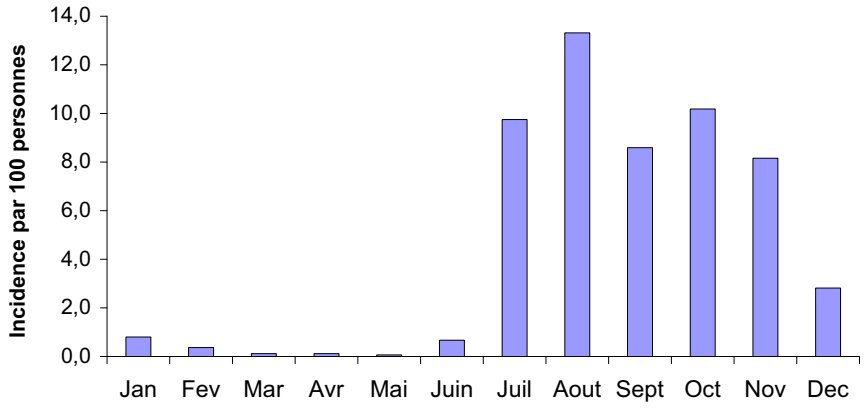
Au Mali les résultats d'une large étude de cohorte (n = 2350) d'enfants de 0-10 ans

Figure 3: Distribution des cas de maladies Etude de cohorte Bancoumana , 1999



d'âge conduite en 1999 à Bancoumana, un village situé dans la zone nord soudanienne du Mali ont montré que le paludisme était de loin la maladie la plus diagnostiquée, représentant 83% de la morbidité totale (Figure 3) et que qu'environ 91% des épisodes de paludisme maladie survenaient au cours des 5 mois de la saison des pluies de juillet à novembre (Figure 4). De même nous avons trouvé que le traitement par SP juste avant la saison de transmission différait le délai médian jusqu'au premier épisode de paludisme à 68 jours [4]. Dans de telles conditions il nous semblait que deux traitements préventifs à la SP à deux mois d'intervalle pouvaient réduire substantiellement l'incidence du paludisme maladie pendant la saison de transmission.

Figure 4: Incidence du paludisme clinique pour 100 personnes par mois



L'objectif principal de cette première partie de notre travail, était de tester l'hypothèse que la stratégie basée sur deux traitements préventifs intermittents à la SP à 8 semaines d'intervalle pendant la saison de transmission du paludisme réduisait l'incidence des accès palustres sans augmenter la résistance de *P. falciparum* à la SP ni s'accompagner d'effet rebond.

Nous avons donc conçu ce projet à mon retour de formation des Etats Unis, sous la supervision du Professeur Ogobara K. Doumbo et nous l'avons soumis à TDR/OMS pour financement dans le cadre des appels à candidature pour les « re-entry grant ». Nous avons obtenu le financement TDR/OMS et nous avons exécuté le protocole sur le terrain, supervisé la collecte et la saisie des données, analysé les données et procédé à la rédaction du manuscrit dont nous sommes le premier auteur et l'auteur à qui les correspondances doivent être adressées. C'est le premier projet que nous avons conçu, exécuté et publié.

1.2 Principaux résultats

Au total nous avons inclus 262 enfants âgés de 6 mois à 10 ans dans le village de Kambila (à 25 km de Bamako) dans cet essai randomisé ouvert à deux bras. Le groupe de traitement a reçu le TPI avec la SP en juillet et septembre 2002, à 8 semaines d'intervalle. Le groupe contrôle n'a pas reçu de TPI.

Au cours des 16 premières semaines de suivi, il y a eu 200 épisodes d'accès palustres dans les deux groupes dont 55 dans le groupe d'intervention et 145 dans le groupe contrôle soit une réduction de 67,5% (IC 95% 55,3% – 76,6%) après ajustement pour l'âge et le sexe. Cette réduction était de 69,4 % (IC 95%, 53,6–79.8) chez les enfants de moins de 5 ans et de 63,4% (IC 95%, 41,5%–77,1%) chez ceux de 6-10 ans après ajustement pour l'âge et le sexe.

Au cours des 12 premiers mois de suivi, il y a eu 352 épisodes d'accès palustres dans les deux groupes dont 130 dans le groupe d'intervention et 221 dans le groupe contrôle soit une réduction de 42,5%; (IC 95%, 28,6%–53,8%) après ajustement pour l'âge et le sexe. Cette réduction était de 46,6% (IC 95% 28,0% – 60,4%) chez les enfants de moins de 5

ans et de 35,9% (IC 95% 11,8% to 53,4%) chez ceux de 6-10 ans après ajustement pour l'âge et le sexe.

Au cours de la saison de transmission suivante, après 12 mois sans intervention, il y a eu 503 épisodes d'accès palustres dans les deux groupes dont 255 dans le groupe qui avait reçu l'intervention l'année précédente et 248 dans le groupe contrôle, avec un ratio de taux d'incidence ajusté pour l'âge et le sexe de 1,07 (CI 95% 0,90–1,27).

Parmi les 351 épisodes d'accès palustres survenus au cours de la première année de l'étude, des tests *in vivo* ont été faits 331 fois (94.3%), avec 119/130 (91.5%) dans le groupe de traitement et 212/221 (95,9%) dans le groupe contrôle. Les fréquences des réponses cliniques et parasitologiques adéquates (RCPA) non corrigées par la méthode PCR étaient comparables dans les deux groupes, 94,1% (112/119) dans le groupe qui avait reçu le TPI avec SP et 92,9% (197/212) dans le groupe contrôle (p = 0,67).

Pendant la saison de transmission suivante, les tests *in vivo* ont été effectués dans 472 des 503 épisodes d'accès palustres (93,8%). Les fréquences des RCPA non corrigées par la méthode PCR étaient comparables dans les deux groupes, 89,6% (208/232) dans le groupe d'intervention contre 87,9% (211/240) dans le groupe contrôle (p =0,56).

Les proportions de RCPA sont aussi similaires dans les deux bras lorsque les données sont analysées par tranches d'âges au cours de chacune des deux périodes.

Notre étude a donc montré que le TPI par SP prise à deux reprises à 8 semaines d'intervalle pendant la saison de transmission réduisait significativement la morbidité palustre, ne s'accompagnait pas d'une augmentation notable de la résistance *in vivo* à la SP (pendant la mise en œuvre de l'intervention et un an après son arrêt) et ne

s'accompagnait pas d'une augmentation du risque de paludisme au cours de l'année suivant l'intervention dans le groupe ayant bénéficié du TPI.

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1.3 Article Dicko et al. *Malar J. 2008*

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Impact of intermittent preventive treatment with sulphadoxine-pyrimethamine targeting the transmission season on the incidence of clinical malaria in children in Mali

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Abstract

Background: Recent studies have shown that intermittent preventive malaria treatment (IPT) in infants in areas of stable malaria transmission reduces malaria and severe anaemia incidence. However in most areas malaria morbidity and mortality remain high in older children.

Methods: To evaluate the effect of seasonal IPT with sulphadoxine pyrimethamine (SP) on incidence of malaria disease in area of seasonal transmission, 262 children 6 months-10 years in Kambila, Mali were randomized to receive either IPT with SP twice at eight weeks interval or no IPT during the transmission season of 2002 and were followed up for 12 months. Subjects were also followed during the subsequent transmission season in 2003 to assess possible rebound effect. Clinical malaria cases were treated with SP and followed to assess the *in vivo* response during both periods.

Results: The incidence rate of malaria disease per 1,000 person-months during the first 12 months was 3.2 episodes in the treatment group vs. 5.8 episodes in the control group with age-adjusted Protective Efficacy (PE) of 42.5%; [95% CI 28.6%–53.8%]. When the first 16 weeks of follow up is considered age-adjusted PE was 67.5% [95% CI 55.3% – 76.6%]. During the subsequent transmission season, the incidence of clinical malaria per 1000 persons-days was similar between the two groups (23.0 vs 21.5 episodes, age-adjusted IRR = 1.07 [95% CI, 0.90–1.27]). No significant difference was detected in *in vivo* response between the groups during both periods.

Conclusion: Two malaria intermittent treatments targeting the peak transmission season reduced the annual incidence rate of clinical malaria by 42.5% in an area with intense seasonal transmission. This simple strategy is likely to be one of the most effectives in reducing malaria burden in such areas.

Trial Registration: Clinicaltrials.gov NCT00623155

Background

Malaria is one of the most common infectious diseases in the world. It is estimated that malaria causes between 300 and 500 million clinical cases and 700,000 to 1.6 million deaths each year with 94% of deaths occurring in sub-Saharan Africa [1,2].

In Mali, malaria is the leading cause of mortality and morbidity in the general population [3]. Like in many other Sahelian west African countries, malaria transmission is highly seasonal occurring during the rainy season, which varies from three to six months. It has been shown that more than 80% of malaria cases occurred during five months of the transmission season in the north savanna area of Mali. In such conditions, a suitable control strategy implemented during this period may have the most impact on the reduction of disease burden.

In the absence of vaccines, early case management and the use of insecticide-impregnated material are the best strategies to control malaria. However, their implementation has been slow and difficult to achieve due to the overarching poverty and illiteracy of the population as well as the insufficiency and inaccessibility of health services in most endemic areas. Home- and self-treatment have been proposed as alternatives to the insufficiency and inaccessibility of the health services, but these strategies have also several disadvantages, including misdiagnoses, lack of compliance with drug regimens, use of inappropriate medicine, which could contribute to development of drug resistance and the non-recognition of the severity of symptoms [4,5]. Consequently in sub-Saharan Africa, the morbidity and mortality of malaria are increasing [6]. There is an urgent need for an easy and simple malaria control strategy.

Recent, randomized controlled trials conducted in areas of perennial malaria transmission have shown that intermittent preventive treatment (IPT) given at the time of childhood vaccinations reduced the incidence of the first episode of malaria and severe anaemia by more than 50% during the first year of life, without there being any rebound in the subsequent year [7,8]. However, in countries such as Mali, where malaria is highly seasonal and prevalent in older children, IPT in infants (IPTi) may not be the optimum way in which to use antimalarial drugs to prevent malaria. An alternative approach is to give intermittent preventive treatment to children at risk only during the rainy season [9]. The primary aim of this study was to evaluate the impact of two seasonal IPT (sIPT) with sulphadoxine-pyrimethamine (SP) given at eight weeks interval on the incidence of malaria disease in children of six months to 10 years of age in an area of seasonal transmission, in Kambila, Mali. Secondary aims were to assess the impact of this strategy on the *in vivo* response of *Plasmodium falciparum* to SP and the potential rebound effect of this strategy on the subsequent transmission season after its cessation.

Study design and methods

Study site

The study was conducted in Kambila, a rural village of about 1,500 inhabitants located about 25 km from Bamako, the capital city of Mali. *Plasmodium falciparum* is hyperendemic with parasitaemia prevalence rates ranging from 40–50% in the dry season (November – May) and 70–85% in the rainy season (June – October). The bed net coverage in the area at the time of the survey was less than 5%. The village was chosen to represent an area with four to five months of seasonal malaria transmission. SP was at the time, the second-line antimalarial drug in Mali, and was recommended for chloroquine failures. In 1995, a previous study found SP efficacy to be greater than 99% in the neighbouring area [10].

Study participants and design

The study was an open randomized controlled clinical trial. After a census of the village population, subjects in the target age group were screened for inclusion and exclusion criteria. Subjects who met inclusion and exclusion criteria were randomized either to receive two intermittent preventive treatments with standard recommended treatment doses of SP or no intermittent preventive treatment. Randomization codes were computer generated using simple randomization technique and treatment allocations were provided within sealed opaque envelopes. The first treatment was given at the beginning of the transmission season in July 2002 and the second treatment eight weeks later in August 2002. Inclusion criteria for the study included: 1) parental or other legal guardian consent; 2) age six months to 10 years; 3) having no chronic illness or symptomatic malaria; 4) agreeing to seek initial medical care for all medical illness in the study clinic during the entire study period; 5) having no plan to travel for a long time during the study period. Specific exclusion criteria included children with a history of allergy to sulpha drugs or SP.

Follow-up

Subjects from both groups were actively and passively followed during the study periods. The active follow-up was done weekly and consisted of: 1) questioning the subjects or parent for presence within the two past days of symptoms consistent with malaria including fever, headaches, body aches, malaise, diarrhoea, vomiting, and abdominal pain; 2) recording the axillary temperature; 3) examining conjunctivae and palms for pallor indicating a profound anaemia. The passive follow-up was done through continuous availability of study clinicians to evaluate any medical complaint at anytime during the entire study period.

Any subject, who complains of symptom consistent with malaria, with an axillary temperature greater or equal to 37.5°C, or with profound anaemia or jaundice, was given a complete medical exam and laboratory assessment including malaria smear and haemoglobin or hematocrit measurement. The criteria for discontinuing further follow-up for a volunteer were: 1) consent withdrawal, 2) volunteer missed three consecutives follow-up visits, 3) assessment of the study physician as to whether being in the study was in the best interest of the volunteer.

Treatment

Subjects who were diagnosed with uncomplicated malaria were treated immediately with standard doses of SP, and those who were diagnosed with severe malaria were treated with quinine according to the guidelines of National Malaria Control Programme. They remained in the study so that multiple episodes of malaria disease could be detected over the course of the study period. *In vivo* response to SP was assessed using the WHO 2003 protocol [11] on uncomplicated malaria cases. Clinical assessments were made on days 1, 2, 3, 7, 14, 21 and 28 following treatment with SP, and blood smears were obtained (by finger prick) on days 3, 7, 14, 21 and 28 to determine the *in vivo* SP treatment failure. At the end of every clinic visit, subjects and their parent/legal guardian were encouraged to return immediately to the study clinic and/or their primary health care system should any other new symptoms appear.

Standard recommended treatment doses of SP (Fansidar®, F. Hoffman-La Roche Ltd, Basel, Switzerland) was given for IPT and for treatment of episodes of uncomplicated malaria (1/4 tablet per 5 kg wt for age ≤12 years). All subjects were observed for at least 60 minutes for vomiting. If vomiting occurred within 30 minutes, the full dose was repeated and if it occurred within 60 minutes, 1/2 of the dose was repeated. Cases of SP failure, and cases of malaria disease occurring within two weeks of a periodic treatment in the IPT group, were treated with chloroquine at 25 mg/kg in three days. Severe and complicated malaria cases were treated with quinine 8 mg/kg three times a day for five days. Serious adverse events were monitored during the duration of the study.

Laboratory methods

Haematocrit was determined using microhaematocrit reading device after centrifugation (IECMicro-MB centrifuge) and haemoglobin concentration was determined using a portable analyzer (Hemocue, Lake Forest, CA). Parasitaemia was assessed by counting the number of asexual *P. falciparum* parasites on Giemsa-stained thick blood films until 300 leukocytes were observed by microscopists unaware of the treatment group of the subject. Parasite densities were converted assuming 7,500 leukocytes/μl. Routine quality control was performed on 10% of the slides, with a second microscopist re-examining the blood films while blinded to the previously recorded result. Differences in parasitaemia of more than 10% were resolved by an expert microscopist.

Study endpoints

The primary end point of the study was the incidence rate of clinical malaria, defined as uncomplicated or severe malaria for the assessment of protective efficacy and the rebound effect. Uncomplicated malaria was defined as signs or symptoms consistent with malaria either leading to treatment-seeking behaviour or reported during weekly follow-up visits accompanied by any level of parasitaemia. These signs and symptoms included fever at the time of evaluation (axillary temperature ≥37.5°C by digital thermometer), profound anaemia (conjunctival or palmar pallor), jaundice, report of fever within the last two days, lassitude, headache, body aches, diarrhoea, or abdominal pain. *Severe malaria* was defined according to the most recent WHO protocols: severe anaemia (defined as haemoglobin <5 g/dL); parasitemia >10% or 500,000/mm^3, prostration, respiratory distress, bleeding, recent repeated seizures, coma or obtundation, inability to drink, or persistent vomiting. The secondary endpoint was the non PCR corrected adequate clinical and parasitological response (ACPR) for the *in vivo* response of *P. falciparum* to SP. ACPR of *P. falciparum* to SP was defined according the WHO 2003 protocol [11] a protocol already available in 2002.

Sample size

The sample size was computed using the primary end point which was the incidence rate of malaria disease. Using normal approximation of square root transformation of Poisson formula, 96 persons-years of follow-up in each group were needed in order to detect a reduction of 40% in the incidence malaria disease, with 90% power and 95% confidence level, based on the estimated incidence of 1.2 episodes per person-year in the non-treatment group. To account for loss to follow up a total of 262 subjects (131 for each group) were enrolled. Using equivalency study of interventions method a total of 120 *in vivo* tests per arm will give more than 80% power for a maximum difference of 7% in ACPR between the two arms assuming ACPR of 95%.

Statistical analysis

Intention to treat analysis was used. The person-time method was used to calculate the incidence rate of malaria disease and to account for variable length of follow-up. The incidence rates were calculated as number of malaria episodes divided by the number of person days of follow-up at risk in each group. The incidence rates in the two groups were compared adjusted for age as continuous var-

iable, using Poisson regression models in overall and for each age category (<5 years and > = 5 years). Subjects were not considered at risk for 28 days after a treatment for malaria disease. Protective efficacy of two intermittent treatments with SP was computed as 1 minus the incidence rate ratio of malaria disease over the first 52 weeks. This was also computed for the first 16 weeks to allow comparison with other studies. Proportions of subjects with ACPR using WHO latest protocol [11] in the two groups were compared during the first year and the subsequent transmission season (53rd to 75th week in December 2003) using Pearson chi square or Fisher exact tests as appropriate. Parasite densities were log transformed and geometric means parasite densities were compared using Student t test. Data were entered and verified using MS Access and then exported to Stata (StataCorp, College Station, Texas, US) for analysis.

Ethical considerations

The protocol was approved by the Ethical Committee of Faculty of Medicine, Pharmacy and Dentistry of the University of Bamako, Mali. Community permission and written individual consent from parent or legal guardian were obtained before inclusion in the study.

Role of the funding source

The study was funded by the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), re-entry grant ID AI0828. Additional funds came from the President Clinton donation to MRTC through NIAID/NIH. The two institutions had no role in the design conduct analysis and reporting of the study. The corresponding author had full access to all the data and had final responsibility for the decision to submit for publication.

Results

Characteristics of the study participants

A total of 262 subjects were enrolled in this study (131 in each arm). Baseline characteristics are summarized in Table 1. There was no significant difference between the two groups at baseline in term of age, gender and malaria parasite prevalence.

Table 1: Baseline characteristics of the study participants

	sIPT + (n = 131)	sIPT- (n = 131)	p
Mean age in years (SD)	5.6 (2.9)	5.1 (2.8)	n.s.
Sex (% of male)	50.4	58.0	n.s.
Parasite prevalence (%)	33.6	37.4	n.s.
Lost to follow up first year (%)			
Week 1 to 52	11.4	11.4	n.s.
Week 53 to 75	5.3	3.8	n.s.

SD = standard deviation
sIPT - = control group
sIPT + = treatment group
n.s. not significant

Loss to follow-up

Of the 262 subjects enrolled, 30 subjects (11.4%) did not complete one year follow up and were equally distributed between the two groups (15 in each group). An additional 12 subjects (4.6%) did not complete the extended follow-up 7 in treatment group and 5 in the control group (Figure 1). Overall proportion of subjects who did not complete the follow up was similar between the two groups (16.8% versus 15.3%, p = 0.73). The reasons for loss to follow-up during the study were: migration to another location (n = 32), missing more three consecutive weekly visits (n = 6), consent withdrawal (n = 3) and death from severe malaria (n = 1).

Incidence rate of malaria disease and efficacy of sIPT after 52 weeks of follow up

The incidence rate and protective efficacy after 52 weeks of follow up are presented in Table 2. Overall 352 episodes of malaria disease occurred, 221 in the control group and 130 in the sIPTc group. The incidence rates per 1000 person-days at risk for the malaria disease were 5.8 and 3.2 in the control and treatment groups respectively, giving a protective efficacy of 44.8% (95% CI 31.5% to 55.6%). After adjustment for age, the protective efficacy was 42.5% (95% CI 28.6 to 53.8%.) (Table 2). When the analysis was stratified by age categories, the incidence rate of malaria disease was 7.3 and 3.9 episodes per 1000 person-days in control and treatment groups respectively with an age-adjusted PE of 46.6% (95% CI 28.0% to 60.4%) in children less than five years of age. In children five years and above, the incidence rate of malaria disease were 4.5 and 2.7 episodes per 1000 person-days respectively in the control and treatment group. Age adjusted PE in this age group was 35.9% (95% CI 11.8% to 53.4%).

Five cases of severe malaria (two cases of cerebral malaria, two cases of hyperparasitaemia, and one case of severe anaemia) occurred during the first 12 months of the follow-up, all in the control group giving an incidence rate of 0.048 episodes per 1000 persons-days at risk and a cumulative incidence of 3.9% in this group.

Incidence rate of malaria disease and efficacy of sIPT after 16 weeks of follow-up

The incidence rate and protective efficacy after the first 16 weeks of follow-up are presented in Table 3. A total of 200 malaria disease episodes occurred during the first 16 weeks of follow-up, 145 in the control group and 55 in the group who received sIPT. The age adjusted PE in this period was 67.5% (95% CI 55.3% to76.6%) and was not significantly different in subjects of less than five years compared to those aged of five years and above.

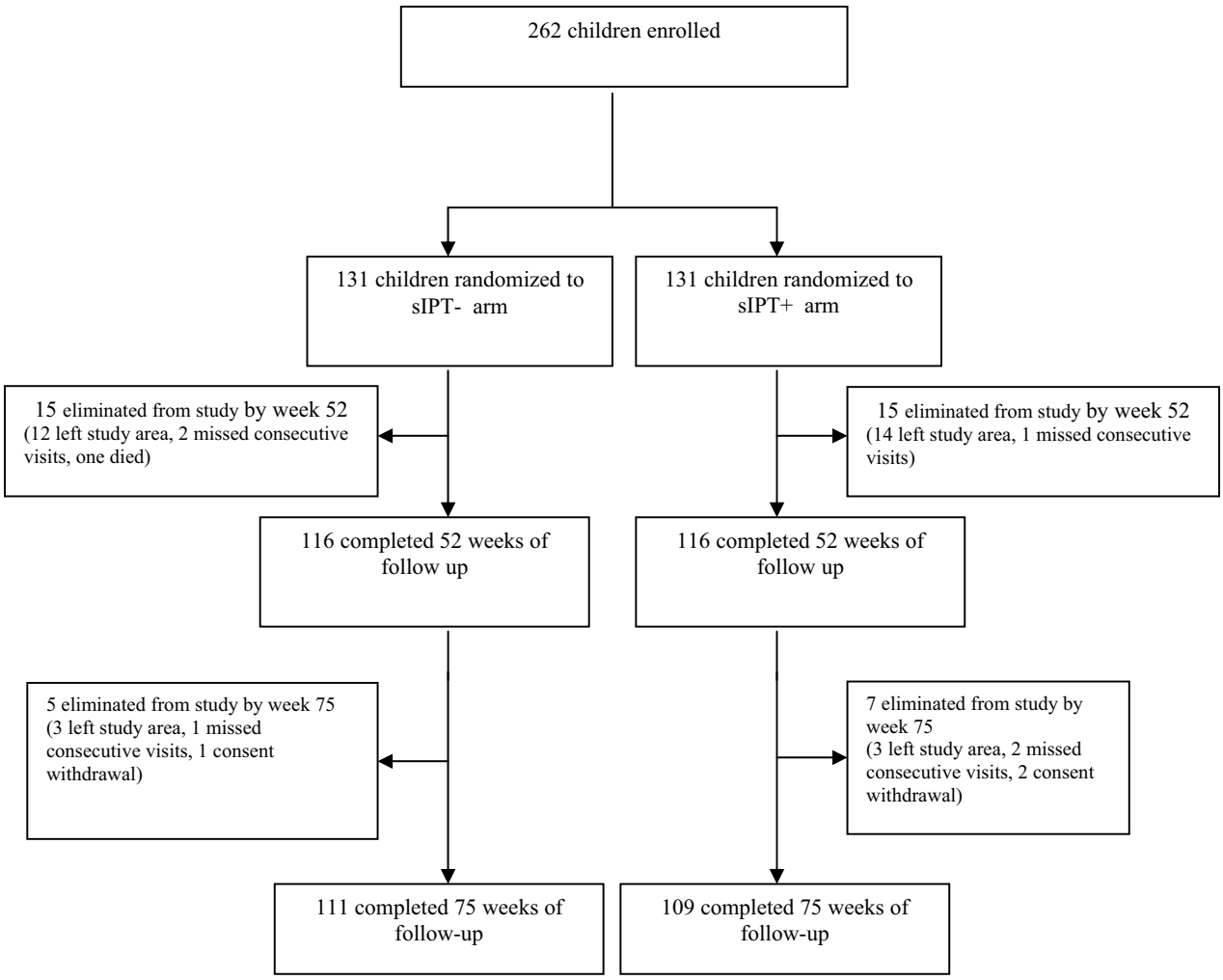

Figure 1
Flow Chart.

