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Déploiement d'une nouvelle stratégie de traitement d'une maladie à transmission vectorielle : application au paludisme, analyse des pratiques thérapeutiques, et conséquences sur l'épidémiologie de *P. falciparum* en Casamance, Sénégal, 1996 - 2009.

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Résumé

Le nombre de cas de paludisme était évalué à 24 millions en 2008 dont 212 millions dans la région africaine. L'utilisation de thérapies antipaludiques est un des moyens de lutte existant.

La disparité des résultats des essais cliniques sur la combinaison AS+AQ a nécessité la réalisation d'une méta-analyse afin d'obtenir une mesure globale de l'efficacité. En outre pour compléter cette preuve expérimentale, il était nécessaire d'étudier l'utilisation des médicaments en pratique courante, dans une approche de médecine factuelle et d'en effectuer le suivi sur plusieurs années.

Pour prendre en charge le paludisme au dispensaire de Mlomp, Sénégal, un programme pilote a été mis en place à partir de l'année 2000 : un traitement avec la combinaison artésunate + amodiaquine après confirmation parasitologique.

Nos travaux ont permis la compréhension des problèmes de santé publique relatifs aux changements de politique de traitement du paludisme en montrant l'efficacité et l'innocuité de la combinaison thérapeutique AS-AQ,. L'inadaptation de la posologie pouvait causer un sur- ou sous-dosage. Cela a mis en exergue les problèmes liés aux formes pharmaceutiques et l'importance du choix du dosage des molécules actives pour la posologie et l'observance au traitement. Une stabilité dans le temps de la résistance à l'AS et l'AQ grâce à une surveillance *in vitro* a été observée, ainsi qu'une baisse de la morbidité palustre parallèlement à une homogénéisation du risque de paludisme par âge.

Ces résultats permettent d'alimenter les politiques de santé publique pour la constitution de recommandations de prise en charge du paludisme au niveau national (PNLP) comme international (OMS).

Mots-clés : politique de santé, paludisme, artésunate-amodiaquine, test parasitologique

Implementing a new treatment strategy for a parasitic disease with vector transmission: the malaria case, therapeutic practices and consequences on the epidemiology of P. falciparum in Casamance, Senegal, 1996-2009.

Abstract

The malaria case number was evaluated to 243 M in 2008 with 212 m in the African region. Antimalarial drugs are one of the mean to combat uncomplicated falciparum malaria. With AS+AQ data accumulating, compiling comprehensive summaries of efficacy and safety is important to better understand the advantages and disadvantages through a pooled estimate. However there were no data on the long term use of AS+AQ and a pragmatic study was required to evaluate the use of AS+AQ in an evidence based medicine approach.

In Senegal, Mlomp started AS+AQ on parasitological diagnosis with operational research support since 2000. The study was conducted at the outpatient clinic of the dispensary.

Understanding of the public health problem related to the malaria treatment policy change was permitted following the result of this work. A high and stable efficacy and safety of AS+AQ was shown in this field study. Dosing accuracy varied markedly with different AS+AQ presentations; specifically a co-blister dosed less accurately and was associated with more mild side effects. The long-term, population-wide implications of the under- and overdosing of antimalarials demonstrate the need for more research.

Malaria prevalence and treatments are decreasing in this rural district but antimalarial treatments continue to be given without parasitological confirmation despite clear policies and training.

These results would give information for policy making related to the treatment of malaria at the national (PNLP) or international level (WHO)

Keywords: health policy, malaria, artesunate-amodiaquine, parasitological test

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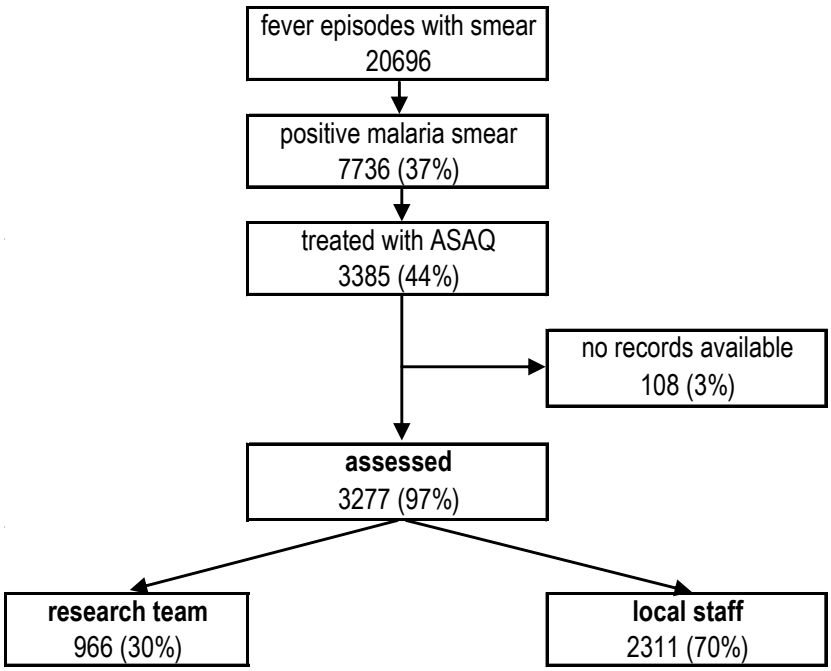

Figure 14.