Table 2: Incidence rate of clinical malaria and protective efficacy after 52 weeks of follow-up per treatment group and age category

	<5 years		5–10 years		All	
	sIPT-	sIPT +	sIPT-	sIPT +	sIPT-	sIPT +
Number of malaria episodes	127	65	94	65	221	130
Person-days (-years) of follow up	17311 (47.6)	16590(45.6)	20799 (57.1)	24025 (66.0)	38110 (104.7)	40615 (111.6)
Incidence rate malaria disease per 1000 person-days (-years) of follow up	7.3 (2.67)	3.9(1.43)	4.5 (1.65)	2.7 (0.98)	5.8 (2.11)	3.2 (1.16)
Unadjusted PE in % (95% CI)	Reference group	46.6 (28.0–60.4	Reference group	40.1 (17.9–56.4)	Reference group	44.8 (31.5–55.6)
Age adjusted PE in % (95% CI)	Reference group	46.6 (28.0 – 60.4)	Reference group	35.9 (11.8–53.4)	Reference group	42.5 (28.6 – 53.8)

sIPT - = control group
sIPT + = treatment group
PE = Protective efficacy

Table 3: Incidence rate of malaria disease and protective efficacy after 16 weeks of follow up per treatment group and age category

	<5 years		5–10 years		All	
	sIPT-	sIPT +	sIPT-	sIPT +	sIPT-	sIPT +
Number of malaria episodes	86	30	59	25	145	55
Person-days of follow up	4797	5474	6207	7875	11004	13349
Incidence rate malaria disease per 1000 person-days of follow up	17.9	5.5	9.5	3.2	13.2	4.1
PE in % (95% CI)	Reference group	68.7 (57.4–77.1)	Reference group	66.6 (46.7–79.1)	Reference group	68.7 (57.4 – 76.1)
Age adjusted PE in % (95% CI)	Reference group	69.4 (53.6–79.8)	Reference group	63.4 (41.5–77.1)	Reference group	67.5 (55.3–76.6)

sIPT - = control group
sIPT + = treatment group
PE = Protective efficacy

Incidence rate of malaria disease after cessation of sIPT during the next subsequent transmission season (weeks 53 to 75)

During the subsequent malaria transmission season in 2003, a total of 503 malaria episodes occurred, with 255 cases in the treatment group and 248 in the control group. The incidence rates of malaria disease were similar between the two groups 23.0 episodes per 1,000 persons-days in the treatment group and 21.5 episodes per 1,000 persons-days in the control group with age adjusted Incidence Rate Ration (IRR) = 1.07 (95% CI 0.90 to 1.27, p = 0.46). During this extended follow up period two cases of severe malaria occurred, both type cerebral malaria and in the control group.

In vivo response of P. falciparum *to SP*

Of the 351 malaria episodes during the first year, valid *in vivo* tests were performed in 331 (94.3%) with 119/130 (91.5%) in the control group and 212/221 (95.9%) in the sIPT group. Table 4 shows that non PCR corrected 28 days Adequate Clinical and Parasitological Response (ACPR) was similar in the two groups in overall (92.9% in the control group vs. 94.1% in the sIPT group) and when the analysis was stratified by age categories. Mean parasitaemia at day 0 was also similar between the two groups with geometric means parasitaemia per μl of 11,480 (95% CI 8,770 to 15,027) in the control group versus 9,478 (95% CI 6,639 to 13,530) in the treatment group, p = 0.39.

During the extended surveillance following cessation of sIPT (53rd to 75th week), valid *in vivo* tests were performed in 472 (93.8%) of the 503 malaria episodes were treated with SP. Mean parasitaemia at day 0 was also similar between the two groups with geometric means parasitaemia per μl of 8,191 (95% CI of 6,367 to 10,538) in the control group versus 9,903 (95% CI 7,704 to 12,730) in the treatment group, p = 0.29.

In vivo responses per treatment and follow up period are presented in Table 5. The ACPR were similar between the two groups (87.9% versus 89.6%). The same similarities were found when the analysis was done in children less than five years of age or in those aged of five years and above.

Table 4: In vivo response of *P. falciparum* to SP during the first year of follow up group age categories and treatment groups

	< 5 years		5–10 years		All	
Outcome	sIPT- (n = 122)	sIPT + (n = 58)	sIPT- (n = 90)	sIPT + (n = 61)	sIPT- (n = 212)	sIPT + (n = 119)
ACPR	93.4	93.1	92.2	95.1	92.9	94.1
ETF	0.0	1.7	0.0	0	0.0	0.8
LCF	0.0	0.0	0.0	0	0.0	0.0
LPF	6.6	5.2	7.8	4.9	7.1	5.0

sIPT - = control group
sIPT + = treatment group
ACPR = adequate clinical and parasitological response
ETF = early treatment failure
LPF = late parasitological failure

Table 5: In vivo response of *P. falciparum* to SP during the extended period of follow up after cessation of sIPT implementation per age categories and treatment group

	1–4 years		5–11 years		All	
Outcome	sIPT- (n = 102)	sIPT + (n = 104)	sIPT- (n = 130)	sIPT + (n = 136)	sIPT- (n = 232)	sIPT + (n = 240)
ACPR	87.3	84.6	88.5	93.4	87.9	89.6
ETF	0.0	1.0	1.5	0.7	0.9	0.8
LCF	1.0	0.0	0.0	0.0	0.4	0.0
LPF	11.8	14.4	10.0	5.9	10.8	9.6

sIPT - = control group
sIPT + = treatment group
ACPR = adequate clinical and parasitological response
ETF = early treatment failure
LPF = late parasitological failure

Overall there was a reduction in the efficacy of SP during the extended follow-up compared to the first year of the follow up, especially in children less than five years of age.

Safety

No SP related serious adverse event was recorded during the study period. No subject was withdrawn because of allergy to SP.

Discussion

This study assessed the impact of two doses of intermittent preventive treatment with SP at 8-week intervals in children of six months to 10 years of age targeting the transmission season in Kambila, Mali and found a reduction of 42.5% in annual incidence of clinical malaria. When SP plus single dose of artesunate was given at monthly intervals in an area with seasonal malaria transmission, Cisse *et al* [12] in Senegal found a reduction of 86% in incidence of clinical malaria children less than five years of age over 13 weeks period. This higher efficacy can be explained by the shorter time interval between treatments (four weeks instead of eight weeks), the number of intermittent treatments (three instead of two) and the shorter duration of the follow up (13 instead 52 weeks) [12]. Furthermore when the analysis of the efficacy of the two doses of intermittent preventive treatment with SP at 8-week intervals was limited to the first 16 weeks of follow up, the protective efficacy was 69.4% in children aged less than five years. Although the incidence rate of clinical malaria was higher in children less than five years of age compared to those of five years and above, the differences in efficacy of the strategy were not significantly different suggesting that IPT is also appropriate for older children.

While several studies have assessed the impact of IPT in pregnant women and infants, few studies have assessed the impact of this strategy in children. Several studies have shown that intermittent treatment given at 3, 6, 9 months of age reduced the incidence of clinical episodes by 20 to 60% with strong variations according to transmission duration and the seasonality [7,8,13,14]. In areas of seasonal malaria transmission, the burden of malaria remains high in older children in addition to infants [15-20]. Furthermore, more than 80% of the malaria cases in non-irrigated areas occurred during the 4–6 months of the transmission season [21], suggesting that sIPT is an appropriate preventive strategy for malaria control.

Unlike the use of other malaria control strategies such as use of insecticide-impregnated material that require daily implementation, this strategy requires only a twice-yearly treatment while offering significant protection against malaria. This simple strategy can be delivered through schools and/or local and national campaigns. Despite the current evidence and recommendation for use of insecticide-treated nets (ITN), this strategy remains largely under-utilized in Mali and many parts of Africa [22-24]. Increasingly, as efforts are being made to expand coverage of ITN it will be interesting to assess the efficacy of sIPT in children in the context of wide use of ITN.

The incidence rates of malaria disease (including severe malaria) reported in this randomized control trial were similar to those reported in previous studies in Mali [18,25]. This suggests an unbiased measure of the outcome despite the fact that the study was not a placebo controlled one, and that similar efficacy can be achieved by sIPTc in these areas.

There was no significant difference in SP efficacy *in vivo* between the treatment and control groups during the two periods of follow up using the WHO 2003 protocol for assessing the *in vivo* efficacy of antimalarials [11]. However there was a reduction in SP efficacy during the extended follow up period compared to the first year of follow up, especially among children less than five years of age. It is difficult to conclude if this reduction in SP in vivo efficacy was due to the sIPTc or was rather the

expected decrease in efficacy due to the use of SP for treatment of malaria episodes in this population. In this study, uncomplicated malaria cases in both arms were treated with SP (2nd line malaria treatment in Mali at the time of the study). While the higher efficacy and the longer prophylactic effect of SP compared to choloroquine (the first line treatment at the time of the study), provide more benefice for the study participants, this may contribute to the lack of difference in *in vivo* response between the two arms and the trend towards a lower efficacy during the extended follow up period.

Cisse *et al* [12] have found variation in frequency of molecular markers to SP resistance according to the season, without clear link with the intermittent preventive treatment. Results of studies undertaken by the IPTi consortium will bring more insight on the impact of IPT on drug resistance and whether this is an acceptable price to pay for the substantive benefit of this strategy and whether the combination with artesunate would be able to reduce the risk of increased resistance.

As shown in other studies [7,8,12] the cessation of the strategy was not associated with an increase of malaria disease during the subsequent transmission season. However in all these studies, IPT was given for a short time usually one year. It would be interesting to assess whether seasonal IPT in children given over longer periods impairs immunity to malaria.

In conclusion two malaria intermittent treatments targeting the peak of the transmission season have substantially reduced the incidence of clinical malaria in this area with intense seasonal transmission without a rebound effect after cessation. The strategy is simple and is likely to be very effective in reducing malaria burden in areas with seasonal malaria transmission.

Completing interests

The authors declare that they have no competing interests.

Authors' contributions

AD was the principal investigator of the study. He drafted the protocol, oversees the conduct of the study and contributed to the data analysis and interpretation. IS monitored the data quality of the study and contributed to the data analysis. MSS assisted with the implementation and coordination of the field activities. Data collection in the field data was done by OG, AID and MK. OBT assisted with the data management. MS contributed in overseeing the study. OKD contributed in the design of the study and in overseeing the data collection, analysis and interpretation. The manuscript was drafted by AD and all the authors contributed to revision and approved the final version.

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CHAPITRE II

Impact de la mise en œuvre du traitement préventif intermittent couplé à la vaccination du PEV sur la fréquence des mutations des gènes *dhfr* et *dhps*, marqueurs moléculaires de la résistance de *P. falciparum* à la Sulfadoxine pyrimethamine

2. Impact de la mise en oeuvre du traitement préventif intermittent couplé à la vaccination du PEV sur la fréquence des mutations des gènes dhfr et dhps, marqueurs moléculaires de la résistance de P. falciparum à la Sulfadoxine pyrimethamine

2.1 Position du problème

Sur la base des résultats des études d'efficacité du traitement préventif intermittent chez les enfants de moins de un an, l'UNICEF en collaboration avec le consortium IPTi (http://www.ipti-malaria.org/) a reçu un financement de la Fondation Bill & Melinda, pour faire des études de mise en œuvre pilote de la stratégie IPTn dans six pays en Afrique subsaharienne dont le Mali. Une des inquiétudes exprimées par les autorités sanitaires était que la mise en œuvre de la stratégie TPI à large échelle risquait d'entrainer une augmentation rapide et importante de la résistance des *Plasmodium* à la Sulfadoxine Pyrimethamine, compromettant ainsi son utilisation non seulement en TPIn mais aussi en traitement préventif intermittent chez les femmes enceintes (TPIp), une politique déjà en cours dans plusieurs pays en Afrique dont le Mali. Cette inquiétude était partagée par la communauté scientifique. En effet les groupes d'experts de l'OMS ainsi que ceux de l'Institut de Médicine des USA qui ont évalué l'ensemble des données disponibles en vue de recommander cette stratégie dans les politiques de santé, ont tous mis l'accent sur le besoin de données additionnelles concernant l'impact de la stratégie sur le développement de la résistance à la SP [1,2]. Il est connu que la résistance à la SP de développe rapidement et il n'y avait qu'une seule étude qui avait examiné l'impact du TPIn sur le développement la résistance à la SP. Cette étude avait été menée dans le cadre d'un essai randomisé et avait un nombre limité de sujets (n= 174). Il y avait donc un besoin urgent d'examiner

cette question et les études de mise en œuvre pilote offraient une excellente opportunité. L'objectif principal de cette partie de notre travail était d'évaluer l'impact de 12 mois de mise en œuvre de la stratégie TPIn sur la fréquence des marqueurs moléculaires de la résistance de *P. falciparum* à la SP. Nous avons modifié de schéma initial de l'étude pour permettre de répondre valablement à cette question. Nous avons ainsi, randomisé les aires de santé, formé le personnel, procédé au lancement, supervisé les activités de mise en œuvre et les enquêtes d'évaluation. Nous avons analysé les données et procédé à la rédaction du manuscrit dont nous sommes le premier auteur et l'auteur à qui les correspondances doivent être adressées. C'est le premier projet pilote de mise en œuvre à une échelle aussi large que nous avons conduit en tant que chercheur principal.

2.2 Principaux résultats

L'étude était réalisée dans le district de Kolokani dans la région de Koulikoro au Mali, un district qui couvre une superficie de 14.380 km2 et qui est divisé en 22 aires de santé. Les 22 aires de santé ont été randomisées suivant un ratio 1:1. Le TPIn a été mise en œuvre dans 11 aires de santé, les 11 autres aires servant de contrôle, pour l'évaluation de l'impact de la stratégie. Les fréquences des marqueurs moléculaires de la résistance ont été mesurées par des enquêtes transversales en début d'intervention et après 12 mois de mise en œuvre dans les deux zones, intervention et contrôle.

En enquête de base, nous avons examiné, 2.437 enfants dont 1.213 dans la zone d'intervention et 1.224 dans la zone contrôle. La proportion d'enfants porteurs de *P. falciparum* sur la goutte épaisse était de 47,2% en zone d'intervention et de 47,7% dans la zone contrôle (p = 0,82).

Les fréquences individuelles des mutations dhfr 51, 59 108 et dhps 437 et 540 étaient similaires entre les deux zones. Il en était de même pour les fréquences de la triple mutation dhfr (codons 51, 59 et 108, 36% en zone d'intervention contre 35% en zone contrôle, p = 0,81) et de la quadruple mutation (triple mutation dhfr + mutation dhps 437, 10,2% en zone d'intervention et 8,1% en zone contrôle, p = 0,47).

Après les 12 mois de mise nous avons fait une deuxième enquête transversale au cours de laquelle 2.453 enfants ont été examinés dont 1.227 dans la zone d'intervention et 1.226 dans la zone contrôle. Il y avait 73,3% des enfants dans la zone d'intervention et 71,5% dans la zone contrôle qui étaient porteurs de *P. falciparum*. Les fréquences individuelles des mutations (dhfr 51, 59 108 et dhps 437, 540) étaient restées similaires dans les deux zones. Il en était de même pour les fréquences de la triple mutation dhfr (codons 51, 59 et 108, 38,5% dans la zone d'intervention et 44,8% dans la zone contrôle) et de la quadruple mutation (triple mutation dhfr + mutation dhps 437, 11,2% en zone d'intervention et 11,6% en zone contrôle, p=0,90). Ces fréquences étaient aussi similaires entre les deux zones lorsque les données étaient analysées par catégories d'âge (2-24 mois et 25-60 mois).

Notre étude n'a donc pas mis en évidence d'impact notable de la mise en œuvre du TPI par SP sur les prévalences des marqueurs moléculaires de la résistance de *P. falciparum* à la SP.

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Molecular markers of resistance to sulphadoxine-pyrimethamine one year after implementation of intermittent preventive treatment of malaria in infants in Mali

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Molecular markers of resistance to sulphadoxine-pyrimethamine one year after implementation of intermittent preventive treatment of malaria in infants in Mali

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Abstract

Background: Intermittent preventive treatment in infants (IPTi) with sulphadoxine-pyrimethamine (SP) given during routine vaccinations is efficacious in preventing malaria disease and shows no interaction with the vaccines. However, there is a fear that IPTi may result in a rapid increase of parasite resistance to SP.

Methods: To evaluate the impact of IPTi on SP-resistance point mutations, the 22 health sub-districts in the district of Kolokani, Mali, were randomized in a 1:1 ratio and starting in December 2006, IPTi with SP was implemented in 11 health sub-districts (intervention zone), while the other 11 health sub-districts served as the control (non-intervention zone). Blood smears and blood dots on filter paper were obtained from children aged 0-5 years, randomly selected in each of heath sub-districts during two cross-sectional surveys. The first survey was conducted in May 2007 before the start of the transmission season to collect baseline prevalence of the molecular markers of resistance to SP and the second in December 2007 after the end of the transmission season and one year after implementation of IPTi. A total of 427 and 923 randomly selected blood samples from the first and second surveys respectively were analysed by PCR for *dhfr* and *dhps* mutations.

Results: Each of the three *dhfr* mutations at codons 51, 59 and 108 was present in 35% and 57% of the samples during the two surveys with no significant differences between the two zones. *Dhps* mutations at codons 437 and 540 were present respectively in about 20% and 1% of the children during the two surveys in both zones at similar proportion. The prevalence of quadruple mutants (triple *dhfr*-mutants + *dhps*-437G) associated with *in-vivo* resistance to SP in Mali after one year implementation of IPTi was also similar between the two zones (11.6% versus 11.2%, p = 0.90) and to those obtained at baseline survey (10.3% versus 8.1%).

Conclusion: This study shows no increase in the frequency of molecular markers of SP resistance in areas where IPTi with SP was implemented for one year.

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Background

Malaria remains a major cause of morbidity and mortality with an estimate of 881,000 deaths per year of which 91% occur in sub-Saharan Africa, mainly in infants and children under five years of age [1]. In the absence of a vaccine, simple and effective control strategies are urgently needed to reduce the malaria burden in sub-Saharan Africa. Several randomized controlled trials have demonstrated that Intermittent Preventive Treatment of malaria in infants (IPTi) with sulphadoxine-pyrimethamine (SP) given during routine vaccinations of the Expanded Programme of Immunization (EPI) at approximately two, three and nine months of age, result in a reduction of the incidence of clinical malaria by 22 to 59% [2-8], without showing interactions with EPI vaccines [2,4,9]. Although the efficacy and safety as well as the lack of interaction with EPI vaccines of IPTi in various endemic areas were well established [10-12], there have been concerns regarding the possibility that this strategy may cause an increase of *Plasmodium falciparum* resistance to SP, jeopardizing the use of SP not only for the prevention of malaria in infants, but also in pregnant women [13-15], an intervention widely adopted in many countries in sub-Saharan Africa. SP is known to rapidly select for mutant SP-resistant parasites [16-19], especially when given in prophylaxis, despite the reassuring predictions from mathematical modeling [13,20]. A report on the impact of IPTi with SP on the resistance of *P. falciparum* to SP in Mozambican children found no difference in the frequencies of *dhfr* and *dhps* mutations in children with a clinical malaria episode two months after the third dose of IPTi [21]. However, this study was conducted in the context of a placebo-controlled randomized trial with a limited number (n = 174) of samples. An evaluation in a context of large-scale implementation was needed to adequately address the impact of IPTi on SP drug resistance. This study evaluated the impact of IPTi on the selection SP-resistant *dhfr* and *dhps* mutations in *P. falciparum* after 12 months of implementation of IPTi in the district of Kolokani, Mali, where UNICEF pilot implementation of IPTi with SP was undertaken as part of a large operational research study in six African malaria endemic countries.

Methods

Study site

The study was conducted in the district of Kolokani, Mali. The district of Kolokani is an administrative subdivision of the region of Koulikoro. The town of Kolokani is located approximately 140 km north of Bamako. The district covers 14,380 km^2 and is divided into 22 health sub-districts. Each health sub-district is composed of several villages and has a health care center under the responsibility of a physician or chief-nurse and is staffed with at least two or three midwives and vaccinators. Three physicians ensure the coordination of health activities at the district level. The total population was 214,509 inhabitants. The children under one year of age represent about 4% of the total population. Malaria transmission is seasonal (July to November) with parasite prevalence in children 0-5 years of 45% at the end of the no-transmission season and above 70% at the end of the transmission season. First-line treatment of uncomplicated malaria was artesunate-amodiaquine or artemether-lumefantrine. Quinine was recommended for the treatment of severe malaria and SP was recommended prophylaxis for pregnant women.

Study participants and design

The study was a cluster-randomized trial. Twenty-two health sub-districts were randomized into two groups in a 1:1 ratio and IPTi was implemented in 11 health sub-districts (intervention zone) while the other 11 health sub-districts served as the control (non-intervention zone). The implementation of IPTi started in December 2006 and consisted of the administration of ½ tablet of SP alongside the DTP2, DTP3 and Measles routine immunizations. Afridoxine® (manufactured by Fourrts Laboratories Pvt. Ltd, Tamil Nadu, India), a breakable and dispersible form of SP was used after quality controls at the Laboratoire National de Santé in Bamako, Mali. Two cross-sectional surveys were performed. The first survey was performed in May 2007 a few months after the start of the IPTi-implementation, but before the start of the transmission season to collect baseline prevalence of the molecular markers of SP resistance. The second cross-sectional survey was conducted after one year of implementation in December 2007 after the end of the transmission season, to assess the impact of IPTi on the selection of SP-resistant *dhfr* and *dhps* mutations in *P. falciparum*. Children aged 0-5 years randomly selected in each of the health sub-districts were screened for malaria parasitaemia using thick smears obtained by finger pricking. Filter paper blood dots were also collected at the same time for the analysis of SP resistance molecular markers.

Laboratory methods

Thick smears were Giemsa-stained and examined by microscopy. Parasite density was determined by counting parasites against 300 leucocytes using a conversion factor of 25, based on an assumed leukocyte count of 7,500/μl. Filter papers samples were air-dried and stored at room temperature in labeled individual envelopes. Preliminary genotyping of one hundred samples demonstrated that samples with a parasitaemia less than 500 parasites/μl did not consistently yield PCR product.

Therefore, only filter paper samples from children with *P. falciparum* mono-infection parasitaemia greater or equal to 500 asexual stages per μl of blood were analysed by PCR for *dhfr* and *dhps* point mutations. Because of a higher than expected number *P. falciparum* mono-infection positive samples, 427 samples of the baseline survey and 923 samples of end of one year implementation survey were randomly selected and tested by PCR. Of the 427 baseline survey samples, 274 samples (150 in the intervention zone and 124 in the non-intervention zone) were successfully genotyped for all three *dhfr* and two *dhps* mutations, while the remaining were successfully tested for one, two, three or four mutations. Of the 923 randomly selected samples of end of one-year implementation survey, 742 samples (379 in the intervention zone and 363 in the non-intervention zone) were successfully genotyped for all three *dhfr* and two *dhps* mutations, and the remaining for one, two, three or four mutations.

A simple DNA extraction method for filter paper strips was used [22]. Briefly, approximately 1 × 2 mm piece of blood-soaked filter paper was placed in 50 μl of methanol for 15 min. The methanol was poured off and the paper heated at 95-100°C in 50 μl of water for 10 minutes. Five microliters of the resulting solution was used as PCR template for the first round of the Nested PCR. One microliter of the product of the first PCR was amplified in the second round of PCR. Samples that failed to yield PCR amplification with the methanol method were re-extracted using an alternative chelex-based method [23]. *Dhfr* and *dhps* genotypes were determined using nested mutation-specific PCR and/or PCR-RFLP according to published methods [16,23]. *Dhfr* mutations at codons 51, 59 and 108 and *dhps* mutations at codons 437 and 540 were analysed. One third of the PCR gels were examined by a senior scientist external to the study. The conformity of the gels with standard criteria for PCR gel validation and the fidelity of the transcription of lab notebook (source documents) with electronic files were checked. Error cases were corrected and PCR analyses repeated as needed.

Study endpoints

The primary endpoint was the frequency of quadruple mutant of molecular markers (*dhfr* 51, 59 and 108 and *dhps* 437) after one year of IPTi implementation. Secondary endpoints were the frequencies of single mutations as well as the triple mutants (*dhfr* 51 + 59 + 108) determined by PCR at the end of one year of implementation and frequencies of these individual and multiples *dhfr* and *dhps* mutations at baseline. The frequency of a particular mutant was calculated as the proportion of the specific mutant samples among the total number of samples successfully analysed for this mutation. Similarly, the frequencies of triple, quadruple and quintuple mutants were determined as the proportion of subjects with the three, four and five mutations among the total numbers of samples tested for the each.

Sample size

Assuming a proportion of the primary endpoint (quadruple mutants, *dhfr* 51, 59 and 108 and *dhps* 437) of 8% in control zone and 14% in IPTi intervention zone, one side p-value 0.025, a power of 80%, and inter class correlation using 0.01, a sample size of 380 children with positive parasitaemia in each group with a minimum of 22 subjects and average of 35 subjects per cluster were needed. Assuming a prevalence of *P. falciparum* parasitaemia of 35% and about 10% lost or unsuccessful samples, about 110 subjects were need to be screened per cluster resulting in a total of 2,420 children for the two arms.

Statistical analysis

Cases of mixed infection (wild type and mutant) were categorized as mutant throughout the analysis. Prevalence of single mutations as well as the triple mutant (*dhfr* 51 + 59 + 108) and quadruple mutant (triple mutant + *dhps* 437) genotypes were determined and compared between the IPTi implementation and control zones with 95% confidence intervals around the odds ratios adjusted for non-independence within health subdistricts using cluster option in Stata (version 9, Houston Texas USA). Proportions of categorical variables were compared using Pearson's Chi square test. Student t test was used for the comparison of mean ages and Mann-Whitney test for the comparison of parasite densities between the two zones.

Ethical considerations

Ethical clearance was obtained from the Ethical Committee of the Faculty of Medicine Pharmacy and Dentistry of the University of Bamako, Mali, and written informed consent from a parent or legal guardian was obtained prior to the enrollment of the child in the survey.

Results

Prevalence of *dhfr* and *dhps* mutations at baseline survey

A total of 2,437 subjects were screened (1,213 in the intervention zone and 1,224 in the non-intervention zone). Among these, 1,157 subjects (573 in the intervention zone and 584 in the non-intervention zone) had *P. falciparum* parasitaemia and 858 (429 in each zone) with parasitaemia equal or above 500/μl were selected. Characteristics of subjects enrolled are presented in Table 1. There were no significant differences between the two groups in terms of age, parasite prevalence, parasite density, and prevalence of parasitaemia equal or above 500/μl.

Prevalence of individual mutations, triple and quadruple mutations in intervention and non-intervention

Table 1 Characteristics of subjects enrolled in the IPTi intervention and non-intervention zone in Kolokani in May 2007.

	No IPTi	IPTi	p
Number screened	**1224**	**1213**	
Mean age (months)	34.1	32.9	0.15
Gender (Male) (%)	53.4	50.9	0.21
Parasite prevalence (%)	47.7	47.2	0.82
Median parasitaemia (per µl)	950	1275	0.44
Prevalence of parasitaemia ≥ 500/µl (%)	35.1	35.2	0.87

zones are presented in Table 2. Prevalence of individual *dhfr* and *dhps* mutations and triple *dhfr* mutation were similar between the intervention and non-intervention zones. The quadruple mutation was present in 9.2% of all the samples with similar frequency in the two zones (10.3% in the intervention zone and 8.1% in the non-intervention zone).

Prevalence of *dhfr* and *dhps* mutations after one year of IPTi implementation

A total of 2,453 subjects were screened (1,227 in the intervention zone and 1,226 in the non-intervention zone). Among these, 1,693 (855 in the intervention zone and 838 in the non-intervention zone) had *P. falciparum* parasitaemia and 1,490 (735 in the intervention zone and 755 in the non-intervention zone) had *P. falciparum* parasitaemia equal or above 500/µl. Characteristics of children sampled in the two zones are presented in Table 3. There were no significant differences between the two groups in terms of age, parasite prevalence, parasite density and prevalence of parasitaemia equal or above 500/µl.

Prevalence of individual *dhfr* and *dhps* mutations as well as multiple mutations (*dhfr* triple mutation and quadruple mutation (triple +*dhps* 437)) are presented in Table 4. There were no significant differences in the frequency of individual mutations between intervention and non-intervention zones. About half of the children harboured one of three *dhfr* mutations in both the intervention and non-intervention zones with a frequency of the triple *dhfr* mutation of 38.5% in the intervention zone compared to 44.8% in the control zone. About 20% of the children with parasitaemia in each zone were positive for parasites with the mutation 437 on *dhps* while only about 1% harboured parasites with the *dhps* 540 mutation. Similar patterns were found when the analysis was done by age categories for 2-24 months and 25-60 months.

The prevalence of the quadruple mutants was also similar between the two zones: 11.6% in the intervention zone versus 11.2% in the control zone, odd ratio = 1.04 (95% CI 0.51-2.12) p = 0.90 (Table 4). When the data were analysed by age categories, similar frequencies of quadruple mutants were found in the two zones in children aged 2-24 months; 9.9% (14/142) in the intervention group versus 8.2% (12/147) in the control group (p = 0.62). The corresponding figures in children aged 25-60 months were 12.9% (29/236) in the intervention group and 13.2% (29/219) in the control group (p = 0.76).

Three of the overall 742 samples (0.8%) successfully genotyped for all three *dhfr* and two *dhps*, harboured quintuple mutants; all three in the non-intervention zone, with one in the younger age group (less or equal to 24 months) and the other two in the older age group (25-60 months).

Discussion

This study showed no difference between intervention and non-intervention zones in the frequency of individual mutants in the *dhfr* and *dhps* genes, as well as in the triple *dhfr* and quadruple *dhfr dhps* mutants, suggesting that the implementation of IPTi with SP for one year does not result in an increase in the frequency of these molecular markers at the community level. This is consistent with the predictions using mathematical modelling that have indicated that IPTi is unlikely to appreciably shorten the useful life of the drug used [20],

Table 2 Prevalence of molecular markers associated with *P. falciparum* resistance to SP in IPTi intervention and non-intervention zone in Kolokani at baseline in May 2007.

	No IPTi		IPTi		p
Molecular Markers	**n**	**Prevalence**	**n**	**Prevalence**	
DHPS 437	333	20.1%	315	17.5%	0.39
DHPS 540	265	0.4%	313	1.6%	0.22
DHFR 51	209	35.4%	216	38.4%	0.52
DHFR 59	220	48.2%	206	47.6%	0.90
DHFR 108	223	43.0%	242	38.0%	0.27
Triple DHFR mutation	179	34.6%	184	35.8%	0.81
Quadruple mutation	161	8.1%	174	10.3%	0.47

Table 3 Characteristics of subjects enrolled in the IPTi intervention and non-intervention zone in Kolokani in December 2007 after one year of implementation.

	No IPTi	IPTi	p
Number screened	1226	1227	
Mean age (months)	32.9	33.0	0.96
Gender (Male) (%)	51.3	50.3	0.63
Parasite prevalence (%)	71.5	73.3	0.32
Median parasitaemia (per µl)	3325	3850	0.06
Prevalence of parasitaemia ≥ 500/µl (%)	62.7	64.8	0.31

or to significantly impact on the spread of highly resistant parasites in areas where partial resistance is already established [13] as it is the case in Mali and in many countries in sub-Saharan Africa. A small scale placebo-controlled randomized trial of IPTi with SP in Mozambican children [21] found no difference in the frequency of *dhfr* and *dhps* mutations during the episodes of clinical malaria that occurred two months after the last dose of IPTi. Another study [24] found a higher frequency of infections exhibiting four mutations in *dhfr* and *dhps* in children of nine months of age, after a single dose of SP compared to those in the control group, but no difference in the rate of infections with isolates with less than four mutations between the two groups. Other small scale placebo-control randomized studies of IPT in children (IPTc) during a highly seasonal malaria transmission season did not find differences in the frequency of *dhfr* and *dhps* mutations [25] or *in vivo* response to SP [26] between those who received IPT with SP versus those who did not. Similar frequency of the *dhfr* triple mutant among antennal care attendees of early gestation who had not received IPT and delivering women who had received IPT with SP in pregnancy (IPTp) was reported in a study in Ghana [18]. As estimated in mathematical models, the drug pressure resulting from IPTi may be relatively small [20] and there is no evidence that IPT will lead to increased resistance to SP that could jeopardize the use of the strategy in many countries in sub-Saharan Africa. However, this study has assessed the impact of IPTi on molecular markers of SP resistance after only one year of implementation, and continuous monitoring of resistance to SP (both molecular and *in vivo*) would be warranted once IPT is to be implemented as policy for longer periods of time. Possible limitations of this study include i) potential bias due to the difference in the spread of resistance between this study target population (children between 0-5 years of age) and the IPTi target population (children less than one year of age), however the sub-groups analysis did not shown any difference between the two zones in the children 2-24 months at the time of the second cross-sectional survey, targeted by the intervention and ii) the possibility of cross-contamination between intervention and non-intervention zones. However, the use of IPTi in non-intervention zones is unlikely to bias our results as only a very small proportion (less than 1%) of children 0-23 months had received IPTi in the non-intervention zone, and a high coverage of IPTi (>90% based on health records) was achieved in the intervention zone. Furthermore, the frequency of quadruple mutants at baseline was 10.3% compared to 11.6% at the end of first year of intervention and 8.1% compared to 11.2% in the control group. Rapid selection under drug pressure

Table 4 Prevalence of molecular markers associated with *P. falciparum* resistance to SP in IPTi intervention and non-intervention zone in Kolokani in December 2007 after one year of implementation.

	No IPTi		IPTi		OR	95% CI	p
Molecular Markers	**n**	**Prevalence (95% CI)**	**n**	**Prevalence (95% CI)**			
DHPS 437	432	19.4% (15.1 - 24.6)	479	21.7% (17.4-26.7)	1.15	0.59- 2.24	0.68
DHPS 540	428	0.9% (0.2-3.1)	474	1.0% (0.3-3.1)	1.13	0.16-7.99	0.90
DHFR 51	379	56.5% (50.1-62.6)	402	51.0% (44.9-57.1)	0.80	0.52-1.23	0.32
DHFR 59	376	55.3% (48.9-61.5)	402	51.0% (44.9-57.1)	0.84	0.50-1.40	0.51
DHFR 108	375	56.8% (50.4-63.0)	392	47.7% (41.5-53.9)	0.69	0.47-1.03	0.07
Triple DHFR mutation (DHFR 51, 59 and 108)	368	44.8% (38.5-51.3)	384	38.5% (32.6-44.8)	0.77	0.50-1.19	0.24
Quadruple mutation (DHFR 51, 59 and 108 + DHPS 437)	367	11.2% (7.6-16.0)	379	11.6% (8.1-16.4)	1.04	0.51-2.12	0.90

of pre-existent resistant strains at the community level has been reported [27]. However, in the absence of structured *P. falciparum* populations, the flow of parasites between areas that are very close could have diluted *P. falciparum* populations that were selected under SP pressure in the intervention zones by unselected *P. falciparum* populations coming from non-intervention zones [28]. The use of threshold parasitaemia equal or above 500/μl is unlikely to bias our results since the prevalence of parasitaemia above this threshold was similar between the two zones, and it is unlikely that mutants parasite were more prevalent below this threshold in one zone compared to other.

In line with other studies in Mali and countries in West Africa [29-32], this study shows relatively high levels of individual *dhfr* mutations in the study area with half of the parasitemic children harbouring mutant genotypes and a prevalence of triple mutations of 44.5%. This may reflect the long history of usage of sulpha drugs including SP across the countries. Although the *dhfr* triple mutation was prevalent in 34.6 to 44.8% of infections, it is well established that this genotype does not correlate with *in vivo* SP failure in West Africa [31-33]. The quadruple mutation genotype most associated with *in vivo* SP failure in Mali (our unpublished data) and in Ghana [33] was similar between the two zones and present in about 11% of infections in overall at the end of one year of implementation. This is also consistent with rates for this genotype found by us in various parts of Mali and by others in the West African sub-region [31,34,35]. Similar to other reports from the sub-region, the *dhps* 540E was very rare in this study [31,33]. However, here are reported the first cases of *dhps* 540E found in Mali.

In conclusion, this study shows low prevalence of quadruple mutants (triple *dhfr* mutants + *dhps* 437G) associated with *in vivo* resistance to SP in Mali and no evidence of increased frequency of molecular markers of SP resistance in areas where IPTi was implemented for one year.

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Authors' contributions

AD was the principal investigator of the study. He contributed to the study design, oversaw the study conduct, the data collection and contributed to the data analysis and interpretation. IS contributed to the study design and conducted the data collection. AAD contributed to the study design and oversaw the molecular analysis of the samples and interpretation of the data. Data collection in the field was done by ST, MT, SD, AID, AB, MD. OMC contributed to the study conduct and data collection. CR contributed to the study design and data analysis. AS contributed to the study design. OKD contributed to the design of the study and overseeing the data collection, analysis and interpretation. The manuscript was drafted by AD and all the authors contributed to revision and approved the final version.

Competing interests

The authors declare that they have no competing interests.

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CHAPITRE III

Impact de la mise en œuvre du traitement préventif intermittent couplé à la vaccination du PEV sur la couverture des vaccins du PEV

3. Impact de la mise en œuvre du traitement préventif intermittent couplé à la vaccination du PEV sur la couverture des vaccins du PEV

3.1 Position du problème

Le programme élargi de vaccination est déjà utilisé pour délivrer des interventions additionnelles telles que la supplémentation en Vitamine A et la distribution des MII pour l'amélioration de la survie de l'enfant. Le traitement préventif intermittent chez les enfants de moins un an qui consiste à administrer une dose curative de SP à l' âge de 3, 4 et 9 mois au moment de la prise de la deuxième et la troisième dose de DPTP et du vaccin contre la rougeole, est une addition logique au PEV puisque le paludisme constitue la première cause de morbidité et de mortalité chez les enfants dans la plupart des pays d'endémie palustre en Afrique sub-saharienne. Cependant l'addition continue des interventions au paquet du PEV pourra augmenter la charge et le temps de travail des agents de santé et entrainer une réduction de la couverture des vaccins PEV. Ceci est encore plus à craindre dans des pays comme le Mali ou le nombre d'agents de santé est en dessous des normes souhaitées et la couverture vaccinale reste en dessous de 50% et donc loin des 80% souhaités [1,2]. Cependant l'addition du TPI au paquet PEV peut aussi augmenter la couverture vaccinale en améliorant la participation des populations si ces dernières comprennent le bénéfice (notamment immédiat) qu'elles peuvent tirer de l'intervention. Une évaluation de l'impact de la stratégie sur la couverture vaccinale s'imposait donc avant une mise en œuvre du TPI dans la politique sanitaire du Mali. L'objectif principal de cette partie de notre travail était d'évaluer l'impact de 12 mois de mise en œuvre de la stratégie TPIn sur la couverture des vaccins du PEV.

Nous avons modifié de schéma initial de l'étude pour permettre de répondre mieux à cette question un choisissant un schéma de randomisation par grappe au lieu d'une évaluation avant et après l'intervention, comme initialement envisagé. Nous avons donc randomisé les aires de santé, formé le personnel, procédé au lancement, supervisé les activités de mise en œuvre et les enquêtes d'évaluation. Nous avons analysé les données et procédé à la rédaction et à la soumission du manuscrit le 30 Juin 2010 à *BMC Public Heath.* Nous sommes le premier auteur de cet article et l'auteur à qui les correspondances doivent être adressées.

3.2 Principaux résultats

Cette étude a été réalisée dans le district de Kolokani au Mali. Comme indiqué en chapitre II, les 22 aires de santé du district ont été randomisées suivant le ratio 1:1, le TPIn a été mise en œuvre dans 11 aires de santé, et les 11 autres aires ont servi de contrôle pour l'évaluation de l'impact de la stratégie sur la couverture vaccinale. La couverture des vaccins du PEV et des autres interventions associées au PEV a été mesurée avant la mise en œuvre du TPI et après 12 mois de mise en œuvre, dans les zones d'intervention et de non intervention.

Au total 1.050 enfants de 0-23 mois ont été vus en enquête de base avant la mise en œuvre de la stratégie dont 71,2% avaient leur carte de vaccination au moment de l'enquête. Comme attendu, les couvertures des vaccins du PEV et autres interventions associées au PEV étaient similaire dans les deux zones au moment de cette enquête de base. La proportion des enfants de 9-23 mois complètement vaccinés dans le district était 36,7% (IC 95% 25,3% -48,0%).

L'enquête après les 12 mois de mise en œuvre du TPIn a porté sur 2.106 enfants de 0-23 mois dont 1.051 dans la zone d'intervention et 1.055 dans la zone de non-intervention. Les proportions des enfants qui avaient leur carte de vaccination au moment de l'enquête étaient similaires dans les deux zones (78,0% contre 80,1% ; p = 0,65). Dans la zone d'intervention, à l'exception du vaccin contre l'hépatite B, la couverture de tous les autres vaccins a augmenté significativement en comparaison à celle de l'enquête de base. Même dans la zone de non intervention on a noté une augmentation significative de la couverture de certains des vaccins : la proportion des enfants âgés de 9-23 mois complètement vaccinés est passée de 33,3% (IC 95% 16,2% - 50,4%) pendant l'enquête de base à 53,8% (IC 95% 43,4% - 64,1%) après la période d'intervention. Cette augmentation est encore plus importante dans la zone d'intervention où la proportion des enfants complètement vaccinés est passée de 39,6% (IC 95% CI 24,8% - 54,3%) au cours de l'enquête de base à 69,5% (IC 95% 64,2% - 74,7%) après les 12 mois d'intervention.

Les proportions des enfants de la classe d'âge cible ayant reçu le TPIn associé à chacune des trois vaccinations DTCP2, DTCP3 et vaccin contre la rougeole étaient de 89,2% (IC 95% 85,9%-92,0%), 91,0% (IC 95% 87,6% -93,7%) et 77,4% (IC 95% 70,7% - 83,2%), respectivement. Dans les zones de non-interventions ces proportions étaient inferieures à 3%.

Dans cette étude pilote menée en milieu rural au Mali, la mise en œuvre du TPIn couplé au PEV a significativement amélioré le taux de couverture de ce dernier. L'adjonction d'une intervention comme le TPIn au PEV peut donc avoir un effet bénéfique sur la couverture vaccinale.

3.3 Références

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2. Enquête Démographique et de Santé (EDSM-IV), Mali http://www.malariafreefuture.org/projects/mali/docs/dhs.pdf (consulté le 23.10.2010).

3.4 Article Dicko et al. under review *BMC Public Health*

Impact of the implementation of malaria intermittent preventive treatment with Sulfadoxine –pyrimethamine (IPTi-SP) in EPI vaccine coverage in the district of Kolokani, Mali: A cluster randomized control trial. *under review BMC Public Health*

TITLE

Impact of the implementation of malaria intermittent preventive treatment with Sulfadoxine – pyrimethamine (IPTi-SP) in EPI vaccine coverage in the district of Kolokani, Mali: A cluster randomized control trial.

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ABSTRACT

Background

Even though the efficacy of Intermittent Preventive Treatment in infants (IPTi) with Sulfadoxine-Pyrimethamine (SP) against clinical disease and the absence of its interaction with routine vaccines of the Expanded Immunization Programme (EPI) have been established, there are still some concerns regarding the addition of IPTi, which may increase the work burden and disrupt the routine EPI services especially in Africa where the target immunization coverage remains to be met. However IPTi may also increase the adherence of the community to EPI services and improve EPI coverage, once the benefice of strategy is perceived.

Methods

To evaluate the impact of IPTi on the coverage of routine immunizations, 22 health areas of the district of Kolokani were randomized at a 1:1 ratio to either receive IPTi-SP or to serve as a control. The EPI vaccines coverage was assessed using cross-sectional surveys at baseline in November 2006 and after one year of IPTi pilot-implementation in December 2007.

Results

At baseline, the proportion of children of 9-23 months who were completely vaccinated (defined as children who received BGG, 3 doses of DTP/Polio, measles and yellow fever vaccines) was 36.7% (95% CI 25.3% -48.0%). After one year of implementation of IPTi-SP using routine health services, the proportion of children completely vaccinated rose to 53.8% in the non-intervention zone and 69.5% in the IPTi intervention zone ($p < 0.01$).

The proportion of children in the target age groups who received IPTi with each of the 3 vaccinations DTP2, DTP3 and Measles, were 89.2% (95% CI 85.9%-92.0%), 91.0% (95% CI 87.6% -93.7%) and 77.4% (95% CI 70.7%-83.2%) respectively. The corresponding figures in non-intervention zone were 2.3% (95% CI 0.9% -4.7%), 2.6% (95%CI 1.0% -5.6%) and 1.7% (95% CI 0.4% - 4.9%)

Conclusion

This study shows that high coverage of the IPTi can be obtained when the strategy is implemented using routine health services and implementation results in a significant increase in coverage of EPI vaccines in the district of Kolokani, Mali.

Keys words: malaria, intermittent preventive treatment, sulphadoxine-yrimethamine, expanded program of immunization.

Trial Registration number: ClinicalTrials.gov NCT00766662

INTRODUCTION

Malaria is one of biggest killer of infants and children in sub-Saharan Africa. In the absence of a vaccine against malaria, the intermittent preventive treatment of malaria in infant (IPTi) has been developed to reduce the burden of malaria in infants. IPTi consists on the administration of a curative dose of antimalarials at the time of EPI vaccination during the first year of life regardless of the presence of symptoms or infection [1]. Several randomized control studies of IPTi with Sulfadoxine-pyrimethamine (SP) in different parts of Africa have shown that the strategy is efficacious in preventing clinical episodes of malaria, severe anemia and hospital admission [2-8]. It has also been established that the administration of SP at the time of EPI vaccines shows no negative interaction with these vaccines [2,4,9] and no safety issue was found [10,11]. A consortium was established in 2003 to generate scientific evidence required to inform and speed up the process of going from strategy into policy [1]. Even though the efficacy, the absence of interaction with EPI vaccines and safety of IPTi are well established, there are still concerns that addition of IPTi will result in an increase in the work burden and disrupt the routines EPI services especially in Africa where the target EPI vaccines coverage remains to be met. However IPTi may also increase the adherence of the community to EPI services and improve EPI coverage, once the benefit of the strategy is perceived [12, 13].

Mali has one of the highest infant morality rate in the world estimated at 119 per 1000 (http://www.unicef.org/french/infobycountry/mali_statistics.html) along with a high malaria burden. Malaria is reported by the health centers as a primary cause of the consultations and death both from routine health reports [14] as well as carefully designed cohort studies [15, 16]. Proportion of children completely vaccinated with EPI vaccines in Mali remained below 50% according to health surveys in 2001 and 2006 [17, 18]. This study aimed to evaluate the impact of the IPTi-SP implementation on the coverage of EPI vaccines after one year of intervention in a context of a large pilot implementation of health services in the district of Kolokani, Mali.

METHODS

Study site

The study was conducted in the district of Kolokani, Mali. The district of Kolokani is an administrative subdivision of the region of Koulikoro, in Mali. The town of Kolokani is located at about 140 km north of Bamako. The district covers 14,380 km^2, divided into 22 health subs districts. Each health sub-district is composed of several villages. The total population was 208,317 inhabitants with children under 1 year representing about 4% of the total population. Malaria is hyperendemic in the region with parasite prevalence in children under 5 years of 45% during the dry season and above 70% during the rainy season [19]. Heath services are provided in the district through 6 physicians, 2 midwifes, 17 nurses 19 matrones and 18 vaccinators.

Study Participants and Design

The study was a cluster-randomized trial. The 22 health areas (sub-districts) were randomized in a 1:1 ratio with the intervention in 11 health areas and the other 11 serving as controls for the assessment of the impact of IPTi implementation on EPI vaccines and other health interventions coverage as well as its impact on the resistance to SP. The intervention consisted on the administration to infants of ½ tablet of SP along with EPI vaccines (DTP2, DTP3 and Measles/Yellow fever vaccine) from December 2006 to December 2007. Two cross-sectional surveys were performed, one at baseline in November 2006 prior to the beginning of IPTi implementation and another in December 2007 after one year of IPTi implementation. Thirty clusters were selected using a random sampling with probability proportional to the size of the population size. In each cluster (location), a sample of about 35 children in November 2006 and about 70 children in December 2007 aged 0-23 months were randomly selected and surveyed using the WHO method of evaluation of vaccine coverage [20]. An interviewer-administered questionnaire was used to obtain data using modified UNICEF multiple indicators cluster surveys questionnaire (http://www.childinfo.org/mics2_questionnaire.html). Parents or guardians of children aged 0-23 months in selected households were questioned about the EPI vaccines and other health interventions. Information on EPI vaccination cards was recorded for

each child and in its absence, questions regarding the EPI vaccines were asked and responses recorded. The interviews were conducted in local language and by two interviewers in each household. Main indicators included child immunizations and IPTi coverage, use of insecticide-impregnated bed net (ITN) during the previous night, and child receiving vitamin A supplementation. Filled questionnaires were verified at the end of each day by a supervisor and corrected if necessary.

Sample size

Sample size for the baseline survey was estimated using the following assumptions. Based on a precision of 6% and alpha error of 5% and DTP3 coverage of two thirds (67%), a sample of 472 children was selected using a cluster effect of 2. This sample size was doubled to take into account analysis for specific age categories and increased by 10% to take into account missing information, making a total sample size of 1,050 children aged 0-23 months.

During the second survey after one year of implementation, the same number of 1,050 children was sampled in each zone (intervention and non intervention) to allow estimates of vaccine coverage in each zone and comparisons between them.

Statistical analysis

Data were double entered and reconciled using Epi Info version 6 (CDC Atlanta). The cleaned database was exported to Stata 9 (Houston Texas, USA) for the analysis. Vaccine coverage based on the information on the vaccination card was computed as the proportion of children in target age groups who received a particular dose of the vaccine among those with vaccination card. Coverage based on vaccination card and interview was the proportion of children who receive the vaccine based on the information on the card or the vaccination history provided by mothers or guardians among the total number of children surveyed in the target age group. Target age groups were defined as age ranges starting from the age at which the vaccine is given according to EPI vaccination schedule up to 23 months of age (i.e. 0-23 months for BGG and Polio0, 2-23 months for Polio1, DTP1, HB1, 3-23 months for Polio2, DTP2, HB3, 4-23 months for Polio3, DTP3, HB3, and 9-23 months for measles and yellow fever vaccines). Children of 9-23 months were considered completely vaccinated if they had had BCG, at least 3 doses of DTP, Polio, measles and yellow fever vaccines.