1 Introduction

1.1 Le paludisme

Le paludisme est une parasitose due à des protozoaires appelés hématozoaires du genre *Plasmodium*, transmise par des moustiques du genre *Anopheles*.

Le paludisme est transmis à l'homme par la piqûre d'un moustique du genre *Anopheles* au moment de son repas sanguin. Seule la femelle, hématophage, transmet la maladie. Elle ne pique qu'à partir du coucher du soleil avec un maximum d'activité entre 23 heures et 6 heures.

Les larves d'anophèles se développent dans les collections d'eau. La nature des sols, le régime des pluies, la température et donc l'altitude, la végétation naturelle ou l'agriculture, rendent les collections d'eau plus ou moins propices au développement des espèces vectrices. Certaines espèces ont ainsi pu s'adapter à des milieux particuliers comme le milieu urbain. Le développement et la longévité des anophèles dépendent de la température avec un optimum entre 20 et 30°C pour une durée de vie de l'ordre de 30 jours.

Il existe plus de 140 espèces de *Plasmodium*, touchant diverses espèces animales mais seulement quatre de ces espèces sont généralement retrouvées en pathologie humaine. Il s'agit de *Plasmodium falciparum*, *P. vivax*, *P. ovale* et *P. malariae*. Une cinquième espèce initialement restreinte aux primates non humain, P. knowlesi, a récemment été associée à l'homme dans de nombreux cas de paludisme en Asie du Sud-Est [1]. Les espèces diffèrent les unes des autres par des critères biologiques, cliniques, par leur répartition géographique et par leur capacité à développer des résistances aux antipaludiques. *P. falciparum* est l'espèce qui est la plus largement répandue à travers le monde. Elle est responsable des formes cliniques potentiellement mortelles et a développé depuis les années 60 une résistance croissante aux monothérapies antipaludiques.

Dans les régions équatoriales, le paludisme est transmis toute l'année avec cependant des variations saisonnières. Dans les régions subtropicales, il ne survient qu'en période chaude et humide. La transmission s'interrompt lorsque la température chute en dessous de 18°C. C'est pourquoi le paludisme n'est plus transmis en altitude quelle que soit la latitude (au dessus de 1500 mètres en Afrique et 2500 mètres en Amérique et en Asie).

Pour *P. falciparum* le temps de latence entre une piqure infectante et l'apparition des symptômes est environ de 7 à 12 jours. Contrairement aux autres espèces, aucune rechute tardive comme n'est observée comme avec les autres espèces. *P. falciparum* est responsable des formes cliniques graves causées par la séquestration des globules rou-

ges infectés par les cellules endothéliales des micro-vaisseaux, entrainant des thromboses au niveau de plusieurs organes, notamment le cerveau (neuropaludisme).

Si P. vivax est beaucoup plus rarement observé en Afrique, il est très largement répandu en Amérique du Sud et en Asie. La transmission s'arrête en dessous de 15°. La période d'incubation est de 11 à 13 jours et on peut observer des rechutes pendant 3 à 4 ans (accès de reviviscence causés par des formes retard, appelées hypnozoïtes, au niveau hépatique). L'affection par *P. vivax* est généralement considérée comme bénigne (fièvre tierce bénigne due à un cycle érythrocytaire de 48 heures) mais peut avoir des répercussions graves sur l'état de santé des populations, notamment par l'intermédiaire d'anémies chez l'enfant. Des formes graves à *P. vivax* ont été notifiées [2,3].

P. ovale sévit en Afrique intertropicale du Centre et de l'Ouest (et dans certaines régions du Pacifique) et provoque une fièvre tierce bénigne, comme *P. vivax* dont il est très proche. L'incubation est de 15 jours au minimum. Son évolution est bénigne mais des rechutes tardives (5 ans) ont également été observées.

P. malariae est rencontré en Afrique, de manière beaucoup plus sporadique. Il se différencie des autres espèces par une incubation plus longue (15 à 21 jours), par une périodicité différente de la fièvre (cycle érythrocytaire de 72 heures responsable d'une fièvre quarte) et surtout par sa capacité à entraîner des reviviscences très tardives (20 ans ou plus après le retour de la zone d'endémie). Les mécanismes physiopathologiques responsables de ces reviviscences tardives interviennent généralement après une phase d'immunosuppression (maladies, greffes, …). L'infection est bénigne dans la grande majorité des cas mais quelques complications rénales ont été rapportées [4,5].