Impact of IPTi implementation on EPI and other health intervention's coverage was assessed by comparing the coverage of each vaccine dose in intervention and non intervention zones using information on vaccination cards and/or interviews, before and after one year of IPTi pilot implementation with 95% confidence intervals adjusted for cluster design using cluster option in Stata (version 9, Houston Texas USA).

Proportions of children who received IPTi relative to the three EPI vaccinations (DTP2, DTP3, and measles vaccines) were computed as the number of children who had documented doses 1, 2 or 3 of IPTi, divided by the number of children with documented DTP2, DTP3, and measles vaccines respectively. Coverage of IPTi1 and IPTi2 were computed for children aged 4-15 months and coverage of IPTi3 for those aged 9-15 months. Coverage of ITN was defined as the number of children who slept under ITN the night before divided by the number of children surveyed, and coverage of vitamin A supplementation was defined as the number of children 6 -23 months who received a vitamin A supplementation divided by the number of children of this age group surveyed.

Ethical considerations

Ethical clearance was obtained from the Ethical Committee of the Faculty of Medicine Pharmacy and Dentistry of the University of Bamako, Mali, and informed consent from a parent or legal guardian was obtained prior to the enrollment of the child in the survey.

RESULTS

Results of baseline survey

A total of 1050 children were surveyed, 51.9% were male. The proportion of children with EPI vaccination cards was 71.2% (95% CI 64.5% - 77.9%) in Kolokani. The coverage of vaccines, vitamin A and ITN usage based on the information on the vaccination card, (with and without information from interviews) is presented in Table 1. There were no significant differences between the intervention and non intervention regarding coverage of vaccines and other intervention at baseline. Based on the vaccination card, the EPI vaccines coverage varied from 46.2% (95% CI 34.0% - 58.4%) for yellow fever vaccine to 82.4% (95% CI 76.1% - 88.7%) for DTP1. Similar figures were found for BCG, measles and

yellow fever vaccines coverage, when the information from interviews were used in addition to those in vaccination card. Based on information from interviews and vaccination cards, the proportion of children 4 -23 months who received 3 doses of DTP/Polio was 54.4% (95% CI.43.3% - 65.5%) and for children aged 6 months and above with Vitamin A supplementation it was 74.4% (95% CI 67.3% - 82.5%). Sleeping under ITN was reported for 49.7% (95% CI 39.4% - 59.6%) of children aged 0-23 months. The proportion of children 9-23 months who were completely vaccinated (defined as children who received BGG, 3 doses of DTP/Polio, measles and yellow fever vaccines) was 36.7% (95% CI 25.3% - 48.0%).

Impact of IPTi implementation on EPI vaccines and other health interventions' coverage

A total of 1051 children were surveyed in the intervention and 1055 in the control zone. About 50.7% in the intervention zone were male and 52.8% in the control zone (p = 0.34). The proportion of children with EPI vaccination cards was similar between intervention and control areas; 78.0% (95% CI 69.4% -86.6%) versus. 80.1% (75.6% - 84.6%), p = 0.65). The coverage of vaccines and other health interventions based on the information on the vaccination card, with and without information from interviews in pre and post-intervention per zone are presented in Table 2**.** In the intervention zone, with the exception of Hepatitis B vaccines the coverage of the EPI vaccines given with IPTi increased significantly in post-intervention compared to baseline. Even in the non-intervention zone, there was also a significant increase in coverage of polio, measles and yellow fever vaccines in post-intervention compared to baseline. The proportion of children completely vaccinated increased significantly from 33.3% (95% CI 16.2%- 50.4%) at baseline to 53.8% (95% CI 43.4% - 64.1%) in post intervention in the non intervention zone. The increase was even higher in the intervention zone from 39.6% (95% CI 24.8% - 54.3%) at baseline to 69.5% (95% CI 64.2% - 74.7%) in post intervention.

Coverage of IPTi

The proportion of children in the target age groups who received IPTi with each of the 3 vaccinations DTP2, DTP3 and Measles, were 89.2% (95% CI 85.9%-92.0%), 91.0% (95% CI 87.6% -93.7%) and 77.4% (95% CI 70.7%-83.2%) respectively. The corresponding figures in non-intervention zone were 2.3% (95% CI 0.9% -4.7%), 2.6% (95%CI 1.0% -5.6%) and 1.7% (95% CI 0.4% - 4.9%).

DISCUSSIONS

This study showed a significant increase in the proportion of children completely vaccinated in post-intervention compared to baseline in both intervention and non-intervention zones. The increase was more pronounced in the intervention zone (29.9%) compared to the non-intervention zone (20.5%). In the intervention zone the increase in vaccine coverage occurred for all the vaccines given with IPTi with the exception of Hepatitis B vaccine for which several shortages were documented in the district during the implementation period as well as for the other health interventions (vitamin A supplementation and use of ITN). In the non-intervention zone, a significant increase was noted for Polio, measles and yellow fever vaccines, but not for DTP and Hepatitis B vaccines. Possible reasons for this higher coverage in the IPTi intervention zone include more adhesion of the community because of the high acceptability of the intervention, the additional motivation of the health workers and increase in supervision of EPI activities due to the introduction of IPTi-SP.

Mothers' knowledge of immunization or other interventions is known to be positively correlated with full immunization rates [17, 18, 21] and it is possible that the adhesion of the community through information and sensitization was responsible for this increase in EPI vaccines coverage compared to baseline levels for all the vaccines and for the fact that this increase was more marked in the intervention zone compared to non-intervention zone. Acceptability surveys conducted in Mali (our unpublished data) and other countries in sub-saharan Africa [13] have found that mothers like IPTi and come more to health centers because i) they are concern with malaria; ii) they think it is an anti-pyretic (and post-vaccinal fever is one of the major bottlenecks of EPI coverage) and iii) it is free. Even in the context of maternal illiteracy, educating mothers about the vaccines and vaccine-preventable diseases was reported to be highly effective in increasing the immunization coverage [22]. Positive mother's perceptions about IPTi have been reported in previous studies [3, 23].

Because in many countries in sub-Saharan Africa including Mali there is a critical shortage of health service providers [24]**,** the concern that addition of IPTi-SP to the vaccination package could result in a reduction in the coverage of the EPI vaccines and other interventions already delivered through EPI was legitimate. However, there is an insufficient use of the staff time and efforts in Mali so that

addition of IPTi to the current EPI package is possible without significant uptake of EPI services as reported in Ghana [3].

As indicated in a previous study, the strategy is well accepted by the health staff and the time used for IPTi implementation is acceptable, with a median time of 12.4 min ranging from 1.6-28.9 minutes per nurse per vaccination clinic [25] and representing about 11% of health workers' time [26] which is compatible with the current health staff and activities in Mali.

The relatively closer and more regular supervision, especially at the beginning of the intervention, may have also contributed to increased staff adhesion and motivation that could have resulted in an increase in EPI vaccine coverage compared to baseline.

In line with other reports [17, 18, 27] the proportion of children aged 9-23 months completely vaccinated was only 36.7% in November 2006. However this proportion increased significantly to 53.8% in non intervention and 69.5% in the intervention zone after one year of IPTi implementation, indicating an improvement of vaccine coverage in the district and suggesting that i) low vaccination coverage should not be criteria preventing the introduction of IPTi into existent health systems; ii) IPTi can be easily added to the routine immunizations of EPI without impairing its coverage but on the contrary with the possibility of boosting it; iii) at a coverage twice as high of immunizations and IPTi the current health systems can efficiently deliver these services; and iv) cost and effectiveness studies [28, 29] and assessment of the role of IPTi-SP in infants [30-32] should consider a positive interaction between IPTi and EPI vaccine coverage.

It is possible that the staff adhesion and the enthusiasm toward this new intervention have resulted in the observed increase of EPI vaccination coverage in the control zone compared to baseline. Although there is a trend toward improvement of EPI vaccine coverage in Mali [18] with a change from 29% in 2001 to 48% five years after, the increase from 36.7% to 53.8 % in the non-intervention zone in one year is likely due to IPTi implementation in other parts of the district. The proportion of children completely vaccinated after the IPTi implementation in the intervention zone (69.5%) was consistent with a previous report in the district of Kita in Mali, after three years of intense EPI vaccination priority program [33].

To the best of our knowledge, this is the first study to assess the impact of IPTi-SP on EPI vaccine coverage using a cluster-randomized design. Additional studies undertaken as part of the UNICEF pilot

implementation of IPTi-SP using pre-and post-implementation surveys, although with more potential for bias, will provide further insight on the impact of the strategy on the EPI vaccines coverage. If the increase in EPI coverage is confirmed in other settings, along with the positive recommendations of the Institute of Medicine [30] and recent the WHO's recommendations [34], decision-makers in sub-Saharan Africa should consider rapid adoption and implementation of the strategy.

As expected, the coverage of EPI vaccines was similar when assessed among subjects who had vaccination cards and when interview data for those who did not had their vaccination card were added. Other studies have reported a high concordance between vaccination coverage obtained by vaccination cards and interviews with parents [21, 35].

The use of ITN remained similar between the two zones and compared to baseline, suggesting that the IPTi implementation did not lower people's attitudes on malaria prevention.

Limitations of this study include the lack of control intervention and blinding, and the fact that the strategy was implemented for only one year. In addition, because of the natural of the deployment, one cannot control for "implementation slips" generated by the lack of borders of community sensitization. Like with any new intervention of its kind, continuous monitoring of its impact on the EPI vaccine coverage will be needed along with the implementation of the strategy over longer period of time.

CONCLUSIONS

In summary, this study shows that high coverage of IPTi can be obtained using routine health services and IPTi-SP implementation resulted in a significant increase in coverage of EPI vaccines in the district of Kolokani, Mali, suggesting that the cost-effectiveness of the intervention may be higher than reported and that low coverage of the EPI vaccine should not prelude the implementation of IPTi-SP.

LIST OF ABREVIATIONS

BGG: Bacillus Calmette-Guérin vaccine

DTP: Diphtheria-Tetanus-Pertussis vaccine

DTP1: First dose of Diphtheria-Tetanus-Pertussis vaccine

DTP2: Second dose of Diphtheria-Tetanus-Pertussis vaccine

DTP3: Third dose of Diphtheria-Tetanus-Pertussis vaccine

EPI: Expanded Immunization Programme

HB: Hepatitis B vaccine

HB1: First dose of Hepatitis B vaccine

HB2: Second dose of Hepatitis B vaccine

HB3: Third dose of Hepatitis B vaccine

IPTi: Intermittent Preventive Treatment in infants

ITN: Insecticide-impregnated bed net

Polio: Poliomyelitis vaccine

Polio1: First dose of Poliomyelitis vaccine

Polio2: Second dose of Poliomyelitis vaccine

Polio3: Third dose of Poliomyelitis vaccine

SP: Sulfadoxine-Pyrimethamine

UNICEF: United Nations of International Children's Emergency Fund

WHO: World Health Organization

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AUTHORS' CONTRIBUTION

AD was the principal investigator of the study. He contributed to the study design, oversaw the study conduct, the data collection and contributed to the data analysis and interpretation. IS, MSS, ATD, AS contributed to the study design and in overseeing the IPTi implementation. Data were collected by or under the supervision of ST and MT. CR, RS and OKD contributed to the study design and data analysis and interpretation. Data Management and quality control was performed under supervision of OT.

The manuscript was drafted by AD and all the authors contributed to revision and approved the final version.

CONFLICT OF INTEREST

None declared.

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Table 1: Vaccines and other health interventions coverage at baseline based on the information on the vaccination card from interview in Kolokani in November 2006.

	No IPTi		IPTi			Overall	
Vaccines	**n**	**Coverage**	**n**	**Coverage**	**p**	**n**	**Coverage**
Based on information on vaccination card							
BCG	346	66.2% (51.1-81.2)	402	73.4% (62.9-83.9)	0.42	748	70.1% (60.9 – 79.2)
POLIO 0	346	50.9% (37.7- 64.0)	402	57.5% (42.9-72.0)	0.50	748	54.4% (44.4 – 64.4)
POLIO 1	332	76.8% (65.4-88.2)	368	70.4% (57.2- 83.6)	0.45	700	73.4% (64.5 – 82.3)
POLIO 2	311	64.0% (49.5– 78.5)	344	65.4 (51.9 -78.9)	0.88	655	64.5% (54.8 – 74.6)
POLIO 3	289	53.6% (34.8– 72.4)	330	59.7% (45.6-73.8)	0.60	619	56.9% (45.2 – 68.5)
DTP1	332	83.7% (76.4-91.1)	368	81.3% (71.3-91.1)	0.68	700	82.4% (76.1 – 88.7)
DTP2	311	66.9% (54.3-79.5)	344	74.4% (61.8-87.1)	0.40	655	70.8% (61.8 – 79.9)
DTP3	289	57.1% (39.8-74.4)	330	67.9% 54.2-81.5)	0.32	619	62.8% (51.7 – 73.9)
HB1	332	78.9% (68.8-89.0)	368	78.3% (67.4-89.1)	0.93	700	78.6% (71.1 – 86.0)
HB2	311	65.3% (53.5-77.0)	344	72.1% (59.0-85.2)	0.44	655	68.9% (59.9 – 77.8)
HB3	289	54.3% (37.6-62.4)	330	65.4% (51.6-79.3)	0.30	619	60.3% (49.3 – 71.2)
Measles	212	47.6% (31.1-64.2)	249	56.2% (40.1 -73.4)	0.45	461	52.3% (40.5 – 64.0)
Yellow Fever	212	42.5% (24.0-60.9)	249	49.4% (33.5-65.3)	0.57	461	46.2% (34.0 – 58.4)
Using information on vaccination card and interview							
BCG	410	67.3% (53.7-80.9)	457	74.2% (65.0-83.4)	0.39	867	70.9% (62.7 – 79.1)
3 doses of DTP	332	55.7% (38.3-73.1)	377	66.6% (54.3-78.8)	0.34	709	61.5 (50.8 – 72.2)
3 doses de Polio	340	51.8% (42.0-74.3)	375	59.2% (55.4-79.6)	0.50	715	55.7% (44.6- 66.7)
3 doses of DTP/Polio	331	51.7% (33.7-69.6)	373	56.8% (43.5-70.2)	0.47	704	54.4% (43.3 – 65.5
Measles	263	51.0% (35.1-66.9)	297	58.6% (44.9 –72.3)	0.46	560	55.0% (44.4 – 65.6)
Yellow Fever	263	46.8% (29.1-64.4)	296	52.7% (39.9-66.5)	0.59	599	49.9% (38.7 – 61.1)
Completely vaccinated	249	33.3% (16.2-50.4)	288	39.6% (24.8 – 54.3)	0.58	537	36.7% (25.3 - 48.0)
Vitamin A supl.	347	69.2% (57.5-80.7)	373	79.4 (70.2-88.6)	0.17	720	74.4% (67.3 – 82.5)
Use of ITN	523	44.2% (27.5 – 60.8)	516	55.2% (45.3-65.2)	0.26	1039	49.7% (39.4 – 59.6)

Tableau 2: Coverage of EPI vaccines given with IPTi and other health interventions based on the information on vaccination card and from interview in pre and post-intervention in intervention and non intervention **zone in Kolokani**

	No IPTi					IPTi				
	Pre-intervention		Post-intervention		Δ	Pre-intervention		Post-intervention		Δ
Vaccines	n	Coverage	n	Coverage		n	Coverage	n	Coverage	
Based on information on vaccination card										
POLIO 2[§¥]	311	64.0% (49.5– 78.5)	720	81.1% (73.8 – 88.4)	17.1%	344	65.4 (51.9 -78.9)	740	91.4% (87.0– 95.7)	26.0%
POLIO 3[§¥]	289	53.6% (34.8– 72.4)	671	67.2% (56.4 – 78.0)	13.6%	330	59.7% (45.6-73.8)	692	83.4% (77.4 – 89.3)	23.7%
DTP2[¥]	311	66.9% (54.3-79.5)	720	75.1% (65.8 – 84.4)	8.2%	344	74.4% (61.8-87.1)	740	90.4% (86.0 – 94.8)	16.0%
DTP3[¥]	289	57.1% (39.8-74.4)	671	62.0% (50.2 -73.8)	4.9%	330	67.9% 54.2-81.5)	692	82.7% (77.4 – 87.9)	14.8%
HB2	311	65.3% (53.5-77.0)	720	72.9% (63.0 – 82.9)	7.6%	344	72.1% (59.0-85.2)	740	74.3% (61.3 – 87.4)	2.2%
HB3	289	54.3% (37.6-62.4)	671	60.7% (49.2 – 72.1)	6.4%	330	65.4% (51.6-79.3)	692	70.2% (58.1 – 82.4	4.8%
Measles[§¥]	212	47.6% (31.1-64.2)	482	73.4% (67.1 – 79.8)	25.8%	249	56.2% (40.1 -73.4)	454	85.0% (81.0 -89.1)	28.8%
Yellow Fever[§¥]	212	42.5% (24.0-60.9)	482	72.8% (66.7 – 79.0)	30.3%	249	49.4% (33.5-65.3)	454	83.7% (79.2 – 88.2)	34.3%
Using information on vaccination card and interview										
3 doses of Polio[¥]	332	55.7% (38.3-73.1)	733	65.6% (55.4 – 75.8)	9.9%	377	66.6% (54.3-78.8)	776	79.3% (72.9 – 85-6)	12.7%
3 doses of DTP[¥]	332	55.7% (38.3-73.1)	725	62.2.0% (51.2 – 73.2)	4.1%	377	66.6% (54.3-78.8)	766	80.7.0% (75.5- 85.8)	13.1%
Measles[§¥]	331	51.7% (33.7-69.6)	544	73.9% (67.6 – 80.1)	22.2%	373	56.8% (43.5-70.2)	528	83.3% (78.8 – 87.9)	26.5%
Yellow Fever[§¥]	263	51.0% (35.1-66.9)	544	73.2% (67.2 – 79.1)	22.2%	297	58.6% (44.9 –72.3)	529	82.6% (77.8 – 87.4)	24.0%
Completely vaccinated[§¥]	249	33.3% (16.2-50.4)	519	53.8% (43.4- 64.1)	20.5%	288	39.6% (24.8 – 54.3)	511	69.5% (64.2 – 74.7)	29.9%
Vitamin A suplementation[§]		69.2% (57.5-80.7)	723	83.7% (77.1- 90.3)	14.5%		79.4 (70.2-88.6)	691	85.2% (78.0-92.5)	5.8%
Use of ITN	523	44.2% (27.5 – 60.8)	1045	52.7% (41.3 – 64.1)	8.5%	516	55.2% (45.3-65.2)	1045	52.3% (43.7 – 61.0)	-2.9%

[§] *Statistical significant increase in control zone*
[¥] *Statistical significant increase in intervention zone*

CHAPITRE IV

Evaluation de l'impact du Traitement Intermittent Préventif à la Sulfadoxine-Pyrimethamine sur la mortalité infantile dans le cercle de Kolokani

Ce chapitre sert de base à la rédaction en anglais d'un article qui sera soumis pour publication

Evaluation de l'impact du Traitement Intermittent Préventif à la Sulfadoxine-Pyrimethamine sur la mortalité infantile dans le cercle de Kolokani

4.1 Introduction

Le paludisme est l'une première cause de morbidité et de mortalité dans le monde avec près de un million de décès chaque année dont 91% en Afrique sub-saharienne [1]. En absence de vaccin, il y a un besoin urgent pour une stratégie facile et simple de contrôle de cette maladie.

Le traitement préventif intermittent (TPI) défini comme l'administration d'un antipaludique à dose curative à des intervalles de temps prédéfinis réduit l'incidence du paludisme et apparaît aujourd'hui comme une des stratégies les plus prometteuses. La stratégie a été évaluée et est aujourd'hui recommandée par l'OMS pour la prévention du paludisme chez les femmes enceintes avec une mise en application dans la plupart des pays d'endémie palustre. Des études au cours des dernières années ont montré que le TPI couplé au Programme Elargi de Vaccination (PEV) chez les enfants de moins de 1 an TPIn; notamment à 2, 3 et 9 mois) réduit l'incidence du paludisme maladie de 20 à 60% sans interaction négative avec les vaccins du PEV [2-7]. L'absence d'interaction avec les vaccins PEV et l'efficacité de cette stratégie pour prévenir les épisodes de paludisme maladie ont été bien établies dans le cadre d'essais cliniques randomisés et bien suivis dans différentes zones géographiques [8,9]. La stratégie vient d'être récemment approuvée par l'OMS comme un

nouvel outil additionnel dans la lutte contre le paludisme [10]. Cependant, à notre connaissance, il n'existe pas d'étude publiée sur l'impact de cette stratégie sur la moralité. L'étude de la mise en œuvre pilote de cette stratégie à une large échelle au Mali a offert une bonne opportunité d'évaluation de son impact sur la mortalité. L'objectif de notre étude était donc d'évaluer l'impact du TPIn à la SP sur la mortalité des enfants de 4 à 18 mois lorsqu'il est mis en œuvre dans le système de santé du Mali.

4.2 Méthodologie

Site d'étude

L'étude a été réalisée dans le district sanitaire de Kolokani dans la région de Koulikoro au Mali. La ville de Kolokani est située à 140 km au nord de Bamako. Le district couvre une superficie de 14.380 km2 divisée en 22 aires de santé. Chaque aire de sante couvre plusieurs villages. La population totale est estimée a 208 317 habitants dont environ 4% sont âgés de moins de un an. Le paludisme est hyper endémique dans la zone avec une prévalence du paludisme infection de 45% pendant la saison sèche, et plus de 70% pendant la saison des pluies. Les aires de sante sont dirigées par des médecins ou des infirmiers d'état, auxquels s'ajoutent des infirmiers de premier cycle, des matrones et des aide-soignants. Le centre de sante basé à Kolokani sert de centre de référence pour les 22 aires de sante. Il est équipé de salles de consultations, de salles d'hospitalisations et d'un bloc opératoire. Une équipe composée de médecins, sages femmes, infirmiers et gestionnaires assure les activités au niveau du centre ainsi que la coordination et la supervision des activités au niveau des aires de santé.

Schéma de l'étude

C'est une étude randomisée par grappes. Les 22 aires de santé du district Kolokani ont été randomisées suivant un ratio 1:1, le TPIn étant mis en œuvre dans 11 aires de santé, les 11 autres aires servant de contrôles, pour l'évaluation de l'impact de la stratégie comme décrite précédemment [11]. Le TPIn –SP était administré à la dose de ½ comprimé de SP au moment où les enfants recevaient des vaccins du PEV : la deuxième et la troisième dose de DTCP, à l'âge de 3 et 4 mois respectivement, et les vaccins contre la rougeole et la fièvre jaune à l'âge de 9 mois. La mise en œuvre a commencé en décembre 2006 après la formation du personnel et la livraison de la SP dans les centres de santé. Cette mise en œuvre était supervisée de décembre 2006 à décembre 2007 par un médecin d'appui du Malaria Research and Training Center (MRTC) de l'Université de Bamako détaché au Centre de Santé de Référence et visitant l'ensemble des aires de santé au début de la mise en œuvre. La SP était fournie au Centre de Santé de Référence de Kolokani par le MRTC. Le médecin d'appui s'est assuré de la disponibilité de la SP pour l'ensemble des aires de santé d'intervention jusqu'en décembre 2007. La mise en œuvre a continué dans les zones d'intervention jusqu'en décembre 2008 sous la seule responsabilité du Centre de Santé de Référence avec l'appui de l'UNICEF pour la fourniture de la SP. L'impact de la mise en œuvre de la stratégie a été évalué en avril 2009 au cours d'une enquête transversale.

Taille de l'échantillon et échantillonnage

Le calcul de la taille de l'échantillon a été fait en utilisant l'approximation arc-sinus en assumant un taux attendu de mortalité de 106 pour 1000 par mois chez les enfants âgés de 4 à 18 mois dans le but de pouvoir détecter une réduction de 30% de la mortalité avec un risque alpha de 5% et une puissance de 80%. L'effectif calculé était d'environ 1263 enfants par zone si les individus étaient choisis par tirage au sort direct. En supposant un effet

grappe de 1,5 pour tenir compte de l'échantillonnage en grappe et une proportion de données incomplètes ou manquante de 5%, nous avons estimé le nombre de sujets nécessaires à 3989.

Avec une population totale estimée à 214.509 habitants et une population d'enfant âgés de 4 à 18 mois de 4% (8580), nous avions besoin de recruter environ la moitié de la population, soit une localité sur deux en vue d'assurer une taille minimum de 4.000 enfants de 4-18 mois. Nous avons donc tiré au sort 141 localités sur les 281 du district de Kolokani. Les enquêteurs ont visité chacune des maisons des 141 localités sélectionnées. Ils ont interrogé les parents sur les enfants ayant atteint l'âge de 4 mois entre le 1er décembre 2006 et le 1er décembre 2008 (i.e. nés entre le 1er aout 2006 et le 1er aout 2008 et ayant vécu au moins 4 mois) dans la zone d'étude (i.e. susceptibles d'avoir été exposés à l'intervention entre le 1 décembre 2006 et le 31 décembre 2007) et sur le statut vital de ces enfants (i.e. vivants ou décédés) au 31 mars 2009. Les informations ont été collectées sur des questionnaires. Elles comprenaient la date du décès.