De façon similaire à celui des autres parasites, le cycle se déroule successivement chez l'homme (phase asexuée chez l'hôte intermédiaire) et chez l'anophèle (phase sexuée chez l'hôte définitif) (Figure 1). Chez l'homme le cycle est divisé en 2 phases : la phase hépatique ou pré-érythrocytaire (= exo-érythrocytaire) qui correspond à la phase d'incubation, cliniquement asymptomatique, et la phase sanguine ou érythrocytaire : elle correspond à la phase clinique de la maladie.

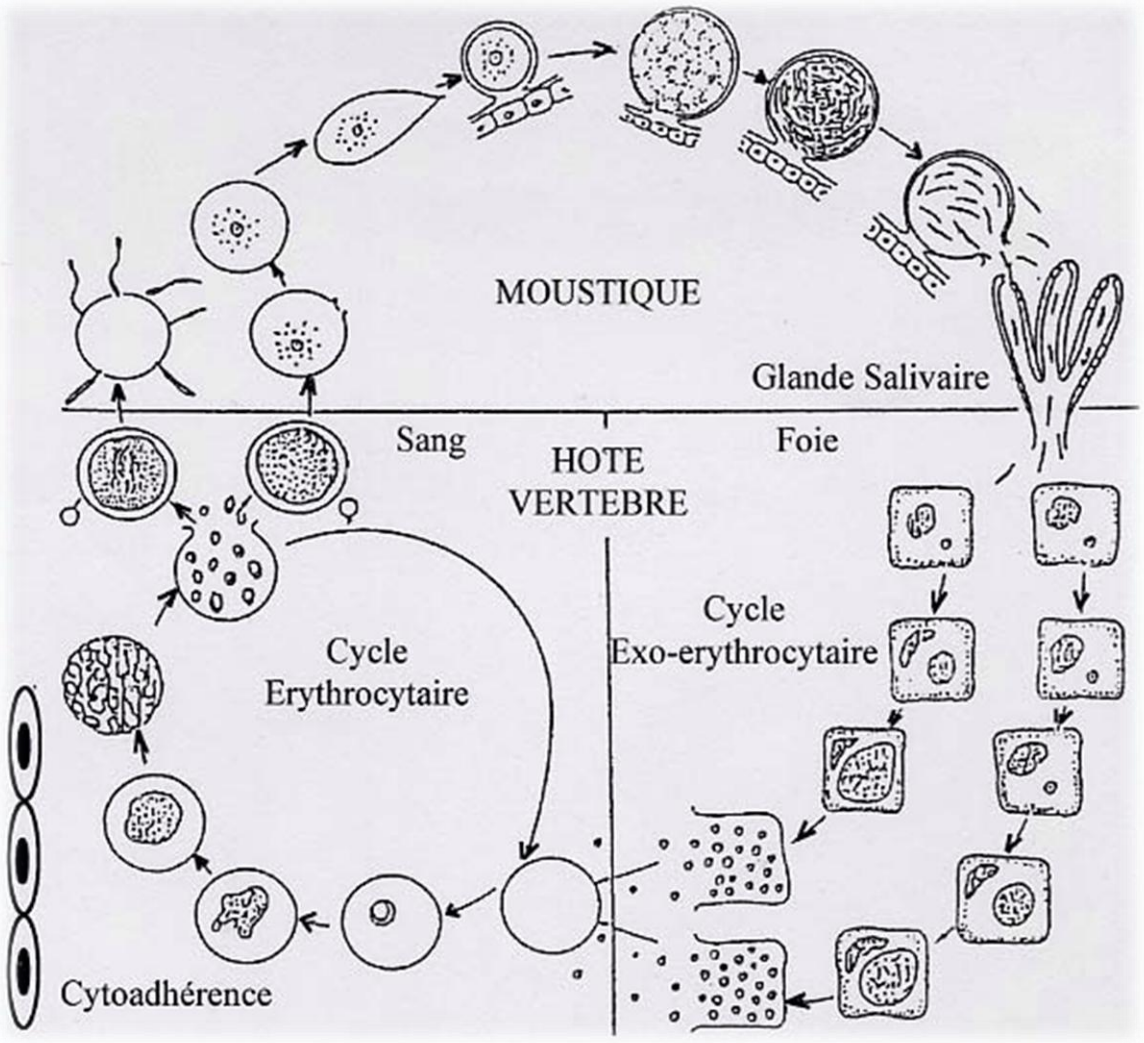

Figure 1. Cycle de reproduction de *Plasmodium falciparum* [6]

Chez l'homme comme chez tout hôte vertébré, lors de la schizogonie pré-érythrocytaire, les forme parasitaires infestantes sont les sporozoïtes, inoculés par l'anophèle femelle lors de son repas sanguin. Ils restent pendant une trentaine de minutes maximum dans la peau, la lymphe et le sang. Beaucoup sont détruits par les macrophages mais certains parviennent à gagner les hépatocytes. Ils vont y poursuivre leur développement, la schizogonie exo-erythrocytaire. Ils se transforment en schizontes pré-érythrocytaires ou « corps bleus » (formes multinucléées) qui, après quelques jours de maturation, éclatent et libèrent les mérozoïtes dans le sang (10 000 à 30 000 mérozoïtes en fonction des espèces) qui initieront la phase erythrocytaire. La schizogonie hépatique est unique dans le cycle, la cellule hépatique ne pouvant être infectée que par des sporozoïtes.

Figure 2.Femelle du genre *Anopheles* gambiae se gorgeant [7]

Dans les infections à *P. vivax* et P. ovale, une schizogonie hépatique retardée (hypnozoïtes) peut entraîner la libération dans le sang de mérozoïtes plusieurs mois après la piqûre du moustique, expliquant ainsi les reviviscences tardives observées avec ces 2 espèces. Les hypnozoïtes n'existent pas dans l'infection à *P. falciparum* et ils n'ont pas non plus été mis en évidence dans l'infection à *P. malariae*.

Lors de la schizogonie érythrocytaire, les mérozoïtes pénètrent très rapidement dans les globules rouges. La maturation en trophozoïte puis en schizonte prend 48 ou 72 heures (en fonction de l'espèce). Ce cycle conduit à la destruction du globule rouge hôte et à la libération de 8 à 32 nouveaux mérozoïtes. Ces mérozoïtes vont alors parasiter de nouveaux globules rouges et débutent un nouveau cycle de réplication. C'est la phase clinique : la parasitémie s'élève, le sujet devient fébrile, c'est l'accès palustre. En l'absence de traitement, tous les parasites évoluent progressivement au même rythme (on dit qu'ils deviennent synchrones), tous les schizontes érythrocytaires arrivent à maturation au même moment, entraînant la destruction d'un grand nombre de globules rouges de manière périodique, toutes les 48 heures (fièvre tierce de *P. falciparum*, *P. vivax* ou P. ovale) ou toutes les 72 heures (fièvre quarte de *P. malariae*). En pratique on observe que la fièvre tierce due à *P. falciparum* est rarement synchrone.

Après un certain nombre de cycles érythrocytaires, certains mérozoïtes subissent une maturation particulière d'une dizaine de jours, aboutissant à une différenciation sexuée : ils se transforment en gamétocytes mâles et femelles, seul élément aptes à se développer chez l'anophèle femelle.

Chez l'anophèle femelle, les gamétocytes, ingérés par le moustique lors d'un repas sanguin sur un sujet infecté, se transforment en gamètes mâles et femelles qui fusionnent en un œuf libre, mobile appelé ookinète. Cet ookinète quitte la lumière du tube digestif, se fixe ensuite à la paroi externe de l'estomac et se transforme en oocyste. Les cellules pa-

rasitaires se multiplient à l'intérieur de cet oocyste, produisant des centaines de sporozoïtes qui migrent ensuite vers les glandes salivaires du moustique. Ces formes infestantes que sont les sporozoïtes sont prêtes à être inoculées avec la salive du moustique, lors d'un repas sanguin sur un hôte vertébré. La durée du développement sporogonique des *P.* varie en fonction des conditions climatiques : entre 9 et 20 jours pour *P. falciparum* (entre, respectivement, 30°C et 20°C), un peu plus rapide pour *P. vivax* à températures équivalentes, plus long pour *P. malariae*.

1.2 Situation épidémiologique du paludisme dans le monde

A la suite des campagnes d'éradication menées depuis les années 50, le paludisme a disparu de la majeure partie de l'Europe et d'une grande partie de l'Amérique centrale et du sud.