Gestion des données et analyse statistique

Les questionnaires ont été transférés à Bamako pour une double saisie sur MS Access. Les données ont ensuite été réconciliées. Elles ont été analysées en utilisant le logiciel Stata version 10 (Houston Texas, USA). Le critère de jugement principal était le taux de mortalité toutes causes confondues, entre le 1 décembre 2006 et le 31 mars 2009, chez i) les enfants âgés de 4 à 18 mois et ii) les enfants âgés de 4 à 12 mois. Les taux d'incidence observés dans les deux bras ont été comparés par un test exact de Fisher unilateral. Un modèle de Cox a été utilisé pour contrôler l'effet du sexe en analyse multivariée et un modèle de Cox stratifié par trimestre a été utilisé pour contrôler l'effet potentiel de la saisonnalité (i.e. variation du

risque de base en fonction du temps) de la transmission et de la mortalité par paludisme. La date d'origine était la date à laquelle l'enfant atteignait l'âge de 4 mois (i.e. début d'exposition à l'intervention) et la date de dernière nouvelle était la date la plus précoce parmi les suivantes : date à laquelle l'enfant atteignait l'âge de 18 mois (ou 12 mois), date du décès ou le 31 mars 2009. La durée de suivi de chaque individu (i.e. période à risque) était le délai entre la date d'origine et la date des dernières nouvelles. Le nombre de décès et les durées de suivi ont été sommés par village et par sexe. Ces données cumulées ont ensuite été analysées dans un modèle de Poisson en systèmes d'équations d'estimation généralisées (GEE) avec une matrice de corrélation échangeable pour estimer le risque relatif de décès entre les deux bras en tenant compte de l'effet du sexe et de l'agrégation des observations par village.

Considération Ethique

Le protocole initial et les amendements successifs ont été approuvés par le comité d'éthique de la Faculté de Médecine de Pharmacie et d'Odonto-stomatologie de l'Université de Bamako. L'autorisation des parents était demandée avant l'interview.

4.3 Résultats

Au total 5882 enfants ayant atteint l'âge de 4 mois entre le 1er décembre 2006 et le 1er décembre 2008 dans la zone d'étude ont été enquêtés dont 2869 dans 75 villages de la zone d'intervention et 3013 dans 75 villages de la zone de non intervention. La distribution par sexe était comparable entre les deux bras (50,5% de garçons en zone d'intervention contre 51.6% en zone de non intervention, p = 0,43). La date de décès n'a pas pu être établie chez deux enfants (un garçon en zone d'intervention né le 15 février 2007 et une fille en zone de non intervention née le 10 janvier 2008) qui ont été exclus de l'analyse.

Parmi 5880 enfants, il y a eu 198 décès survenus entre l'âge de 4 et 18 mois dont 86 (2,86%) dans la zone d'intervention et 112 (3,91%) dans la zone de non intervention (test exact de Fisher ; unilateral, p =0,015, bilateral, p=0,030), et 119 décès survenus entre l'âge de 4 et 12 mois dont 51 (1,69%) dans la zone d'intervention et 68 (2,37%) dans la zone de non intervention (test exact de Fisher ; unilateral, p =0,040, bilateral, p=0,078).

Les taux de mortalité, les ratios des taux de mortalité et les réductions des taux de mortalité sont présentés en Tableau 1. Une estimation de Kaplan Meier des taux de survie est présentée dans la figure 1.

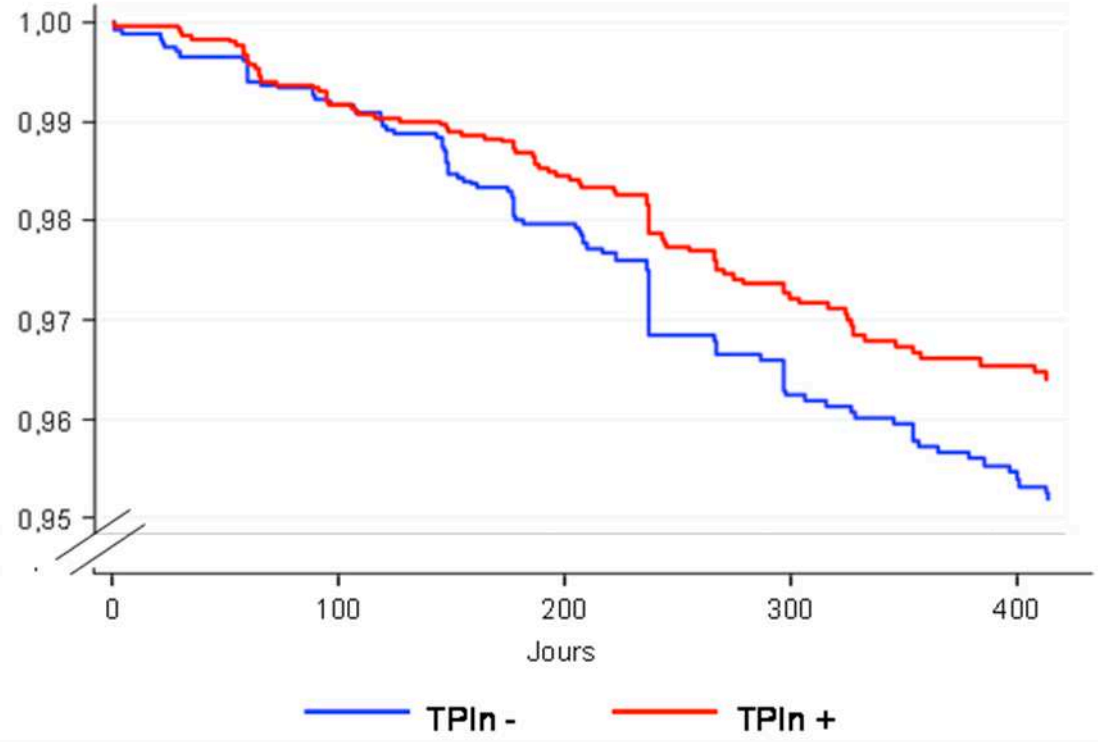
Tableau 1: Les taux de mortalité, les ratios des taux de mortalité et les réductions des taux de mortalité dans les deux cohortes.

	Cohorte 4-18 mois		Cohorte 4-12 mois	
	TPIn +	TPIn -	TPIn +	TPIn -
Décès	86	112	51	68
Personne-jours	1.018.825	971.905	688.294	652.688
Taux mensuel de mortalité pour 1000 enfants	2,532	3,456	2,223	3,126
RR non ajusté (IC 95%)	0,73 (0,55-0,98)		0,71 (0,48-1,04)	
p (unilateral / bilateral)	0,015 / 0,030		0.033 / 0,066	
RR ajusté*, Cox (IC 95%)	0,73 (0,55-0,97)		0,71 (0,49-1.02)	
p* (bilateral)	0,029		0,064	
Réduction en mortalité* en %	27 (3-45)		29 (0-49)	
RR ajusté**, Poisson (IC 95%)	0,73 (0,55-0,99)		0,72 (0,49-1,06)	
p**	0,041		0,093	

*ajusté pour le sexe dans un modèle de Cox
**ajusté pour le sexe et le plan de sondage par village dans un modèle de régression de Poisson en système d'équations d'estimation généralisées (GEE)

Les résultats de l'analyse en modèle de Cox stratifié différaient peu ou pas de ceux obtenus dans un modèle de Cox simple (avec ajustement sur le sexe, RR= 0,73, IC95% : 0,55-0,96, p=0,026 dans la cohorte 4-18 mois ; RR= 0,71, IC95% : 0,49-1,01, p=0,059 dans la cohorte 4-12 mois).

Figure 1 : Courbe de survie de Kaplan-Meier après l'âge de 4 mois



4.4 Discussions

Nous avons estimé l'impact de deux ans de mise en œuvre du TPIn sur la mortalité des enfants cibles et nous avons trouvé une réduction de 27 et de 29% de la mortalité des enfants de 4-18 mois et 4 -12 mois, respectivement, dans la zone d'intervention comparée à la zone de non-intervention. L'utilisation des moustiquaires imprégnés d'insecticide dans les essais randomisés a entrainé une réduction de 17% de réduction la mortalité globale [12]. La mise en œuvre du TPIn associé au PEV a donc eu un impact important sur la mortalité des enfants, comparable ou supérieur à celui des moustiquaires imprégnées d'insecticide.

L'évaluation de l'impact d'une stratégie sur la mortalité palustre est toujours difficile à cause du nombre important de sujets nécessaires et des nombreux facteurs pouvant confondre la relation entre l'intervention et la mortalité. L'impact de l'utilisation des moustiquaires imprégnées d'insecticide sur la mortalité palustre n'a pas pu être démontré au cours de large essais randomisés [13,14]. Il a fallu les résultats des méta-analyses pour mieux préciser l'importance de leur impact sur la mortalité attribuée au paludisme [15]. L'attribution d'un décès au paludisme est problématique et les méthodes indirectes comme celle des autopsies verbales peuvent manquer de sensibilité et de spécificité. L'impact sur la mortalité globale, toute cause confondues, a été plus facilement démontrée [12-14,16] malgré le grand nombre de sujets nécessite et les nombreux facteurs pouvant confondre la relation entre l'intervention et la mortalité globale. Le diagnostic de décès « toute causes confondues » ne pose pas de problème de sensibilité ou de spécificité. C'est pourquoi dans cette étude nous avons limité notre objectif à l'évaluation de l'impact du TPIn sur la moralité toute cause confondue. Une autre raison qui nous a amené à nous limiter à cet objectif, c'est le fait que les informations ont été collectées de façon rétrospective, à distance des

évènements (plusieurs mois après), ne permettant pas de faire une évaluation précise des causes des décès, même en utilisant la méthode des autopsies verbales.

Dans notre étude, la réduction de la mortalité pourrait être due à une combinaison de l'impact direct du TPIn sur le paludisme et de son impact indirect sur d'autres affections des enfants pouvant entrainer la mort. Les évaluations antérieures ont montré que la mise en œuvre du TPIn a entrainé une augmentation significative de couverture des vaccins du PEV et que cette augmentation était plus marquée dans la zone d'intervention [17]. Par ailleurs, il est connu qu'une part notable de la mortalité par paludisme est due à une mortalité indirecte (*i.e.* non directement attribuable au paludisme) [18]. Ainsi, l'impact de l'élimination du paludisme sur la mortalité générale a souvent dépassé ce qui était attendu. Il est possible que les infections plasmodiales altèrent les capacités des individus à survivre à d'autres pathologies.

Le TPIn est maintenant adopté par l'OMS comme politique de lutte contre le paludisme malgré l'absence de données sur l'impact de la stratégie sur la mortalité [9, 10]. Nous avons voulu saisir l'opportunité de la mise en œuvre pilote de la stratégie au Mali pour essayer de répondre à un certain nombre de questions parmi lesquelles l'impact de la stratégie sur la mortalité. Notre étude a plusieurs points forts. Premièrement, c'est une étude randomisée assurant ainsi une meilleure comparabilité des bras de l'étude. Deuxièmement la mise en œuvre de la stratégie a été faite dans le contexte du système de santé reflétant ainsi la réalité de l'utilisation de la stratégie. Troisièmement l'évaluation de la mortalité a été faite par des enquêteurs qui ne distinguaient pas les localités avec intervention de celles sans intervention, minimisant ainsi les risques de biais.

Le taux de mortalité observé était plus bas qu'attendu dans la zone (40,8 pour mille en zone de non-intervention contre 106 pour mille selon les statistiques nationales), réduisant ainsi la puissance de l'étude avec des intervalles de confiances plus large. Une autre limite de notre étude est la durée relativement courte de l'intervention (deux ans). Il est possible que l'impact sur la mortalité augmente avec la durée de la mise en œuvre (*e.g.* par la réduction du réservoir des *Plasmodium* entrainant une réduction de leur transmission). Il est important que l'impact sur la mortalité continue d'être évalué, sur des périodes plus longes, au cours de la mise en œuvre en œuvre de cette stratégie.

4.5 Conclusion

La mise en œuvre du TPI au cours du PEV a été associée à une réduction de la mortalité générale des enfants. Nos résultats sont en faveur de l'adoption de cette stratégie et de sa mise en œuvre dans le cadre de la politique de lutte contre le paludisme.

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CHAPITRE V

Efficacité du traitement préventif intermittent ciblant la saison de transmission sur les épisodes de paludisme maladie chez les enfants dormant sous moustiquaires imprégnées d'insecticides au Mali

5. Efficacité du traitement préventif intermittent ciblant la saison de transmission sur les épisodes de paludisme maladie chez les enfants dormant sous moustiquaires imprégnées d'insecticides au Mali

5.1 Position du problème

L'utilisation des MII réduit l'incidence des accès palustre d'environ 50% et la mortalité palustre d'environ 20% [1]. C'est une des stratégies de choix dans la lutte contre le paludisme et de gros efforts ont été faits au cours de ces dernières années dans des nombreux pays pour augmenter leur utilisation. En conséquence il y a une augmentation importante de la couverture en MII dans la plus part des pays d'endémie palustre en Afrique [2]. Si cette tendance se maintient, beaucoup de pays vont atteindre l'objectif de 60% de couverture voir celle de couverture universelle dans les prochaines années. Le traitement préventif intermittent chez les enfants ciblant la saison de transmission est une stratégie très prometteuse. Nous avons démontré dans la première partie de cette thèse que deux doses de SP en traitement intermittent réduit les épisodes d'accès palustre pendant la période d'intervention de 69% chez les enfants de moins de 5 ans [3]. Une autre étude effectuée au Sénégal, a montré que SP + Artesunate à un mois d'intervalle en traitement intermittent réduit les épisodes d'accès palustre de 86% [4]. Dans les évaluations des différentes combinaisons d'antipaludiques en traitement intermittent chez les enfants, SP + Amodiaquine s'est montré la plus efficace. Cependant ces études d'efficacité de la stratégie ont été conduites dans des contextes de très faible utilisation de MII et il n'était pas établi que des niveaux d'efficacité aussi importants seraient obtenus dans un contexte de large utilisation des MII.

L'objectif principal de cette partie de notre travail, était de déterminer le degré de protection contre le paludisme associé à l'administration du TPIe chez des enfants dormant sous des MII au Mali.

Nous avons participé à la conception du projet que nous avons soumis à la Fondation Bill & Melinda Gates sous la direction du Professeur Brian Greenwood de l'Ecole de Santé Publique de Londres. Nous avons obtenu le financement et exécuté le protocole sur le terrain au Mali, supervisé la collecte et la saisie des données. Nous avons analysé les données et procédé à la rédaction du manuscrit dont nous sommes le premier auteur et l'auteur à qui les correspondances doivent être adressées. C'est l'essai clinique le plus large que nous avons conduit avec un échantillon de plus 3000 enfants.

5.2 Principaux résultats

Cette étude a été réalisée dans trois localités du cercle de Kati (Djoliba, Siby et Ouelessebougou). Au total, 3.065 enfants ont été criblés, 3.017 enfants répondant aux critères d'inclusion ont été inclus et randomisés, 1.508 dans le bras TPIe (groupe de traitement) et 1.509 dans le bras placebo (groupe placebo).

La proportion des participants qui ont participé à la totalité de l'étude était 98,1% dans le groupe de traitement et 98,5 % dans le groupe placebo.

Au cours de la période d'intervention le taux d'utilisation des MII évalué chez 150 enfants tirés au sort chaque semaine était supérieur à 99% et était comparable dans les deux groupes (99,3% dans le groupe de traitement et de 99,7% dans le groupe placebo, p = 0,41).

Au cours de la période d'intervention, les taux d'incidence des accès palustres (définis comme l'association de fièvre ou d'antécédent de fièvre au cours des dernières 24 heures avec une parasitémie $\geq$ 5000 formes asexuées de *P. falciparum* par µL de sang)

étaient de 1,90 (IC 95% 1,76-2,05) épisodes par personne-an dans le groupe placebo contre 0,34 (IC 95% 0,29 – 0,41) dans le groupe de traitement. Ceci correspond a une réduction des épisodes des accès palustres de 82% (IC 95% 78% -85%, p <0,001).

L'incidence des accès palustres définis comme l'association de fièvre ou d'antécédent de fièvre au cours des dernières 24 heures avec une parasitémie positive pour les formes asexuées de *P. falciparum* (> 0/ µl de sang) était aussi plus basse dans le groupe de traitement que dans le groupe placebo (2,4 épisodes par enfant par an dans le groupe placebo contre 0,41 épisodes par enfant par an dans le groupe de traitement) avec une efficacité protectrice de 83% (IC 95% 80%-86%, p<0.001).

Les analyses du temps au premier épisode d'accès palustres indiquaient également une forte protection du TPIe quelque soit la définition du paludisme maladie utilisée (p < 0,001).

Lorsque les données étaient analysées par localité, l'efficacité protectrice de la stratégie était similaire entre les localités, quelle que soit la définition des accès palustres utilisée, malgré la différence de taux d'incidence des accès palustres entre les trois localités.

Il y a eu au total 17 épisodes de paludisme grave selon la définition de l'OMS, 15 dans le groupe placebo et 2 dans le groupe de traitement, soit une efficacité protectrice de 87% (IC 95% 42% - 99%, p = 0,001).

Au cours de la période d'intervention, la prévalence du paludisme infection a été réduite dans le groupe de traitement de 88% (IC 95% 78%- 94%), p <0,001. A la fin de la période d'intervention, la prévalence de l'anémie modérée (définie comme taux d'hémoglobine < 8g/dL) a été réduite dans le groupe de traitement de 48% (95%CI: 16% – 68%), p<0.001.

Les indices nutritionnels (Poids/âge, Poids/Taille et Taille/âge en Z score) étaient similaires dans les deux groupes avant et après l'intervention.

Sur le plan de la tolérance, les fréquences des événements indésirables étaient similaires entre les deux groupes mais il y avait une tendance a une fréquence plus élevée des vomissements et de l'anorexie chez les enfants qui ont reçu le TPIe (4,0% contre 1,9%, p = 0,06, pour les vomissements et 1,9% contre 0,8%, p = 0,08 pour l'anorexie). Il n'y a pas eu d'événement indésirable grave attribuable au TPIe.

Les fréquences des marqueurs moléculaires de la résistance de *P. falciparum* à la SP et à l'AQ (dhfr 51, 59, 108, dhps 437, 540, pfcrt 76, pfmdr1-86) étaient similaires avant et après l'intervention. Deux des marqueurs moléculaires de la résistance à la SP (mutations des codons dhfr 59 et dhps 437) étaient plus fréquents chez les parasites obtenus à la fin de l'intervention dans le groupe de traitement que dans le groupe placebo. Il en était de même pour la triple mutation dfhr et la quadruple mutation (triple dhfr + dhps 437), même si les fréquences des ces deux groupes de mutations étaient similaires avant et après l'intervention. La quintuple mutation (triple dhfr +dhps437 + dhps540) a été détectée seulement après l'intervention dans des proportions similaires entre les deux groupes (4, 1% dans le groupe de traitement et 1,4 % dans le groupe placebo, p = 0,34).

Conclusion : Le TPI donné pendant la saison de transmission du paludisme confère une protection contre les accès palustres, la parasitémie et l'anémie chez les enfants utilisant des moustiquaires imprégnées d'insecticide à longue durée d'action. La combinaison SP+AQ est bien tolérée. Ces résultats indiquent que le TPI peut utilement contribuer au contrôle du paludisme en association avec des mesures de lutte anti-vectorielle dans les zones de transmission saisonnière.

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5.4 Article Dicko et al. *Plos Med sous presse*

A randomised, placebo-controlled trial of intermittent preventive treatment of malaria with sulphadoxine -pyrimethamine plus amodiaquine in children protected by a long lasting insecticide treated net in Mali.

Plos Med sous presse

Title

A randomised, placebo-controlled trial of intermittent preventive treatment of malaria with sulphadoxine - pyrimethamine plus amodiaquine in children protected by a long lasting insecticide treated net in Mali.

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Keywords

Malaria, intermittent preventive treatment, children, Mali.

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ABSTRACT

Background

Previous studies have shown that in areas of seasonal malaria transmission, intermittent preventive treatment of malaria in children (IPTc), targeting the transmission season, reduces the incidence of clinical malaria. However, these studies have been conducted in communities with a low coverage with insecticide treated nets (ITNs). Whether IPTc provides additional protection to children sleeping under an ITN has not been established.

Methods

To assess whether IPTc provides additional protection to children sleeping under an ITN, we have conducted a randomised, double blind, placebo-controlled trial of IPTc with sulphadoxine pyrimethamine (SP) plus amodiaquine (AQ) in three localities in Kati, Mali. After screening, eligible children aged 3-59 months were given a long lasting insecticide treated net (LLIN) and randomised to receive three rounds of active drugs or placebos. Treatments were administered under observation at monthly intervals during the high malaria transmission season in August, September and October 2008. Adverse events were monitored immediately after the administration of each course of IPTc and throughout the follow up period. Clinical episodes of malaria were recorded through passive surveillance by study clinicians available at all times during the follow-up. Cross-sectional surveys were conducted in 150 randomly selected children weekly and in all children at the end of the malaria transmission season to assess usage of ITNs and the impact of IPTc on the prevalence of malaria, anaemia and malnutrition. Cox regression was used to compare incidence rates between intervention and control arms. The effects of IPTc on the prevalence of malaria infection and anaemia were estimated using logistic regression.

Results

Three thousand and sixty-five children were screened and 3017 (1508 in the control and 1509 in the intervention arm) were enrolled in the study. One thousand, four hundred and eighty-five children (98.5%) in the control arm and 1481 (98.1%) in the intervention arm completed follow-up. During the intervention period, the proportion of children reported to have slept under an ITN was 99.7% in the control and 99.3% in intervention arm (p = 0.48). A total of 672 episodes of clinical malaria defined as fever or a history of fever and the presence of at least 5000 asexual forms of *Plasmodium falciparum* per µl (incidence rate of 1.90; 95%CI: 1.76 – 2.05 episodes per person year) were observed in the control arm versus 126 (incidence rate of 0.34; 95%CI: 0.29 – 0.41 episodes per person year) in the intervention arm, indicating a protective efficacy of 82% (95% CI: 78% – 85%)(p<0.001). There were 15 episodes of severe malaria in children in the control arm compared to 2 in children in the intervention group giving a PE of 87% (95% CI 42% – 99%)(p=0.001).

IPTc reduced the prevalence of malaria infection by 88% (95%CI: 78%- 94%)(p<0.001) during the intervention period and by 46% (95% CI 31% - 68%) (p<0.001) at the end of the intervention period. The prevalence of moderate anaemia (Hb<8g/dl) was reduced by 48% (95%CI: 16% – 68%)(p<0.001) at the end of intervention period. The frequencies of adverse events were similar between the two arms. There was no drug-related serious adverse event.

Conclusions

IPTc given during the malaria transmission season provided substantial protection against clinical episodes of malaria, malaria parasitaemia and anaemia in children using an LLIN. SP+AQ was safe and well tolerated. These findings indicate that IPTc could make a valuable contribution to malaria control in areas of seasonal malaria transmission alongside other interventions.

Trial Registration number: ClinicalTrials.gov NCT 00738946

BACKGROUND

Malaria remains a major cause of morbidity and mortality and is estimated to cause 881,000 deaths per year, 91% of which occur in sub-Saharan Africa, mainly in children under 5 years of age [1]. Thus, in the absence of a vaccine, simple and effective control strategies are urgently needed to reduce the malaria burden in sub-Saharan Africa. Vector control, using insecticide treated bednets (ITNs), insecticide treated curtains or indoor residual spraying (IRS), can reduce mortality and morbidity from malaria substantially [2] but, in high transmission settings, these interventions provide only partial protection and additional control measures are needed.

Intermittent preventive treatment (IPT) is a new approach to the prevention of malaria in infants and older children. Several, randomised, controlled trials have demonstrated that intermittent preventive treatment of malaria in infants (IPTi) with sulphadoxine-pyrimethamine (SP) given during routine vaccinations at approximately two, three and nine months of age, reduces the incidence of clinical malaria by 22% to 59% [3] and this strategy has been shown to be safe and cost effective. However, in many regions of Africa, the main burden of malaria falls not on infants but on older children [4]. In parts of Africa, such as much of the Sahel and sub-Sahel, where malaria transmission is very seasonal, the incidence of severe malaria currently peaks at two to three years of age. As the overall incidence of malaria decreases in Africa in response to enhanced control efforts, an effect already being seen in some countries, it can be anticipated that the mean age of cases of malaria will increase further. For these reasons, trials have been undertaken in areas of seasonal malaria transmission to determine whether intermittent preventive treatment in children (IPTc) could be used as an effective malaria control tool in older children. In Mali, a 69% reduction in the incidence of clinical malaria was seen in children 0-5 years old when two doses of SP were given eight weeks apart during the malaria transmission season [5]. In Senegal, SP plus a single dose of artesunate (AS), administered on three occasions at monthly intervals during the peak malaria season, reduced the incidence of clinical malaria by 86% [6]. A subsequent trial of different drug regimens showed that IPT with SP and amodiaquine (AQ) was even more effective than SP + AS, providing approximately 95% protection [7]. A further study, conducted in an area of Ghana with more prolonged transmission, found that AS + AQ monthly was more effective than AS + AQ or SP alone given every two months, suggesting that for drugs such as SP and AQ, monthly administration is needed to achieve effective IPTc [8]. Bednet coverage among young children was low at each of the sites where these trials were conducted and use of ITNs was very

uncommon. Use of ITNs is now a favoured approach to the control of malaria in most parts of Africa and major efforts are being made to scale up their use. With international support, ITN coverage is increasing in many malaria endemic countries in sub-Saharan Africa [9] and it is expected that almost universal coverage with ITNs in high risk groups, as called for in the Global Malaria Action Plan [10], will be achieved in many malaria endemic countries. Thus, following on the initial encouraging results obtained with IPTc, an issue that needs to be addressed urgently is whether IPTc can provide significant added benefit to the protection against malaria provided by ITNs to warrant its use as a malaria control tool in areas with seasonal transmission of malaria and a high use of ITNs. It was initially planned to address this question simultaneously in each of the three countries Mali, Burkina Faso and Ghana, using a similar design and methods. However, the site in Ghana had to be abandoned due to delays in obtaining regulatory approval for the use of SP + AQ, the drug combination chosen for the study on the basis of the results of previous trials and knowledge of the sensitivity of *Plasmodium falciparum* to these drugs in the proposed study areas. Very similar protocols were used for the studies conducted in Burkina Faso and Mali.