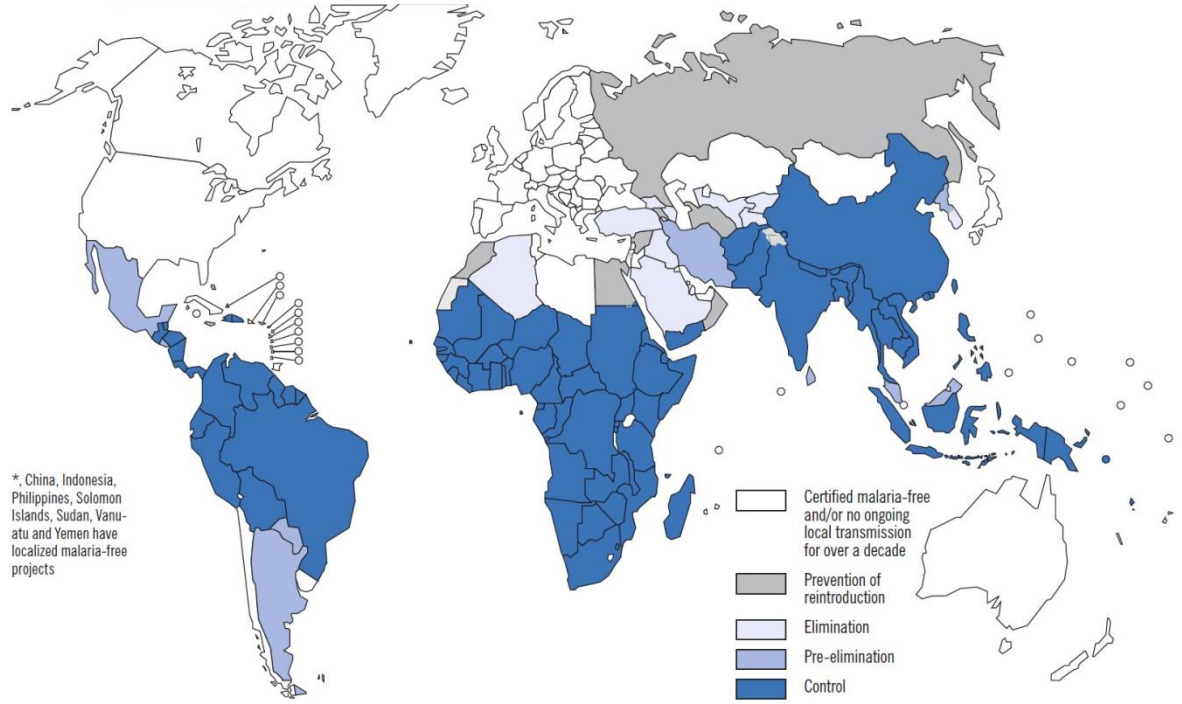

Figure 3. Répartition mondiale du paludisme en fonction de l'endémicité et de la phase de contrôle [8].

Le paludisme est très largement répandu dans toute l'Afrique sub-saharienne où coexistent *P. falciparum* (nettement prédominant), *P. ovale* et de manière plus sporadique *P. malariae*. L'espèce *P. vivax* peut être retrouvé en Afrique de l'Est. Il existe une transmission, faible, en Afrique du Nord (Algérie et Maroc), essentiellement due à *P. vivax*, ainsi qu'au Cap-Vert et à l'Ile Maurice. L'Ile de la Réunion est indemne ; en revanche la transmission est intense à Madagascar où coexistent les 4 espèces.

Le paludisme reste une cause majeure de morbidité et de mortalité dans de nombreuses régions du monde, plaçant la moitié de la population mondiale à risque. 3.3 Milliard

d'individus, soit 40% de la population mondiale, étaient exposés à la pathologie en 2006 [9]. Le nombre d'épisodes était évalué à 247 millions dont 212 millions se sont produits dans la région africaine (86%). En 2008, Il était estimé 243 millions de cas de paludisme dans le monde entier [8]. 85% des cas survenaient en Afrique, 10% dans le sud-est asiatique et 4% dans la région est-méditerranéenne [8]. 109 pays sont endémiques pour le paludisme en 2008, 45 étant situé en Afrique [9]. Le paludisme serait responsable du décès de 1.1 à 2.7 millions de personnes dans le monde, dont environ 1 million d'enfants de moins de 5 ans résidant en Afrique subsaharienne. Ces décès représentent quelques 25% de la mortalité juvéno-infantile générale en Afrique en l'an 2000 [10]. En excluant les neuropaludismes et les anémies 17.5% des causes de décès des enfants de moins de 5 ans en Afrique sont dues au Paludisme en 2000 [11]. En 2008, ce chiffre est estimé à 16% [12].

Tableau 1. Estimations du nombre de cas cliniques de paludisme entre 1995 et 2009

	An	Cas cliniques estimés	
		Monde	Afrique
Bull. WHO 1999 [13]	1995	272.2 M	221 M
	1998	273 M	245.7 M
Rapport n°892 du Comité d'experts du Paludisme	2000	500 M	
World Health Report 2002	2001	396 M	342 M
Hay et al, Lancet Infectious Diseases 2004 [14]	2001	632 M	334 M
snow et al, Nature 2005 [15]	2002	515.05 M (297.59-658.55)	364.98 M (215.82-373.95)
World Malaria Report 2005 [16]	2004	350-500 M	210-300 M
World Malaria Report 2008 [9]	2006	247 M	212 M
hay et al, PLoS Medicine 2010 [17]	2007	450.83 M (348.76-552.22)	270.88 M (241.13-300.56)
World Malaria Report 2009 [8]	2008	243 M	206 M

Toutefois des incertitudes résident dans l'estimation de la morbidité et mortalité palustre. Le système de surveillance des maladies infectieuses en place jusqu'en 2004-2005 étant déficient, les chiffres de la région Afrique (OMS AFRO) ont été obtenus à partir de la compilation d'études prospectives de détection active des cas dans les populations vivant sous différents degré de transmission [13]. En revanche, hors de la région AFRO, les estimations ont été faites à partir d'une détection passive même dans les pays à faibles ressources, conduisant à une sous-estimation de la morbidité palustre [14,15]. A partir de 2006, les données provenant des systèmes d'information de Santé nationaux (HMIS) ont fait l'objet d'une procédure d'ajustement [9]. Motivés par la variabilité de l'épidémiologie du paludisme, Hay et al estimaient à l'aide d'approches cartographiques [18,19] à 451 Millions le nombre de cas de paludisme dans le monde en 2007, à comparer aux 247 Millions estimés par l'OMS sur la base des systèmes de surveillance nationaux [8,9].