METHODS

The protocol of the trial (appendix 5) and CONSORT checklist are available as supporting information (appendix 1).

Objectives

The primary objective of the study was to determine the degree to which IPTc given during the malaria transmission season reduces the incidence of clinical malaria in children who sleep under a Long Lasting Insecticide Treated Net (LLIN). Secondary objectives were determination of the impact of this strategy on severe malaria, all cause hospital admissions, anaemia, nutrition (wasting, stunting and being underweight), parasite prevalence and molecular markers of resistance to SP and AQ.

Study sites.

The study was conducted in two rural villages, Djoliba and Siby, and the small town of Ouelessebougou situated in the district of Kati in the savannah region of Mali. Djoliba and Siby are located 40 and 30 km south west of the capital city Bamako respectively and Ouelessebougou is located 80 km south of Bamako. In Djoliba and Siby, community health centres are staffed with a physician and nurses. In Ouelessebougou, the community health centre is staffed by an assistant physician and nurses, but located less than 100 m from a district health centre staffed by 4 physicians and 6 nurses. A research team composed of physicians

and medical residents was established in each of the three sites to follow up and provide health care to the study participants.

Malaria transmission in the study area is highly seasonal and 80-90% of malaria cases occur between August and November. The Entomological Inoculation Rate (EIR), as described in supplementary information appendix 2, was 9.4 and 6.6 infective bites per person per season respectively in Siby and in Ouelessebougou, two localities far from any river and 37.**3** infective bites per person per season in Djoliba located on the bank of the Niger river. The coverage of ITNs at baseline was 33.4% (312/935) in Siby, 84.7% (563/665) in Djoliba and 89.8% (2207/2458) in Ouelessebougou.

Study design and participants

The study was designed as an individually randomised, placebo controlled, trial of IPTc with SP + AQ in children who received a long-lasting ITN**.** Children aged 3 – 59 months were enumerated and given a census identification number including a house number to facilitate their identification at screening, enrolment and follow-up. Recruitment was started in Djoliba followed by Siby. In these communities all available children in the target age group who were not selected for the baseline survey of drug resistance were screened and enrolled if they met the inclusion criteria. In the larger community of Ouelessebougou, children were screened for enrolment on a first come first served basis until the required sample size was met. Children were eligible to join the study if they were aged 3-59 months at the time of enrolment and permanent residents of the study area with no intention of leaving during the study period. Exclusion criteria were the presence of a severe, chronic illness, such as severe malnutrition or AIDS, and a history of a significant adverse reaction to SP or AQ. Cases of an acute illness, such as malaria, were not excluded. Such cases were treated appropriately and the child randomised and retained in the trial.

Ethics

The study protocol was reviewed and approved by the Ethical Committee of the Faculty of Medicine, Pharmacy and Dentistry, University of Bamako, Mali and by the Ethics Committee of the London School of Hygiene and Tropical Medicine. Community consent was obtained at meetings with leaders, heads of families and other community members of each locality prior to the start of the study. Individual, written, informed consent was obtained from a parent or guardian of each child prior to screening and enrolment. A Data and Safety Monitoring Board (DSMB) was established and monitored the trial with the support of a local

medical safety monitor. GCP monitoring of the trial was performed by PharmaClin http://www.pharmaclin.com).

Interventions

Every child who was screened was provided with a LLIN (Permanet®, Vestergaard Frandsen) that was marked with the child's identification number regardless of whether or not the child was enrolled. Instructions were given to the parent or guardian on how to use the net and the importance of using the net regularly was emphasized. Monitoring of utilisation of ITNs by study participants was made in 150 randomly selected children each week and in all study children during the cross-sectional survey conducted at the end of the malaria transmission season.

Eligible children were treated with a course of SP + AQ or matching placebos on three occasions at monthly intervals during the malaria transmission season, starting in August 2008. SP and AQ were manufactured by Kinapharma Limited, Accra, Ghana and quality control checks on the drugs for solubility and content were performed at the London School of Tropical Medicine and Hygiene, prior to their use in the trial. Tablets met internal standards for drug solubility and content. Doses of SP and AQ were based on weight with children stratified into one of the three weight categories (5- 9 kg, 10-18 kg and 19 or > kg). SP was given at a dose of 175/8.75 mg to children 5-9 kg, 350/17.5 mg to children 10-18 kg and 550/26.25 mg to those who weighed 19 kg and above. The corresponding doses for AQ were 70 mg, 140 mg and 220 mg respectively. AQ was given over three days. Drugs were pre-packaged to facilitate administration and put in envelopes with colour codes, one for each weight group. Within each weight stratum, children were individually randomised using a computer-generated random number sequence and blocks of varying length. Treatment allocations were provided within sealed, opaque envelopes.

Drugs were given under direct observation at a research clinic by study staff. Children were observed for 30 minutes after drug administration. If vomiting occurred during this 30-minute period, drugs were re-administered. If vomiting occurred on a second occasion, this was noted but the drugs were not given again. Such children were not excluded from the trial and they were eligible to receive drugs on the subsequent two days and during subsequent monthly IPT rounds. If a child missed the day set for treatment, a home visit was made to enquire why the child had not been brought for treatment and the reason was recorded. If the family wished to continue with treatment but was unable to attend on the specified day, then treatment was re-offered within an interval of 7 days of the designated date. Children with an acute malaria episode were

treated with artemether-lumefantrine (AL) and did not receive IPT with SP+AQ if the treatment for acute malaria was received within 7 days of the scheduled date of IPT. Such children were eligible for treatment in future treatment rounds

Outcomes

The primary endpoint of the study was the incidence of clinical malaria. This was defined as the presence of fever (axillary temperature ≥ 37.5 ^{o}C) or a history of fever in the past 24 hours and the presence of *P. falciparum* asexual parasitaemia at a density greater or equal to 5,000 parasites per µl. Secondary endpoints were i) the incidence of clinical malaria defined as the presence of fever or a history of fever in the past 24 hours and the presence of *P. falciparum* asexual parasitaemia at any density; ii) incidence of severe malaria defined according to the WHO criteria [11], iii) malaria parasitaemia defined as the presence of asexual parasitaemia and iv) mild, moderate or severe anaemia defined as an haemoglobin (Hb) concentration <11 g/dL , < 8 g/dL and < 5 g/dL respectively, v) hospital admission defined as a stay of at least 24 hours in hospital for treatment, vi) anthropometric indicators including wasting, stunting and being underweight as defined by WHO [12] and vii) safety and tolerability measured by the occurrence of non serious and serious adverse events.

Passive surveillance for clinical malaria started at the time of the administration of the first dose of IPTc in August 2008 and continued until the end of the malaria transmission season in November /December 2008, 6 -7 weeks after the last round of IPTc. Parents were encouraged to bring their child to a study health centre, where medical staff were available 24 hours a day and 7 days a week, if the child became unwell. A finger prick blood sample was be obtained from all study children with fever (an axillary temperature of 37.5 ^{0}C or higher) or a history of fever within the previous 24 hours for preparation of a blood film, measurement of Hb concentration and for a rapid diagnostic test (RDT) OPTIMAL_IT® (Diamed AG, Cressier FR, Switzerland) for malaria. Children who had a positive RDT for malaria were treated immediately with AL. Severe cases were admitted to the health centre or referred to the paediatric ward of the Gabriel Touré Hospital in Bamako. Causes of death were assessed within a month of death using a modified version of the INDEPTH post-mortem questionnaire (http://www.indepth-network.org/index.php?option=com_ content&task =view&id= 96&Itemid=184).

Use of a LLIN was assessed by asking if a child had slept under an LLIN the previous night and the presence of the net was checked by field staff. During these home visits, the axillary temperature of each

child was taken and a blood film obtained regardless of whether or not the child had fever. A RDT was performed if a child had measured fever or a history of fever within the previous 24 hours and if this was positive, treatment with AL was given according to national guidelines. At the end of the malaria transmission season, a cross sectional survey was undertaken at which every child was examined, their height and weight recorded and a finger prick blood sample obtained for determination of Hb concentration, preparation of blood films and collection of a filter paper sample for subsequent molecular studies. Safety and tolerability of SP and AQ were monitored passively during the study period in all the children and actively in a subset at the time of the administration of IPT (days 0, 1 and 2) and one day after the last dose of treatment (day 3) at each round.

Assessment of molecular markers of drug resistance

Monitoring of the frequency of molecular markers of resistance to sulphadoxine, pyrimethamine and amodiaquine was performed in two cross-sectional surveys, the first at baseline in August 2008 and the second during the survey undertaken at the end of malaria transmission season. The baseline survey was conducted in 256 children randomly selected from the screening list. These children were not enrolled in the placebo control trial. Subjects enrolled in the placebo control trial were surveyed about six weeks after the third course of IPTc, at the end of malaria transmission season, to assess whether administration of IPT with SP+AQ had lead to an increase in molecular markers of resistance to these drugs. Thick and thin blood smears and blood blotted onto filter papers were collected during both surveys for molecular analysis as described below.

Laboratory methods

Thick blood films were air dried, stained with Giemsa and examined for malaria parasites by two well-trained technicians. One hundred high power fields were counted before a film was declared negative. Parasite density was determined by counting the number of parasites present per white blood cell (WBC) on a thick smear and assuming a WBC count of 8,000 per µl. In the case of a discrepancy (positive/negative or a difference in parasite density greater than 30%), a third reading was done. The median parasite density of two or three readings was used. An external quality control of slide reading performed by the Malaria Diagnosis Centre of Excellence (MDCoE) of the Walter Reed/Kenya Medical Research Institute, in Kisumu, Kenya, showed an overall concordance of more than 90% on parasite detection and 100% on species identification (supplementary information appendix 4) . Haemoglobin concentrations were measured using a haemoglobin analyzer (Hemocue® HB 301, Angelholm, Sweden) on blood obtained by finger prick.

Filter paper samples from children with a mono-infection of *P. falciparum* on blood smears were analysed by nested PCR for mutations at codons 51, 59 and 108 of the *dhfr* gene, 437 and 540 of the *dhps* gene, 76 of mutations in the *P. falciparum* chloroquine transporter gene (*pfcrt*) and 86 of the *P. falciparum* multidrug resistance gene one (*pfmdr1*) according to published methods [13-15]. Cases of mixed infection (wild type and mutant) were categorized as mutant.

Sample size

Calculation of sample size was based on the assumptions that the clinical attack rate measured by passive surveillance would be 1.0 - 2.0 attacks per child per year in unprotected children aged 3 – 59 months living in the study areas and that sleeping under an LLIN would reduce this attack rate by half to 0.5 to 1.0 clinical episode per child per year. Assuming that children experienced an average of 0.5 clinical episodes per child per year of sufficient severity to present to a health facility, to detect a 20% reduction in this incidence (i.e. from 0.5 to 0.4 attacks per child per year) in children who receive IPTc, the smallest reduction that would be likely to make IPTc a worthwhile investment, and allowing for a 20% loss to follow-up, we estimated that approximately 2,000 children (1,000 in each arm) were required for a study with 90% power at the two-sided 5% level of significance [16]. After the site in Ghana was dropped, the sample size was increased to 1,500 subjects per arm, which would have 80% power to detect a two-thirds reduction in the incidence of severe malaria, assuming an incidence of 2% in children in the control arm. The study was not powered to detect a smaller reduction in the incidence of severe malaria but the analysis plan included provision for combination of the results of this trial with those of a parallel study conducted in Burkina Faso to provide sufficient size to allow detection of a smaller impact of IPTc on this end point.

Data management and analysis

Data were collected on standardized forms, double-entered and verified using MS Access and then exported to Stata (StataCorp, College Station, Texas, US) for additional cleaning and analysis. A data analysis plan was written and submitted to the DSMB prior to analysis. The final, cleaned database was locked and a copy sent to the DSMB. An intention-to-treat analysis was performed. Incidence rates of clinical malaria, severe malaria and hospital admissions were calculated by dividing the number of episodes by the total child days at risk. Children were not considered at risk for 21 days after each type of a malaria episode and these days were not included in the calculation of the child days at risk. The incidence rates in the two treatment groups were compared using Cox regression to estimate the incidence rate ratio, with adjustment for age, gender

and locality, and using a robust standard error to allow for the lack of independence among repeated episodes in the same child. The protective effect (PE) of IPTc was computed as 1 minus the incidence rate ratio. Time to first episode of clinical malaria in the two arms was examined using Kaplan-Meier plots and compared using Log rank test. Anthropometric data at enrollment and at the end of season cross-sectional survey were converted into weight-for-age, height-for-age and weight-for-height z-scores using WHO's anthropometric software (www.who.int/childgrowth/software/en). Underweight, stunting and wasting were defined as z-scores of < -2 for the relevant indicator [12]. Changes in weight and height between the two groups were compared using Student t test. Frequencies of single mutations as well as the triple mutant (*dhfr* 51 + 59 + 108) and quadruple mutant (triple mutant + *dhps* 437) genotypes were determined and compared between treatment arms and between the beginning and end of the study. Proportions of children with binary outcomes were compared between the two groups using Pearson's Chi square test or generalized linear models adjusted for age, gender and locality.

RESULTS

Trial profile and baseline data

The trial profile is summarised in Figure 1. A total of 3,065 children were screened of whom 3,017 (1509 in the IPTc arm and 1508 in the placebo arm) (98%) were enrolled. Reasons for exclusion are shown in Figure 1. The proportion of children who completed the follow up to day 42 after the last round of IPTc was similar in the control and in the intervention arms (98.5% and 98.1% respectively). The reasons for withdrawal were withdrawal of consent (n=29), migration to another location (n= 15), a history of allergy to study drugs (n=4 with two cases confirmed) and death (n=3). There were no significant differences between intervention and control groups with regard to their age and gender distribution, nor in the prevalence of fever, wasting or stunting at the time of enrolment (Table 1).

LLIN usage

Usage of LLINs was assessed for 590 children in the control group and for 591 children in the intervention group during weekly home visits, undertaken without prior warning, during the course of the intervention period. Usage of an LLIN was high in each of the three study localities and similar between the two groups (99.7% in the control group versus 99.3% in the intervention arm; p = 0.41).

The impact of IPTc on malaria

Among children with fever or history of fever who had an RDT positive result, 8.8% (112/1277) turned out to have negative parasitaemia after microscopical diagnosis of malaria. The impact of IPTc on episodes of malaria detected through passive surveillance is presented in Table 2. The incidence of episodes of uncomplicated malaria (fever or a history of fever in the last 24 hours and asexual parasitaemia >=5000/µl) was much lower among children in the IPTc arm than among those in the control arm (0.34 episodes per child/year vs 1.9 episodes per child/year. The protective efficacy against malaria adjusted for age, gender and location was 82% (95% C: 78%-85% (p<0.001). An analysis of time to the first episode of clinical malaria, defined as above, also indicated a strong protective effect of IPTc (p <0.001) (Figure 2). The incidence of malaria defined as fever or a history of fever in the last 24 hours and positive asexual parasitaemia of any density was also much lower in children in the IPTc arm compared to those in the control arm (0.41 episodes per child/year vs 2.4 episodes per child/year), giving a protective efficacy of 83% (95% CI 80%-86%) (p<0.001 Only seventeen cases of severe malaria occurred during the follow up period, fifteen in the control group and two in the intervention group (Table 2) giving a protective efficacy of 87% (95% CI 42% - 99%) (p=0.001). The two cases of severe malaria in the intervention arm, one of whom died, occurred more than three weeks after the third course of IPT.

Incidence rates and the PE of IPTc against clinical malaria by locality and age category are presented in Table 3. Although the incidence of clinical malaria varied substantially between the three study localities, the protective efficacy of IPTc was similar in all three areas regardless of the definition of clinical malaria used. PE was higher in the lower age groups (3-11 months and 12-23 months) compared to the older age groups (above 24 months) when the definition of clinical malaria that incorporated the presence of parasitemia >=5000/µl or any parasitaemia was used (test for effect modification p=<0.001 and p =0.003 respectively).

The percentage of children with malaria infection detected at weekly active surveillance visits was 13.2% (74/563) in the control group compared to 1.9% (11/575) in the intervention group, giving a protective efficacy of 85%, (95% CI 73% - 92%) (p< 0.001). At the end of the transmission season, 13.2% (188/1423) of children in the control group were parasitaemic compared to 7.2% (101/1405) in the intervention group giving a protective efficacy of 46.%, (95% CI 31% - 68%) (p<0.001).

The impact of IPTc on anaemia

At the end of the malaria transmission season, the proportion of the children with anaemia (Hb < 11 g/dL), was significantly higher in the control group compared to the intervention group (61.1% [875/1433] versus 53.9% [766/1422]) (PE = 12%; 95% CI 3% - 20%) (p<0.001). The relative difference was larger for moderate anaemia (Hb <8 g/dL) with a prevalence of 3.5% (50/1433) versus 1.9% (27/1422) in the control and intervention groups respectively (PE= 47%; 95% CI 15% - 67%) (p=0.007). No cases of severe anaemia (Hb <5 g/dL) were observed in either treatment group at the time of the post intervention survey. However, during the follow –up period, a total of eight cases of severe anaemia occurred, two in the intervention arm and six in the control arm. The two subjects in the intervention group who developed severe anaemia had not received a complete course of IPT at the time that they developed their severe anaemia.

The impact of IPTc on nutritional indicators

The impact of IPTc on nutritional indicators is presented in Table 4. The proportions of children with wasting, stunting and being underweight at the end of the malaria transmission season were similar between the control and intervention arms However, weight gain during the intervention period was 97g (95% CI 37g – 157g) more among children in the intervention arm compared to that recorded among children in the control arm. Changes in height were similar between the two arms with an increase of 2.3 cm (95% CI 2.2 cm - 2.5 cm) in children in the intervention arm compared to an increase of 2.4 cm (95% CI 2.2 cm -2.5 cm) in children in the control arm.

The impact of IPTc on molecular markers of antimalarial drug resistance

The frequencies of molecular markers associated with resistance to SP and AQ in the two groups at baseline and post intervention are presented in Table 5. The frequencies of individual and multiple *dhfr* and *dhps* mutations in the placebo group were similar in pre- and post-intervention periods. The frequencies of all individual *dhfr and dhps* and of the triple *dhfr* (51, 59, 108) and quadruple *dhfr* (51,59,108) + *dhps* 437 mutations were higher in the intervention than in the control group at the end of the surveillance period and, for the *dhfr* 59, dhps 437, triple and quadruple mutations, differences between groups were statistically significant. Frequencies of the *pfcrt* 76 and *pfmdr1* 86 did not change significantly over time and were similar post-intervention in the intervention and control groups.

The impact of IPTc on hospital admissions and death.

Hospital admissions and deaths that occurred during the study period are listed in Table 6. Nineteen hospital admissions of at least 24 hours were recorded; 9 of these were recorded in children in the control arm and 10 in children in the intervention arm. The incidence rates of hospital admissions per child/year were 0.0225 episodes in the control group versus 0.0251 in the intervention arm ($p = 0.81$). There were 5 deaths, 2 in the control arm and 3 in the intervention arm. Two of the five deaths were due to malaria (one in each group). Both occurred during hospitalisation while the remaining three deaths occurred at home. On the basis of the results of a verbal autopsy these were thought to be due to poisoning by traditional medicines, meningitis and anaemia and secondary bleeding following a circumcision respectively.

Safety and Tolerability.

There was no serious adverse event related to the study drugs. The frequencies of adverse events following the administration of IPTc with SP + AQ or placebo, using active surveillance are summarized in Table 7. The frequencies of adverse events were similar between the control and intervention arms. However, there was a tendency toward a higher frequency of vomiting and of loss of appetite in the intervention arm compared to the control arm (4.0% versus 1.9%, $p = 0.06$ for vomiting and 1.9% versus 0.8%, $p = 0.08$ for loss of appetite). Proportions of children with skin rash and itching on at least at one occasion were similar between the two arms. Four subjects in the intervention arm were withdrawn from the study because of reactions to study drug versus none in the control arm. Two of these children had a documented skin rash at physical examination (one after the first dose of IPT and the other after the second dose of IPT) and these were assessed as being related to study drugs. Both were moderate in intensity, did not involve bullous eruptions and resolved within 2 days. The parent of the third subject reported itching. Physical examination was normal but the subject was withdrawn from the study on precautionary grounds. The fourth subject had an acute respiratory infection at the time of administration of the first dose of IPT. No adverse event was recorded at the time of routine surveillance but the parents requested withdrawal of their child from the study at the time of the 2nd round of IPTc.

DISCUSSION

This study has shown that three doses of IPTc with SP +AQ given at monthly intervals during the peak transmission season reduced the incidence of uncomplicated and severe malaria by 80% in children 3-59 months of age who slept under an ITN in three localities in Mali despite the difference in ITN use at baseline.

This is a similar level of protective efficacy to that reported in a previous trial conducted in an area of Senegal with a coverage of ITNs of less than 1% [6], suggesting that the efficacy of IPTc is not reduced by the use of an ITN at the time of the intervention. Two studies have shown that in pregnant women, IPT adds little benefit to the protection afforded by an ITN, at least in multigravidae [17, 18]. This is not the case for IPTc in children, as the strategy remained highly efficacious even when deployed in a community with a high usage of ITNs.

Despite the large difference in background incidence of malaria in the three sites, suggesting high variability in transmission intensity, the protective efficacy of IPTc against clinical malaria was high and similar between the three sites. This suggests that similar efficacies of IPTc against clinical malaria can be expected in areas with different transmission intensities and baseline ITN coverage. Surprisingly, Siby and Ouelessebougou, which had a low EIR (less than 10 infective bites per person/ season), had a higher malaria attack rate than Djoliba which had a higher EIR (37 infective bites per person/ season). High malaria infection and attack rates have been reported previously in the context of a low EIR in Mali [19, 20] but this apparently anomalous result could have been due to sampling error in the determination of a EIR, which can vary markedly with time and space. Early detection and treatment of malaria cases is known to reduce hospital admission and deaths due to malaria [21, 22]. Early detection and prompt treatment was available in our carefully controlled study and the protective effect of IPTc on severe malaria or death might be more marked than we observed if IPTc was deployed in a community which did not have such ready access to health care. Parasite prevalence, as assessed by weekly surveys during the intervention period was reduced by 85% in children who received IPTc but this difference dropped to 46% at the end of the intervention period suggesting that the prophylactic effect of the last dose of SP + AQ had begun to decline six weeks after administration, as has been found in studies of IPTi [23].

We observed a 47% reduction in the proportion of children with moderately severe anaemia (Hb <8g/dL) as a result of administration of IPTc. This impact on anaemia is consistent with the reduction of 45% in incidence of anaemia observed when AS +AQ was given at monthly intervals over six months in Ghana, although in the Ghanaian study there was no difference in the proportion of children with anaemia at the end of the 6 months intervention [8]. We did not detect any difference between the intervention and control arms in wasting, stunting or under weight. This is consistent with a previous study in Senegal [24], which did not find evidence of an impact of IPTc on wasting, stunting or being under weight at the end of the transmission season but only on triceps and subcapular skinfold, indicators that were not assessed in our study. However,

in line with the Senegalese study, we found an increase in weight gain in the IPTc arm compared to the control arm during the course of the intervention period. More marked effects on nutritional measurements were found during a parallel study conducted in Burkina Faso [25], perhaps because the force of infection was higher in the Burkina Faso than in the Mali study areas and malaria thus a more important contributor to impairment of weight gain in the Burkina Faso than in the Mali study areas.

SP+AQ was chosen as the drug combination for use in the trial on the basis of the results of previous studies which had shown this to be an effective combination for IPTc. This drug combination was generally well tolerated and no serious adverse event attributable to the study drugs was reported. The proportions of children with mild to moderate adverse events using active surveillance were not significantly different between the two arms, although there was a trend towards a higher frequency of vomiting and loss of appetite in the intervention group. In the parallel study in Burkina using the same drugs, a higher frequency of vomiting was found in the intervention arm [25]. However, even in the placebo group the frequency of vomiting was higher than in this study, suggesting a difference in the way in which minor side effects were solicited in the two study areas. Cisse et al [6] reported a modest increase in vomiting in children who took SP + AS compared to those who took placebo in Senegal, while Kweku et al [8] found no difference in incidence of these adverse events between IPTc intervention and control arms when using SP or amodiaquine. Four withdrawals in the intervention arm were reported to be due to reactions to study drugs. In two cases the presence of a skin rash was confirmed, another child had itching and the final withdrawal followed the occurrence of an acute respiratory tract infection at the time of administration of the first round of IPTc. It is possible that this was considered by the parents as a reaction to the study drugs. The safety of SP and AQ has been a concern in relation to their use for IPTc [26-29]. However, there is a growing body of evidence from studies in the last few years [3, 5, 6, 8, 25, 29] that these drugs are safe when used for IPT in pregnant women, infants or children and no safety concerns have arisen following the use of SP + AQ for IPTc on a large scale in Senegal.

The efficacy of IPTc against clinical malaria has now been demonstrated in a number of studies, including the current trial and a parallel one conducted in Burkina Faso [25]. Is the evidence now strong enough to support the introduction of IPTc into countries with seasonal malaria transmission? Evidence from studies of IPT in infants [30, 31] suggests that prophylaxis is the key protective mechanism of IPT and that long acting drugs are needed for effective IPTc. Currently, the SP + AQ combination meets this requirement in West Africa where both of these drugs are still reasonably effective as has been shown to be the case in the study

area (supplementary information 3). Studies conducted in Senegal and in Ghana [7, 29] have compared different drug combination and regimens and shown that currently SP + AQ at monthly interval is the best combination. However, the continuing efficacy of SP cannot be guaranteed and alternative regimens for IPTc will be required in the future which might include the long-acting drug piperaquine.

Unlike the case of IPT in pregnant women and infants, IPT in children has no established delivery system, raising concerns as to whether it could be implemented as a control measure. However, studies conducted in Ghana, Senegal and Gambia have shown that high coverage with IPTc can be obtained using community health workers [29,30] and this appears the most promising way of delivering this intervention.

Another concern over the widespread deployment of IPTc is that this will enhance the spread of drug resistance. Therefore, we studied the presence of molecular markers associated with resistance before and after the intervention in children in the intervention or control group. The *dhfr 59* and *dhps 437* mutations associated with pyrimethamine and sulphadoxine resistance respectively were found significantly more frequently at the end of the malaria transmission season in parasites obtained from children in the intervention group than in those obtained from children in the control group and this led to higher frequencies of the triple *dhfr* mutants and the quadruple mutant (triple *dhfr* + *dhps 437*) associated with significant resistance to SP in children who had received IPTc. This increase in the frequency of these mutations is consistent with a previous report in Senegal [6]. As in Senegal, the number of children in the intervention group carrying a resistant parasite was less than in children in the control group because of the substantial reduction in the overall prevalence of parasitaemia. Although IPTc may have contributed to the increase in frequency of some of resistant markers in this and other studies, the true impact on the resistance of SP and AQ remains to be established. Despite a prevalence of quadruple mutants of about 37%, the SP + AQ combination was highly effective in clearing parasitaemia from children resident in the study area with asymptomatic parasitaemia (see supplementary information appendix 3).

As is the case with any successful malaria intervention, administration of IPTc to children during several, successive malaria transmission seasons could interfere with the development of naturally acquired immunity, raising concerns that there would be an increased period of risk (rebound malaria) during the period immediately after the intervention was stopped if exposure levels remained high. The risk of malaria for children in this trial in the year after the intervention was stopped has been studied and the results are currently being analysed. However, several years of administration would be needed to define the degree to

which acquisition of natural immunity would be impaired. It is very unlikely that this would outbalance the substantial gains made during the period when the drug was given.

Our study has several strengths. First, the doubled blind, randomised controlled design prevented a number of biases in the selection assignment of the subjects to the two arms as well as in assessing the outcomes. A second strength is that this is the largest IPTc efficacy trial done so far, providing a more precise estimation of the outcomes measured. Third, the trial was conducted in three localities with different malaria incidence rates, allowing the efficacy of this strategy under different levels of malaria transmission to be assessed. The design would have been stronger if a factorial design had been used to assess the individual and combined impact of IPTc and ITN, but such a trial would be unethical as the efficacy of ITN is already established [2] and use of ITNs is policy in Mali. Other potential limitations of the study include the duration of evaluation which focused only on about 15 weeks of follow up during the malaria transmission season. However, it is well established that the in the Sahel region of Mali, 85 -90% of clinical malaria cases occur during the period of August to November, and efficacy of this strategy remained high in a previous, smaller study when efficacy was computed over 12 months period [5, 33].

In summary, IPTc given during the malaria transmission season, provided substantial additional protection against clinical malaria, infection with malaria and anaemia to that provided by ITNs. IPTc with SP+AQ was safe and well tolerated. As the international community moves towards the target of malaria elimination new malaria control tools will be needed [10]. IPT in children targeting the transmission season appears to be one of the strongest available tools to achieve this goal. Our findings support the need for an early review of whether IPTc can now be recommended as a component of malaria control in areas with seasonal malaria transmission.

Contributors

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Conflict of interest

The authors have no competing interests.

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Figure 1. Trial profile.

Figure 2. Time to first episode of clinical malaria defined as fever (temperature >= 37.5 ^{o} C) or history of fever in the last 24 hours and parasitaemia >=5000/µl in the intervention and control arms. Kaplan-Meier survival estimates with pointwise 95% confidence bands.

Table 1: Baseline characteristics of enrolled children at the time of administration of the first dose of IPTc.

	IPTc	Placebo
	% (n/N)	% (n/N)
Age (months)		
3 - 11	18.2 (274/1509)	18.5 (278/1508)
12 - 23	22.5 (339/1509)	20.5 (309/1508)
24 - 35	20.5 (310/1509)	22.0 (332/1508)
36 - 47	20.0 (302/1509)	19.4 (293/1508)
48 – 59	18.8 (284/1509)	19.6 (296/1508)
Gender		
Male	47.7 (720/1509)	50.1 (755/1508)
Female	52.3 (789/1509)	49.9 (753/1508)
Weight (kg)		
5- 9 kg	34.8 (525/1509)	34.7 (523/1508)
10-18 kg	63.1 (952/1509)	63.2 (953/1508)
19 kg +	2.1 (32/1509)	2.1 (32/1508)
Nutritional factors		
Underweight	16.1 (238/1480)	15.1 (223/1477)
Wasting	11.0 (163/1480)	12.5 (185/1477)
Stunting	22.7 (336/1480)	23.8 (352/1477)
Fever	7.2 (105/1460)	7.6 (111/1464)

Table 2. Impact of IPTc on episodes of clinical malaria in children in Mali.

	IPTc			Placebo							
	No of episodes	Years at risk^	Incidence rate* (95% CI)	No of episodes	Years at risk	Incidence rate* (95% CI)	Unadjusted IRRs (95% CI)	P-value	Adjusted** IRRs (95% CI)	PE (95%CI)	P-value
Fever or history of fever & any asexual parasitaemia	149	362.15	0.41 (0.35 – 0.48)	832	345.64	2.40 (2.25 – 2.58)	0.17 (0.14 – 0.20)	<0.001	0.17 (0.14 – 0.20)	83 (80 – 86)	<0.001
Fever or history of fever & parasitaemia >=5000	126	369.41	0.34 (0.29 – 0.41)	672	354.14	1.90 (1.76 – 2.05)	0.18 (0.15 – 0.22)	<0.001	0.18 (0.15 – 0.22)	82 (78 – 85)	<0.001
Severe malaria	2	399.10	0.005 (0.0006 - 0.0181)	15	400.87	0.037 (0.0209 - 0.0617)	0.13 (0.01-0.58)	0.001	-	87 (42 – 99)	0.001

^ Children were not considered at risk for 21 days after each type of a malaria episode
* Incidence rate/child/year. Note the incidence relate refers to only the three month surveillance period and is not an annual rate.
** adjusted for age, gender and location. 95% CI constructed using a robust standard error.

Table 3: Effect of area of residence and age on the protective efficacy of IPTc against clinical episodes of malaria

	IPTc		Placebo						
	Episodes (time at risk)	Incidence rate*	Episodes (time at risk)	Incidence rate*	Unadjusted RR (95% CI)	P-value	Adjusted RR (95% CI)	PE (95%CI)	P-value
Clinical malaria defined as fever or history of fever in the last 24 hours and asexual parasitemia >=5000/µl.									
Locality									
Djoliba	11 (73.77)	0.15 (0.08 - 0.27)	74 (73.49)	1.00 (0.80 - 1.26)	0.13 (0.10 – 0.18)	<0.001	0.15 (0.08 - 0.28)	85 (72 - 92)	<0.001
Siby	70 (93.32)	0.75 (0.59 - 0.94)	308 (90.57)	3.40 (3.04 - 3.80)	0.13 (0.07 – 0.24)	<0.001	0.22 (0.17 – 0.29)	78 (71 - 83)	<0.001
Ouelessebougou	45 (202.55)	0.22 (0.17 - 0.30)	292 (190.20)	1.53 (1.37 - 1.72)	0.21 (0.16 - 0.26)	<0.001	0.14 (0.10 - 0.20)	86 (80 - 90)	<0.001
Age									
3 -11 months	6 (68.64)	0.09 (0.04 - 0.19)	52 (66.60)	0.78 (0.59 - 1.02)	0.11 (0.05 - 0.26)	<0.001	0.13 (0.05 - 0.29)	87 (71 - 95)	<0.001
12 -23 months	12 (83.00)	0.14 (0.08 - 0.25)	134 (72.40)	1.85 (1.56 - 2.19)	0.08 (0.04 - 0.14)	<0.001	0.07 (0.04 - 0.13)	93 (87 - 96)	<0.001
24 - 35 months	38 (76.63)	0.50 (0.36 - 0.68)	173 (77.96)	2.22 (1.91 - 2.58)	0.22 (0.15 - 0.33)	<0.001	0.23 (0.15 - 0.34)	77 (66 - 85)	<0.001
36 - 47 months	36 (72.51)	0.50 (0.36 - 0.69)	153 (67.84)	2.26 (1.92 - 2.64)	0.22 (0.15 - 0.31)	<0.001	0.21 (0.15 - 0.31)	79 (69 - 85)	<0.001
48 – 59 months	34 (66.91)	0.51 (0.36 - 0.71)	156 (66.43)	2.34 (2.00 - 2.74)	0.21 (0.14 - 0.32)	<0.001	0.22 (0.15 - 0.32)	78 (68 - 85)	<0.001
Clinical malaria defined fever or history of fever in the last 24 hours and asexual parasitemia regardless of the density									
Locality									
Djoliba	12 (72.05)	0.17 (0.09 - 0.29)	90 (73.40)	1.22 (1.0 – 1.50)	0.13 (0.10 – 0.18)	<0.001	0.14 (0.10 – 0.18)	86 (82 –90)	<0.001
Siby	83 (90.86)	0.91 (0.74 - 1.13)	372 (86.41)	4.30 (3.88 – 4.76)	0.13 (0.07 – 0.24)	<0.001	0.14 (0.07 – 0.26)	86 (74 –93)	<0.001
Ouelessebougou	54 (199.25)	0.27 (0.21 - 0.35)	370 (185.82)	1.99 (1.80 – 2.20)	0.21 (0.16 - 0.26)	<0.001	0.21 (0.16 - 0.26)	79 (74 - 84)	<0.001
Age									
3 - 11 months	10 (68.15)	0.15 (0.08 - 0.27)	72 (65.96)	1.09 (0.86 - 1.38)	0.13 (0.07 - 0.26)	<0.001	0.14 (0.07 - 0.27)	86 (73 - 93)	<0.001
12 -23 months	15 (81.60)	0.18 (0.11 - 0.30)	153 (71.07)	2.15 (1.83 - 2.52)	0.08 (0.05 - 0.14)	<0.001	0.08 (0.05 - 0.14)	92 (86 - 95)	<0.001
24 - 35 months	47 (75.18)	0.62 (0.47 - 0.83)	206 (76.20)	2.70 (2.36 - 3.10)	0.23 (0.17 - 0.31)	<0.001	0.23 (0.16 - 0.33)	77 (67 - 84)	<0.001
36 - 47 months	39 (70.23)	0.55 (0.40 - 0.76)	200 (65.44)	3.06 (2.66 - 3.51)	0.18 (0.13 - 0.25)	<0.001	0.18 (0.13 - 0.25)	82 (75 - 87)	<0.001
48 – 59 months	38 (65.04)	0.58 (0.42 – 0.80)	194 (63.98)	3.03 (2.63 - 3.49)	0.19 (0.13 - 0.27)	<0.001	0.19 (0.13 - 0.28)	81 (72 – 87)	<0.001

* Incidence rate expressed as number of episodes/child/year. Note that this is based on the three month surveillance period and does not correspond to an annual rate.

Table 4. Effect of IPTc on nutritional indicators in children at the end of the malaria transmission season.

	Placebo		**IPTc**		**Adjusted analysis**	
	%	N	%	N	OR *(95% CI)	P-value
Wasting	5.6	1364	4.3	1360	0.75 (0.53 – 1.07)	0.12
Stunting	25.2	1365	24.6	1361	0.96 (0.81 – 1.15)	0.69
Underweight	12.8	1365	10.9	1361	0.84 (0.66 – 1.06)	0.15

*Adjusted for age, sex and locality

Table 5. Frequencies of molecular markers of resistance to SP and AQ at baseline and at the end of the intervention period in intervention and control arms.

	Baseline		**Post intervention**					**Baseline vs overall post-intervention**
			IPTc		Placebo		P	P
Molecular Markers	**n**	**% mutant**	**n**	**% mutant**	**n**	**% mutant**		
DHFR 51	48	62.5%	78	75.6%	148	66.2%	0.144	0.35
DHFR 59	48	60.4%	78	76.9%	148	59.5%	0.009	0.50
DHFR 108	41	78.0%	76	78.9%	139	71.2%	0.217	0.58
DHPS 437	47	38.3%	83	67.5%	165	43.6%	<0.001	0.09
DHPS 540	45	0%	82	7.3%	165	3.6%	0.205	0.82
Triple DHFR mutations	41	58.5%	76	69.7%	139	54.0%	0.024	0.90
Quadruple mutants (triple DHFR + DHPS 437)	41	22.0%	75	53.3%	139	28.1%	< 0.001	0.07
Pfcrt-76	46	80.4%	79	84.8%	156	75.0%	0.085	0.25
Pfmdr1-86	46	45.6%	76	36.8%	156	34.6%	0.739.	0.19

n = number of subjects with parasitemia at blood smear tested

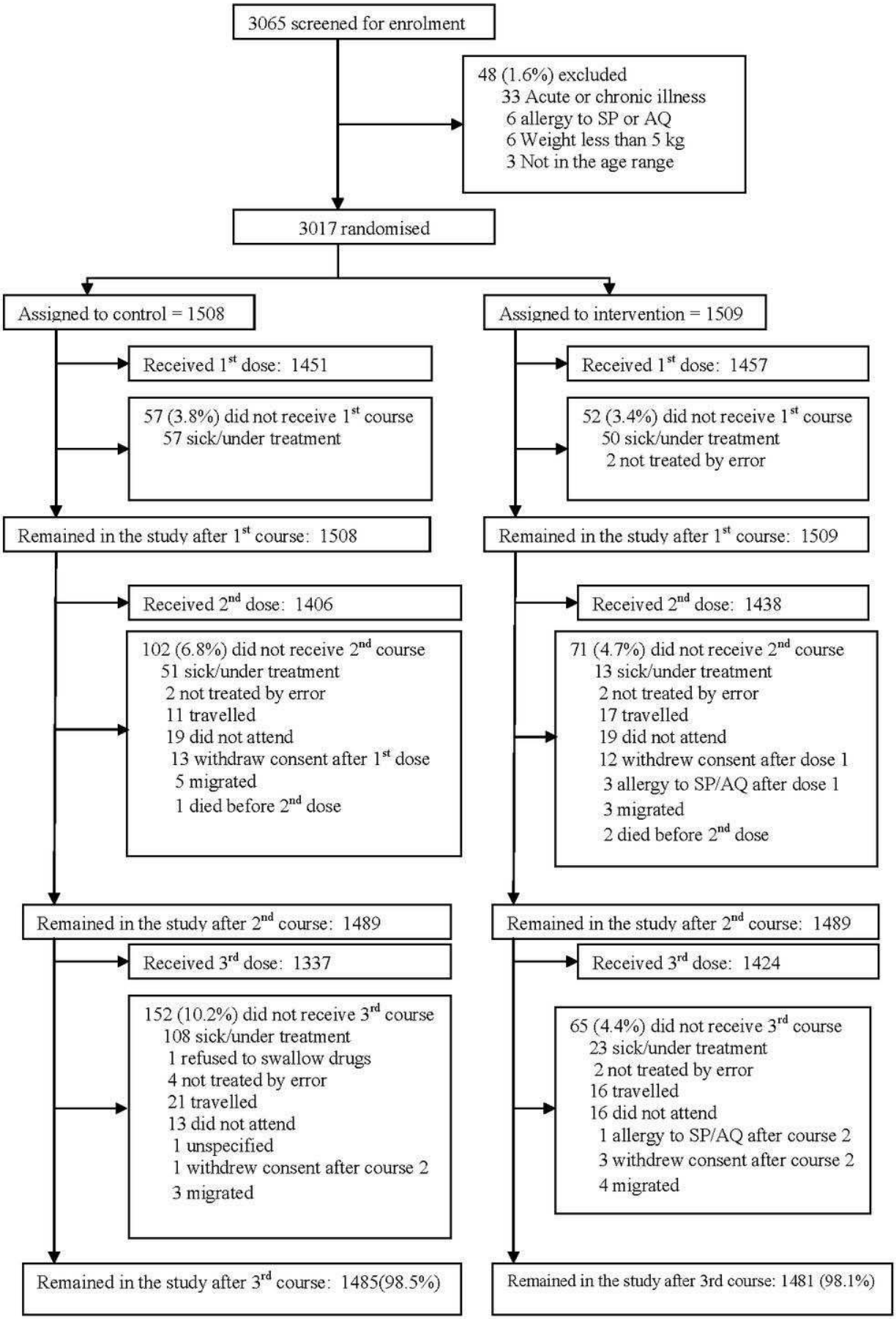
Table 6. Hospital admissions and deaths by treatment arms.

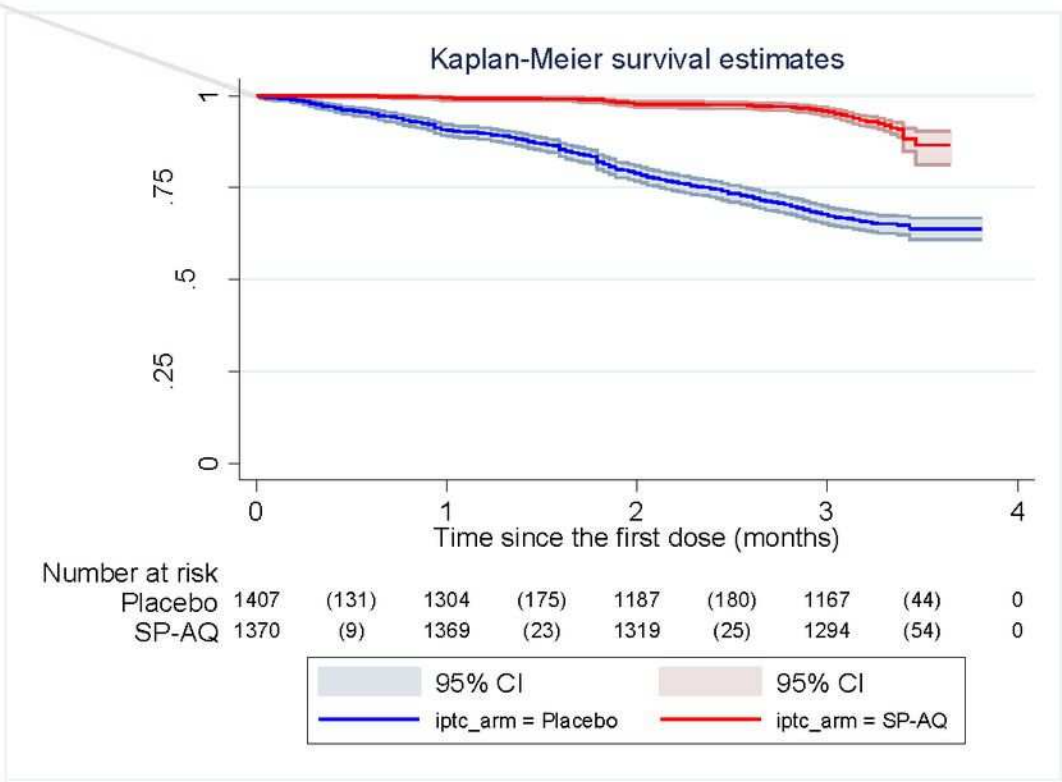
Hospital admission				
#	Treatment arm	Date	Cause	Outcome
1	Placebo	11/8/2008	Severe malaria	Recovered
2	Placebo	10/25/2008	Severe anaemia	Recovered
3	Placebo	9/27/2008	Severe malaria	Recovered
4	Placebo	11/9/2008	Severe malaria	Recovered
5	Placebo	12/7/2008	Severe malaria	Recovered
6	Placebo	9/18/2008	Severe malaria	Recovered
7	Placebo	9/16/2008	Severe malaria	Death
8	Placebo	9/18/2008	Severe malaria	Recovered
9	Placebo	11/16/2008	Severe malaria	Recovered
10	IPTc	11/23/2008	Gastro-enteritis	Recovered
11	IPTc	11/5/2008	Severe malaria	Death
12	IPTc	12/2/2008	Respiratory infection	Recovered
13	IPTc	10/11/2008	Gastro-enteritis	Recovered
14	IPTc	8/13/2008	Severe anaemia	Recovered
15	IPTc	9/21/2008	Asthma	Recovered
16	IPTc	12/3/2008	Severe malaria	Recovered
17	IPTc	11/11/2008	Respiratory infection	Recovered
18	IPTc	11/4/2008	Febrile convulsions	Recovered
19	IPTc	9/19/2008	Respiratory infection	Recovered
Deaths out of hospital*				
1	Placebo	11/08/08	Intoxication to traditional medicines	-
2	IPTc	09/11/08	Meningitis	-
3	IPTc	09/17/08	Anaemia secondary to circumcision	-

* Does not include death occurred following hospital admissions listed above

Table 7: Proportions of children with adverse events on at least one occasion during three rounds of IPTc treatment using the active surveillance.

	IPTc	**Placebo**		
	% (n/N)	**% (n/N)**	**ORs (95% CI)**	**p**
Fever	10.1 (69/686)	9.9 (66/669)	1.02 (0.72 – 1.46)	0.91
Vomiting	4.0 (19/475)	1.9 (9/473)	2.1 (0.96 – 4.80)	0.06
Drowsiness	0.1 (1/686)	0 (0/669)	-	-
Itching	1.0 (7/686)	0.6 (4/667)	1.7 (0.50 – 5.86)	0.39
Diarrhoea	6.7 (46/686)	4.6 (31/669)	1.48 (0.92 – 2.36)	0.10
Skin rash	0.3 (2/686)	0.8 (5/668)	0.39 (0.7 – 2.0)	0.26
Coughing	8.2 (56/686)	6.0 (40/631)	1.40 (0.92 – 2.13)	0.12
Loss of appetite	1.9 (13/686)	0.8 (5/668)	2.56 (0.90 – 7.22)	0.08
Jaundice	0 (0/686)	0.1 (1/667)	-	-


3065 screened for enrolment
48 (1.6%) excluded
33 Acute or chronic illness
6 allergy to SP or AQ
6 Weight less than 5 kg
3 Not in the age range
3017 randomised
Assigned to control = 1508
Assigned to intervention = 1509
Received 1st dose: 1451
57 (3.8%) did not receive 1st course
57 sick/under treatment
Received 1st dose: 1457
52 (3.4%) did not receive 1st course
50 sick/under treatment
2 not treated by error
Remained in the study after 1st course: 1508
Remained in the study after 1st course: 1509
Received 2nd dose: 1406
102 (6.8%) did not receive 2nd course
51 sick/under treatment
2 not treated by error
11 travelled
19 did not attend
13 withdraw consent after 1st dose
5 migrated
1 died before 2nd dose
Received 2nd dose: 1438
71 (4.7%) did not receive 2nd course
13 sick/under treatment
2 not treated by error
17 travelled
19 did not attend
12 withdrew consent after dose 1
3 allergy to SP/AQ after dose 1
3 migrated
2 died before 2nd dose
Remained in the study after 2nd course: 1489
Remained in the study after 2nd course: 1489
Received 3rd dose: 1337
152 (10.2%) did not receive 3rd course
108 sick/under treatment
1 refused to swallow drugs
4 not treated by error
21 travelled
13 did not attend
1 unspecified
1 withdrew consent after course 2
3 migrated
Received 3rd dose: 1424
65 (4.4%) did not receive 3rd course
23 sick/under treatment
2 not treated by error
16 travelled
16 did not attend
1 allergy to SP/AQ after course 2
3 withdrew consent after course 2
4 migrated
Remained in the study after 3rd course: 1485(98.5%)
Remained in the study after 3rd course: 1481 (98.1%)


Kaplan-Meier survival estimates
1
.75
.5
.25
0
0
1
2
3
4
Time since the first dose (months)
Number at risk
Placebo 1407 (131) 1304 (175) 1187 (180) 1167 (44) 0
SP-AQ 1370 (9) 1369 (23) 1319 (25) 1294 (54) 0
95% CI
95% CI
iptc_arm = Placebo
iptc_arm = SP-AQ

SUPPLEMENTARY INFORMATION 2- ENTOMOLOGICAL INVESTIGATIONS.