1.3 Traitements et justification de l'utilisation des combinaisons thérapeutiques à base d'artémisinine (CTA)

L'utilisation de thérapies antipaludiques est un des moyens de lutte existants (dont les plus connues sont la chloroquine ou la quinine). La stra§tégie médicamenteuse est complétée par la lutte contre les moustiques vecteurs du *Plasmodium* grâce à la pulvérisation intra domiciliaire d'insecticides à effets rémanents ou de moustiquaires imprégnées d'insecticides.

l'OMS préconise une couverture exhaustive des populations à risque dans les zones de prévention[8]. Toutefois les moustiquaires imprégnées ne sont pas suffisantes pour obtenir et maintenir une interruption de la transmission du paludisme dans les zones holoendémiques ou hyperendémiques [20]. L'utilisation concomitante de moustiquaires imprégnées et de la pulvérisation intra domiciliaire dans le cadre d'études observationnelles ne s'est pas montrée plus efficace que l'un ou l'autre des moyens de lutte utilisé seul [21]. Malheureusement la résistance aux pyréthrines devient une menace pour la viabilité des insecticides actuels [20,21], rendant nécessaire le suivi de cette résistance et une meilleure gestion des insecticides.

La résistance aux antipaludiques pose un problème majeur de santé publique car elle entrave le contrôle du paludisme dans les régions endémiques. Le développement rapide de la résistance aux médicaments tels que la chloroquine a eu pour effet d'intensifier le suivi de leur efficacité en vue d'effectuer une détection précoce des signes de résistance et de ses changements [8].

Dans ce contexte, le développement de combinaisons thérapeutiques pour le traitement du paludisme est devenu un enjeu majeur [22]. Les recherches se sont dirigées vers les associations à base d'artémisinine en raison de l'action rapide de cette molécule et de sa capacité à contenir voire diminuer la résistance aux antipaludiques [23,24]. En 1998, face à l'inefficacité des antipaludiques utilisés en monothérapies, le programme spécial PNUD/Banque mondiale/OMS de recherche et de formation concernant les maladies tropicales (TDR) a entrepris un vaste programme de recherche clinique sur l'évaluation de combinaisons à base de dérivés de l'artémisinine [22]. Les résultats de 16 différents essais cliniques publiés en 2004 dans le Lancet sous forme d'une méta-analyse montraient que l'addition de 3 jours d'un dérivé de l'artémisinine, l'artésunate, aux traitements standards (chloroquine, sulfadoxine-pyriméthamine, amodiaquine et méfloquine) réduisait fortement les échecs, les recrudescences et les gamétocytes circulants dans le sang [25]. En 1999, Brasseur et al montraient que l'amodiaquine restait efficace en Afrique de l'ouest et en Afrique centrale pour le traitement du paludisme non compliqué [26]. En 2002, en application des recommandations de l'OMS Adjuik *et al* rapportaient

les résultats d'un essai comparatif multicentrique de la combinaison artésunate+amodiaquine versus amodiaquine ayant pour objectif de limiter la résistance à l'amodiaquine [27]. La combinaison améliorait l'efficacité du traitement du paludisme au Gabon et au Kenya, mais ne montrait pas de différence avec l'amodiaquine seule au Sénégal. Les auteurs concluaient qu'artésunate+amodiaquine était une combinaison potentielle pour le traitement du paludisme en Afrique.

En 2005, le Sénégal comme plusieurs pays en Afrique faisant face à la problématique de la résistance antipaludique, abordaient la réflexion en vue du changement des recommandations nationales de traitement du paludisme. Après une phase transitoire de 2003 à 2005 où la chloroquine a été abandonnée au profit de l'association sulfadoxine-pyriméthamine + amodiaquine, la combinaison artésunate+amodiaquine a été adoptée en 2006.

1.4 Le Sénégal et la Casamance

Le Sénégal se trouve à l'extrême ouest du continent africain dans l'hémisphère Nord. Il est situé au sud de la boucle du cours inférieur du Fleuve Sénégal. Sa superficie est de 196 722 km^2. Le Sénégal est limité au nord par la Mauritanie, à l'est par le Mali, à l'ouest par l'Océan Atlantique et au sud par la Guinée Bissau et la Guinée [28]. La Gambie qui est une enclave de terre sur le cours inférieur du fleuve du même nom, est située entre les régions de Kaolack et Ziguinchor [29]. Le pays est encerclé par un relief plat, à sols sablonneux dont l'altitude ne dépasse 100m qu'à l'extrême sud-est [28]. Avec une altitude de 381 m, le mont Assirik qui se situe au sud-est du pays, constitue le point le plus élevé [29]. Son climat se caractérise par l'alternance d'une saison pluvieuse de 3 à 4 mois et d'une saison sèche de 8 à 9 mois [28]. Le réseau hydrographique du Sénégal est constitué par quatre grands fleuves (le Sénégal, la Gambie, la Casamance, le Saloum) et par leurs affluents auxquels s'ajoutent quelques cours d'eau temporaires. Il faut noter la contribution non moins importante du lac Guier au nord du pays [29].

Au niveau administratif, le territoire national est découpé en 14 régions administratives depuis 2007 (11 auparavant). Les régions sont subdivisées en départements, ceux-ci étant découpés en communes (assimilées au milieu urbain) et arrondissements.

Selon le recensement de 1988, la population du Sénégal était évaluée à 6 896 808 habitants, soit en moyenne une densité de 35 habitants au km^2 [30]. Les projections issue de ce recensement estimaient la population à 9 278 618 habitants en 1999, soit une densité moyenne de 47 habitants au km^2 [29]. En 2005, les projections effectuées à partir du recensement 2002 [31] donnaient une estimation de 10.8 millions

Figure 4. Carte administrative du Sénégal et localisation de la région d'Ousouye.

d'habitants, soit 55 au km^2. Cette estimation était de 12, 5 M en 2009. Cependant, cette population est inégalement répartie entre les régions administratives du pays. La région la moins étendue, celle de Dakar, occupe 0.3% de la superficie du territoire national, mais elle abrite près de 23% de la population totale et 53% de la population urbaine totale. La région la plus étendue, Tambacounda (actuelles régions de Tambacounda et de Kédougou) , abrite environ 6% seulement de la population sur 30% de la superficie du pays [32].

La population totale du district est de 39109 habitants et se réparti dans les différents villages comme suit :

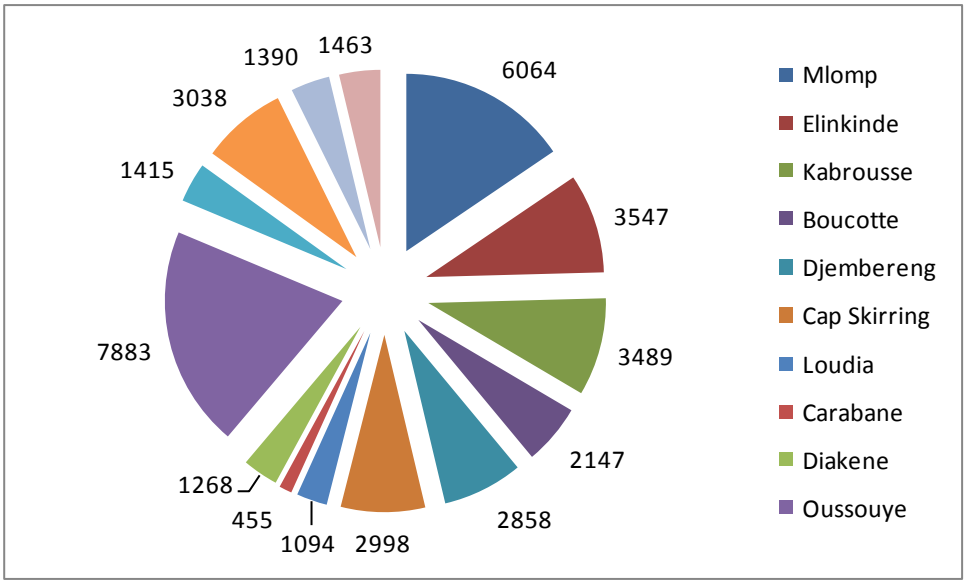

Figure 5. Distribution de la population du district d'Oussouye.