A surprising feature of the study described in this paper is the high malaria attack rate in children who slept under an ITN and who did not receive IPTc. This may have been due in part to the fact ITNs were given only to children in the trial and not to the whole community. However, a number of other studies were undertaken to explore this finding further.

1. Entomological Inoculation Rate in the study area

Introduction

A small study was undertaken in the three localities Djoliba, Siby and Ouelessebougou in the district of Kati in Mali from August to December 2009 to measure the Entomological Inoculation Rate (EIR)

Methods

Catches were performed in 10 houses on two consecutive nights (from 6:00PM to 6:00AM) per week per village using CDC light traps (J. W. Hock Co, Gainesville, Florida, USA).. Trapping bags were emptied the next morning. Mosquitoes collected were then sorted by species, counted and stored in tubes containing silica gel. Tubes were labelled with the following information: village code, date, the mosquito species and numbers by species. Mosquitoes were transported from the field to the laboratory and stored in boxes at room temperature until processing.

Heads and thoraces of mosquitoes caught in the light traps were tested for *Plasmodium falciparum* circumsporozoite protein (CSP) by ELISA essay. A representative sample was tested when the numbers of mosquitoes collected was too large. The EIR was computed for each study site by multiplying the sporozoite rate (proportion of mosquitoes tested positive for CSP by ELISA) by mean vector man biting rate per month.

Results

A total of 10,022 mosquitoes were collected in the three sites (8,444 in Djoliba, 493 in Siby and 1085 in Ouelessebougou) during the 1287 catches (427 in Djoliba, 426 in Siby and 434 in Ouelessebougou) carried out from August to December 2009. The infection rates were 1.1% (35/3282) in Djoliba, 4.9% (17/344) in Siby and 1.8% (16/879) in Ouelessebougou. The cumulative entomological inoculation rates during this five-month period in the three study localities were 37.3 (95% CI -22.3 -44.4), 9.4% (95% CI 5.4-14.6), and 6.6 (95% CI 3.8 -10.7) infective bites per person in Djoliba, Siby and Ouelessebougou respectively.

Conclusions

The EIR in the study sites of 7-37 infective bites per person per season is compatible with an incidence rate of 1-3 clinical attacks of malaria per season in young children in these sites and consistent with previous reports from Mali [1,2] and thus with an attack rate of 0.5 -1.5 clinical attacks per season in children sleeping under an ITN, as observed in this study.

2. Deltamethrin content of treated nets

Introduction

At the beginning of the study, the family of each study child was provided with an LLIN ((PermaNet® Vestergaard) to be used by that child during the course of the study and with instructions on how to use it. To confirm that the ITNs used in the IPTc study had retained chemical activity and were thus likely to be effective, an analysis was made of the deltamethrin content of a random sample of nets being used by study children the end of the malaria transmission season.

Methods

Nets from 49 children randomly selected from the cohort of children enrolled into the study were collected in December 2009 and tested for their content of deltamethrin. Removed mosquito nets were replaced by new ones. Two pieces of netting were obtained from each side of the net and from its roof. The deltamethrin was extracted from the netting samples with solvents and the deltamethrin content of the extract determined by high performance liquid chromatography as previously described [3].

Results

Assays were undertaken on 10 samples (2 per side) obtained from each of 49 nets. Deltamethrin was detected in one sample from all the 49 netting samples tested, confirming that study children had been using an ITN but in some cases only in a few pieces of the net (nets washed >5 times and/or very dirty). However, in most cases content remained in the range likely to be associated with protection.

Conclusions

The results confirmed that the nets used in the study had been treated with an appropriate concentration of deltamethrin. Lower concentrations than expected were found in some samples which may have been due to the fact that the nets had been washed several times, as is common practice in the study area.

3. Pyrethroid resistance

Introduction

A possible explanation for the apparent lack of efficacy of efficacy of the ITNs is that anopheline mosquitoes in the study area have become resistant to deltamethrin and studies were carried out to explore this possibility.

Methods

To assess the resistance of the mosquitoes to the pyrethroids, *An gambiae* s.l., collected in the study areas were tested using the method of WHO (1998). A hundred mosquitoes were exposed to filter paper impregnated with pyrethroids (deltamthrin permethrin) or DDT for one hour and morality after 24 hours was measured. Forty mosquitoes randomly selected among those exposed to each of the three pyrethroids in each of the tree sites (120/site) were tested for the *kdr* gene mutations using the methods described by Martinez-Torres et al. [4] and Ranson et al. [5].

Random samples of 10 long lasting insecticide treated net (LLIN) allocated to study children in each village were removed in October –November 2009 and tested for their killing effect on mosquitoes. Fifty mosquitoes were exposed for three minutes to the LLIN to estimate mosquito knock-down time using the WHO standard protocol [6].

Results

Mortality 24 hours after the exposition to deltamethrin for one hour was 100% in all the three sites. The mortality 24 hours after the exposition to permethrin was 100% in Djoliba, 62% in Ouelessebougou and 46% in Siby. The corresponding figures after exposure to DTT were 100%, 100% and 97% respectively. None of the 360 mosquitoes tested carried the *kdr* gene mutation.

The efficacy of the study nets (defined as mosquitoes mortality 24 hours after exposure to the net) was 100% in Djoliba, 100% in Siby and 94% in Ouelessebougou giving and overall efficacy in the area of was 98% l after an average number of washing of 3.2 (95% CI 2.2- 4.1).

Conclusions

The results of this small study suggest that there is no resistance in *Anopheles gambiae* to deltamethrin in the area where the study was done.

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SUPPLEMENTARY INFORMATION SHEET 3

In vivo efficacy of the sulphadoxine/pyrimethamine + amodiaquine combination used for IPTc in the study area.

Introduction

In order to confirm the ability of the sulphadoxine/pyrimethamine (SP) + amodiaquine (AQ) combination used in the IPTc study to clear malaria parasitaemia in asymptomatic children, a small observational study was undertaken in children aged 1-6 years with proven *Plasmodium falciparum* parasitaemia but no symptoms who were resident in the study area in 2009, the year after the IPTc intervention had been conducted.

Methods

In November/December 2009, available children enrolled in the main IPTc efficacy study were tested for malaria parasite using a Rapid Diagnostic Test (Optimal®). Blood films obtained from the children were examined for asexual malaria parasitemia if the Rapid Diagnostic Test is positive. A total of 247 assymptmatic children with asexual P. falciparum parasitaemia at blood smear were enrolled in this sub-study and treated with SP + AQ. Doses of SP and AQ were given on the basis of weight as in the IPTc study. SP was given once at the doses of 175/8.75 mg, 350/17.5 mg and 550/26.25 mg to children who weighed 5-9 kg, 10 – 18 kg and 19 kg and above. AQ was given over three days at the daily dose of 70 mg, 140 mg and 220 mg to children who weighed 5- 9 kg, 10-18 kg and 19 or > kg, respectively. Children were followed-up for 28 days with clinical examination at days 0, 1, 2, 7, 14, 21, and 28. Blood smears and blood blotted onto filter paper samples were collected at days 0, 7, 14, 21 and 28 and at any unscheduled visit for fever or a history of fever. Thick blood films were air dried, stained with Giemsa and examined for malaria parasites by two well-trained technicians. One hundred high power fields were counted before a film was declared negative. Parasite density was determined by counting the number of parasites present per white blood cell (WBC) on a thick smear and assuming a WBC count of 8,000 per µl. In the case of a discrepancy (positive/negative or a difference in parasite density greater than 30%), a third reading was done. The median parasite density of two or three readings was used. Re-infections were differentiated from recrudescences on the basis of the detection of different msp 1 and msp 2 alleles in paired samples as previously described[1, 2].

Results

Of the one hundred forty seven children with asymptomatic malaria were enrolled and treated, one was excluded on day 1 because of allergy to study drugs and the remainder received the complete

course of SP+AQ. Two subjects were treated with Coartem (on days 7 and 9 respectively) based on the occurrence of clinical features suggestive of malaria and positive RDT although no asexual malaria parasites were seen on a subsequent blood smear. Blood smears for 27 subjects at day 7 were altered and not readable. Three children had a positive blood film for P. falciparum on day 28. However, all three were classified as reinfections by PCR so that the 28 days PCR corrected efficacy of SP+AQ in clearing *P falciparum* in asymptomatic children was 100% (144/144).

Conclusion

The results of this small observational study confirm the *in vivo* efficacy of the SP + AQ combination in clearing malaria parasitaemia from asymptomatic children in the study area. As noted in previous studies of intermittent preventive treatment in pregnant women and in infants, drugs or drug combination may retain high efficacy in clearing low density parasitamemia from asymptomatic subjects after they have lost some of their efficacy in treating clinical infections in young children.

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MALARIA DIAGNOSTICS AND CONTROL CENTRE OF EXCELLENCE (MDCoE)

EXTERNAL QUALITY ASSURANCE OF MALARIA MICROSCOPIC DIAGNOSIS

17 May 2010

Final Malaria Blood Film Concordance Report for LSHTM IPTc/ITNs Project

Background

Following the Quality Assurance/Quality Control (QA/QC) activity carried out on London School of Hygiene and Tropical Medicine (LSHTM) slides from Mali and Burkina Faso in June and July 2009, site results were sent to Malaria Diagnostics Center of Excellence (MDCoE) for comparative analysis. The objective was to determine the concordance between site and MDCoE results in parasite detection (positive/negative), species identification, and parasite quantitation. Results were entered and merged in Microsoft Access. Slides were classified as either positive or negative if two of the initial readers i.e. reader 1 and 2 results agree or any of the initial reader's result agrees with that of the reference reader in case the initial results were discordant. The same criterion was applied in classification of species. Densities were calculated based on parasite counts against white blood cells (WBC) or counts of parasitized red blood cells (RBC) depending on the counts reported. Counts against WBC were converted using World Health Organization recommend count of 8,000 cells per microliter (µL). Counts of parasitized RBC were converted using 4.5 x 10^6 cells/µL. Densities were considered to be concordant if the difference between the paired results was within 0.5 – 2.0 fold.

Summary of Reported Results by Site and MDCoE

Classification	MRTC	MDCoE	Agreement	CNRFP	MDCoE	Agreement
Negative	239	228	221	176	167	162
Positive	50	51	43	110	119	105
P. falciparum	50	51	43	106	111	98
P. malariae	0	0	0	2	1	1
P. ovale	0	0	0	0	3	0
P. falciparum + P. malariae	0	0	0	2	3	1
P. falciparum + P. ovale	0	0	0	0	1	0
Gametocytes	4	1	1	16	34	2

MRTC Results

Of the 289 slides submitted only 279 reads were paired. 10 of the slide could not be read because of poor quality of the smears. MRTC reads reported 86% (239/279) as negative and 18% (50/279) as positive. Of the positive reads, all were (50/50) classified as pure *P. falciparum* infections. Gametocytes were reported on 1% (4/279) of the positive slides. MDCoE classified 82% (228/279) as negative and 18% (51/279) as positive. Of the positive reads, all were reported as (51/51) as pure *P. falciparum* infections. Presence of gametocytes was reported on 0.4% (1/279) of the positive reads. Of the two sets of paired reads (MRTC/MDCoE), 95% (264/279) of the reads were concordant on negative/positive classification. 84% (221/264) were classified as negative and 16% (43/264) as positive. All the positive concordant results (43/43) were classified as pure *P. falciparum* infections and 0.4% (1/264) reported as having gametocytes. Cohen's Kappa on agreement based on classification of the 279 paired reads was almost perfect (estimated Kappa 0.8186). Comparison of densities could not be done because the site did not provide the method used for generation of submitted densities.

CNRFP Results

Of the 300 slides submitted only 286 reads were paired. 9 slides were not read due to poor quality of the smears, 3 did not have legible slide identification numbers and 2 of the results were not provided by the site. CNRFP readers reported 62% (176/286) as negative and 38% (110/286) as positive. Of the positive reads, 96% (106/110) were classified as pure *P. falciparum* infections, 2% (2/110) as pure *P. malariae* infections and 2% (2/110) as mixed infections of *P. falciparum* and *P. malariae*. Gametocytes were reported on 15% (16/110) of the slides. MDCoE readers reported 58% (167/286) as negative and 42% (119/286) as negative. Of the positive reads, 93% (111/119) were classified as pure *P. falciparum* infections, 0.8% (1/119) as pure *P. malariae* infection, 2.5% (3/119) as pure *P. ovale* infections, 2.5% (3/119) as mixed infections of *P. falciparum* and *P. malariae* and 0.8% (1/119) as a mixed infection of *P. falciparum* and *P. ovale*. Gametocytes were reported on 29% (34/119) of the slides. Of the two sets of paired reads (CNRFP/MDCoE), 93% (267/286) were concordant on negative/positive classification. Of the concordant reads, 61% (62/267) were classified as negative and 39% (105/267) as positive. Of the concordant positive reads, 93% (98/105) were classified as *P. falciparum*, 1% (1/105) as pure *P. malariae* and 1% (1/105) as mixed infection of *P. falciparum* and *P. malariae*. Gametocyte presence was concordant on 12% (12/105) of the slides. Cohen's Kappa on agreement based on classification of the 286 paired reads was almost perfect (estimated Kappa 0.8617). Of the 105 positive results, only 98 reads with asexual parasites were paired and of these, 66% (65/98) of the densities were within 0.5 – 2.0 fold difference.

Discussion

Concordance rates in parasite detection between sites and MDCoE were very high (>90%) suggesting site microscopists were highly accurate in classifying the slides. Discrepancies noted were mostly on very low density slides (< 200 parasite/µL) and on poor quality smears. Site microscopists were proficient in classifying *P. falciparum* infections accurately; however, CNRFP site had problems with non-*falciparum* infections. To overcome this problem, continuous training/assessment on identification of non-*falciparum* species must be established. As a rule of thump, concordance on parasite densities should ≥ 70%. Even though agreement between CNRFP and MDCoE density was lower than the rule of thump, binomial approximation suggested differences between 0.5 – 2.0 fold were significantly higher than differences outside the specified limits. It is important to note that it is not easy to achieve acceptable concordance on slides with parasite densities < 200 parasites/µL. It is also important to note that acceptable concordance cannot be achieved on poorly prepared slides. In conclusion, the exercise was successful based on the concordance rates achieved within the target parameters. However, more emphasis is needed on improving the competence levels of microscopists, the quality of prepared smears, and quality assurance/control mechanisms.

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CHAPITRE VI

Discussions générales, conclusions et perspectives

DISCUSSIONS GENERALES, CONCLUSIONS ET PERSPECTIVES

Avec le niveau intolérable de morbidité et de mortalité du paludisme surtout chez les enfants en Afrique, la recherche et la mise en œuvre de nouvelles stratégies de lutte s'imposent. Ceci était d'autant plus important et urgent qu'au début de la décennie 2000, les deux stratégies phares, l'utilisation des MII et la prise en charge des cas connaissaient d'énormes difficultés en raison de la lenteur de la mise en œuvre de la première et du développement de la résistance a la chloroquine en ce qui concerne la deuxième. Le besoin de nouvelles stratégies était donc devenu d'avantage une urgence et une priorité, surtout que statistiques et données des études en cette période indiquaient une augmentation de la mortalité palustre [1,2]. Le traitement préventif intermittent apparaissait donc comme une stratégie prometteuse dans la lutte contre le paludisme.

Nos travaux de recherche ont permis de répondre à un certaines des questions qui se posaient quant à l'utilisation de cette stratégie chez les enfants. Au cours de nos travaux nous avons évalué cette stratégie dans ses deux formes (couplée la vaccination et ciblant la saison de transmission du paludisme) chez les enfants en vue de son utilisation dans la lutte contre le paludisme dans nos pays.

C'est ainsi que nous avons conçu et conduit une des premières évaluations de l'efficacité du TPIe. **En utilisant un schéma de deux traitements intermittents à deux mois d'intervalle, nous avons réduit l'incidence du paludisme de plus de 67% chez les enfants de 6 mois à 10 ans. Cette première étude nous a permis aussi de démontrer que cette efficacité n'était pas suivie d'un effet rebond de morbidité ou de mortalité ou d'une augmentation notable de la résistance *in vivo* de *P. falciparum* à la SP.**

Des études menées par d'autres équipes parallèlement ont permis de montrer que des intervalles plus courts de traitement (un mois) pourraient être utilisé sans entrainer d'effet rebond ou d'augmentation de la résistance à la SP [3] et que la SP + Amodiquine était la combinaison la plus efficace [4]. D'autres études ayant évalué le cout- efficacité de la stratégie TPIe ou comparé différents modes de livraison ont montré que la stratégie a un très bon cout-efficacité surtout si elle est à large échelle [5] et que des couvertures de plus de 90% pourraient être obtenues en utilisant des relais communautaires [6, 7].

Puisque les études initiales d'efficacité du TPie étaient conduites dans des contextes de très faible utilisation des MII, et vu qu'il y a une augmentation importante de la couverture en MII dans la pluspart des pays d'endémie palustre en Afrique au cours de ces dernières années, la question qui se posait était de savoir si le TPIe serait aussi efficace dans un contexte de large utilisation des MII. **Nos travaux ont montré que la stratégie était aussi efficace même dans un contexte de large utilisation des MII**. **Notre étude a aussi démontré pour la première fois que la stratégie réduit l'incidence des formes graves et compliquées de paludisme dans les mêmes proportions que les accès palustres non compliqués**. Des résultats similaires ont été obtenus dans une étude parallèle au Burkina Faso [8].

Au moment de nos travaux, les études d'efficacité du TPIn étaient déjà effectuées ou en cours. Nous avons saisi l'opportunité des études de mise en œuvre pilote pour répondre aux questions clefs qui se posaient. Ainsi nous avons démontré dans **nos travaux pour la première fois: 1) le rôle bénéfique de la stratégie TPIn sur la couverture vaccinale, 2) son impact sur la mortalité globale et 3) l'absence d'impact sur la résistance quand la stratégie est mise en œuvre à large échelle**. Nos résultats ont ainsi contribué à rassurer la communauté scientifique et renforcer les convictions dans l'approbation de l'intégration de

cette stratégie dans les politiques sanitaires au niveau de l'OMS mais aussi au niveau du Mali et de la sous région.

Le TPIn réduit en moyenne les épisodes d'accès palustre de 30% et l'incidence de l'anémie de 20% [9]. **Notre étude a montré une réduction de la mortalité globale de 27%.** La bonne tolérance de la SP et son absence d'interaction avec les vaccins du PEV sont bien établis [9-11]. La stratégie est bien acceptée par les populations et les agents de santé, et elle a un des meilleurs cout-efficacités [12-15]. **La démonstration de l'augmentation de la couverture des vaccins du PEV par la distribution simultanée du TPI est un autre avantage à considérer dans l'évaluation des cout-efficacité et bénéfice global pour la survie de l'enfant**. La stratégie a cependant un certain nombre de limites. Premièrement, elle ne couvre que les enfants de moins de un an, alors que le poids du paludisme reste très élevé même au delà de un an. Dans beaucoup de zones en Afrique, le pic de l'incidence des formes graves et compliquées est observé chez les enfants de 2-3 ans et cet âge risque d'augmenter à cause du retardement de l'acquision d'une immunité anti-palustre liée à la diminution de la transmission des *Plasmodium*.

Deuxièmement, la stratégie est plus indiquée dans les zones de transmission continue periannuelle comme en Afrique de l'Est. Malheureusement c'est dans ces zones que les niveaux de résistance de *P. falciparum* à la SP sont les plus élevés. Plusieurs études ont montré que la SP restait efficace en TPI à des niveaux de résistance relativement élevés (par exemple a des taux d'échec thérapeutique à Jour 14 de 31% pour IPTn et de 26 % pour TPIp) [16, 17]. Cependant une absence d'efficacité du TPIn à la SP a été rapportée récemment en Tanzanie dans une zone où le niveau de résistance *in vivo* à la SP à Jour 28 était de 82% [18]. **Il est donc heureux de constater que la mise en œuvre du TPIn à large échelle n'a pas**

entrainé d'augmentation des marqueurs moléculaires de la résistance de *P. falciparum* à la SP. Cela avait d'ailleurs été prédit par des modèles mathématiques [19, 20].

L'absence de formulation pédiatrique de la SP a été évoquée comme un des points faible. Malheureusement, à nos jours aucune formulation pédiatrique de SP sous forme de sirop ou de comprimés dispersibles n'est encore disponible à notre connaissance en dépit des nombreux efforts déployés. Ceci ne saurait pas être un obstacle cependant puisque des comprimes sécables et rapidement dispersibles sont disponibles.

Le TPIe est une adaptation logique du TPIn aux conditions de transmission saisonnières dans beaucoup de pays en Afrique pour couvrir d'avantage d'enfants à risque. La stratégie offre donc deux avantages majeurs par rapport au TPIe, la possibilité de l'offrir à tous les groupes à risque en fonction du poids de la morbidité et de la mortalité, et la relative courte durée de l'intervention. **La stratégie garde toute son efficacité même dans un contexte de large utilisation des MII**, contrairement au TPIp pour lequel il a été démontré que son ajout additionnel au MII n'avait pas d'impact sur les poids de naissance ou sur l'infection placentaire [21, 22].

La stratégie TPIe devrait jouer un rôle important pour atteindre les objectifs de réduction de morbidité et de mortalité du paludisme voir d'élimination du paludisme notamment dans les zones de transmission saisonnière courte comme la plus part des pays du sahel. Son rôle dans les zones de transmission longue ou periannuelle reste à étudier.

La stratégie est bien acceptée par les communautés et les agents de santé, vu son impact rapide sur l'état de santé des enfants, perceptible même par les communautés. Les études en Gambie et au Sénégal ont montré que la stratégie peut être mise en œuvre à très large échelle, avec des taux de couverture de plus 90%, par des relais communautaires [6, 7]

La SP + AQ s'est révélée la combinaison la plus efficace en IPTe même si dans une étude récente SP + Piperaquine (PQ) a donné des résultats comparables [4, 23].

Le TPIe présente donc un ensemble d'avantages par rapport au TPIn et le TPIp. Comme ces deux dernières stratégies ont été approuvées par l'OMS pour être incluses dans les politiques de santé, il est à espérer que le processus d'évaluation officiellement enclenché en Juillet 2010 pour l'IPTe soit rapide. En absence de vaccin, cette stratégie se présente aujourd'hui comme la meilleure arme dans la lutte contre le paludisme dans les zones de transmission saisonnière.

Si l'ensemble des questions essentielles pour une mise en œuvre du TPIe comme politique semblent avoir être examinées aujourd'hui, il reste à déterminer jusqu' à quel niveau de saisonnalité, la stratégie doit être appliquée et évaluer son impact dans les zones de transmission continue. Le nombre de traitements intermittents pourrait aussi être ajusté en fonction des durées de transmission (deux traitements pourraient être suffisants dans certaines zones alors que d'autres pourraient nécessiter quatre traitements).

Il n'a pas été mis en évidence d'augmentation des niveaux de résistance moléculaire et *in vivo* attribuables à la mise en œuvre du TPI. **En effet la SP +AQ était resté efficace *in vivo* avec des taux de réponse clinique et parasitologique adéquate de 100% à J28** au Mali et de plus de 90% au Burkina Faso dans les localités où la combinaison a été utilisée en TPIe [8]. La durée limitée de ces études et la faible structuration des populations de *P. falciparum* dans les régions d'Afrique où elles ont été menées ne permettent cependant pas de conclure définitivement sur l'absence d'impact de cette stratégie sur les résistances. En effet, l'augmentation des résistances à un niveau détectable peut nécessiter une pression de sélection prolongée (*i.e.* supérieure à un an ou une saison de transmisison), et les flux

continus et importants de populations de *P. falciparum* depuis des zones où la pression de sélection serait plus faible peuvent « diluer » les souches plasmodiales résistantes qui auraient été sélectionnées.

En résumé, il est difficile de trouver une stratégie de lutte contre le paludisme qui marche partout et tout le temps. C'est pourquoi les différentes stratégies doivent être utilisées en fonction des situations locales et des moments. Elles peuvent être combinées lorsqu'elles ont au moins des effets additifs. Au terme de ce travail, nous pensons que le TPIn, malgré ses limites suscités, doit être déployé sans attendre dans les zones de forte endémicité palustre où la SP reste efficace, si on veut atteinte l'objectif du millénaire pour le développement d'une réduction des deux tiers de la mortalité des enfants de moins de 5 ans entre 1990 et 2015. Le TPIe est de toute évidence le meilleur outil aujourd'hui disponible dans la lutte contre le paludisme. Des dispositions doivent être prises rapidement pour sa mise en œuvre dans les zones de transmission saisonnière notamment dans les pays du Sahel où la stratégie pourrait contribuer au contrôle et, plus tard, à l'élimination du paludisme. Les déploiements de cette stratégie, comme toute autre stratégie en matière de santé publique, doivent être accompagnés de travaux de recherche opérationnelle pertinents afin de vérifier leur efficacité sur le long terme et d'optimiser leur utilisation.

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