1.5 Développement du protocole de prise en charge du paludisme au Sénégal

Le Premier problème de santé publique au Sénégal, le paludisme est la première cause de morbidité et de mortalité générale, surtout chez les enfants de moins de 5 ans. Plus de 50% de la demande des services de santé au niveau du pays tout au long de l'année, est lié au paludisme. C'est la raison pour laquelle, le Sénégal a adhéré très tôt à l'Initiative « Faire Reculer le Paludisme » lancée en octobre 1998 par l'OMS, l'UNICEF, la Banque Mondiale et le PNUD [33]. Il a concrétisé cette option en participant en avril 2000 au sommet d'Abuja [34] qui a abouti à un consensus des 43 pays endémiques pour le paludisme. En 1995, le Sénégal a élaboré un Programme National de Lutte contre le Paludisme (PNLP) dont les activités ont été intégrées dans le Plan National de Développement Sanitaire (PNDS) 1998-2007 et le Programme de Développement Intégré de la Santé (PDIS). La mission du PNLP est de mettre en œuvre la Politique de lutte contre le paludisme au Sénégal. Pour le suivi de l'exécution des activités de lutte contre le paludisme dans les districts sanitaires, des membres de l'unité de coordination du PNLP ont été désignés comme points focaux. Chaque point focal a en charge un certain nombre de districts. C'est ainsi qu'en plus de leurs tâches quotidiennes, ils ont pour rôle d'assurer le suivi de la réalisation des activités dans les districts et de répondre aux sollicitations des équipes cadre de districts en vue de leur permettre de résoudre leurs problèmes.

Dans ce district, pour ce qui est de l'accès aux soins, le secteur privé est très peu présent voire absent. Les sites d'investigations sont situés à l'hôpital de référence d'Oussouye,

les postes de santé de Mlomp, Elinkinde, Kabrousse et Diembereng. La population de cette région est très bien suivie dans les postes de santé avec une traçabilité des consultations médicales dans des registres.

Cette configuration permet de garantir les interventions effectuées dans le cadre du projet. La directive OMS de 2000 et du PNLP de 2006 a pu être monitorée. Les taux de mise en place ont été graduels avec une première mise en place à Mlomp en 2000 et une extension ultérieure aux autres centres de santé. La dernière mise en place en 2006 à Elinkinde.

Le déploiement et l'administration d'un nouveau traitement ne peut alors être effectué sans envisager la mise en place concomitante d'une stratégie simple englobant la détection de la fièvre, la confirmation parasitologique de la maladie, les recommandations au patient pour la prise complète de son traitement.

2 Objectifs de la thèse

L'efficacité de la combinaison AS-AQ a été évaluée au travers de nombreux essais cliniques [27,35,36,37,38,39,40,41,42,43,44,45,46,47,48,49,50,51,52,53,54,55,56] réunissant des conditions expérimentales contrôlées. La disparité de leurs résultats imposait toutefois de réaliser une méta-analyse de ces essais cliniques, afin d'obtenir un estimateur agrégé pondéré par l'importance de chaque étude. En outre pour compléter cette preuve expérimentale, dont la qualité des conditions opérationnelles dépasse la réalité, il était nécessaire de produire d'autres preuves basées sur l'utilisation des médicaments en pratique courante, dans une approche de médecine factuelle (Evidence base medicine).

Ces deux approches devraient nous permettre d'alimenter les politiques de santé publique avec de nouvelles recommandations de prise en charge du paludisme à un niveau international (OMS) ainsi que des propositions d'évolution des programmes de lutte contre la pathologie au niveau national.

2.1 Objectif principal

L'objectif de ce travail de recherche est de démontrer l'efficacité réelle de la combinaison AS-AQ.

2.2 Objectifs secondaires

Cette étude propose comme objectifs secondaires :

- La réalisation d'une méta-analyse de l'efficacité de la bithérapie AS-AQ
- L'analyse l'évolution de l'efficacité thérapeutique de l'AS-AQ entre 2000 et 2005
- L'évaluation de l'adéquation entre le dosage effectif de la bithérapie AS-AQ et la plage de dosage thérapeutique préconisée
- La surveillance de la susceptibilité du parasite aux traitements : AS-AQ, QN et CQ.
- L'analyse des conséquences du traitement systématique par AS-AQ sur l'épidémiologie du paludisme

3 Méthodologie

3.1 Zone d'étude

Les travaux présentés dans le cadre de cette étude concernent le dispensaire et le village de Mlomp. Les coordonnées géographiques du site indiquent une latitude variant de 12° 36' à 12° 32' N, et une longitude de 16,33° E à 16,37 (Google Earth®). Le village compte 6064 d'habitants.

3.2 Prise en charge des cas de Paludisme

La prise en charge du Paludisme au dispensaire de Mlomp a suivi les directives du PNLP au Sénégal qui prévoyait le traitement de tous cas de fièvre suspectée d'être un cas de Paludisme par des antipaludiques (chloroquine par voie orale ou Quinine par voie injectable) jusqu'en 2000. A partir de l'année 2000, Suite au changement des recommandations de l'Organisation Mondiale de la Santé, un programme pilote a été mis en place pour favoriser l'utilisation d'un traitement à base de dérivés de l'artémisinine (artésunate+amodiaquine) après confirmation parasitologique.

Ce programme a commencé à petite échelle pour les enfants de moins de cinq ans et pendant la saison humide pour s'étendre à l'ensemble de la population sur la totalité de l'année.

La prise en charge s'est alors déroulée, de manière concomitante, selon deux pratiques, celle de référence correspondant aux directives OMS d'avant 2000 (traitement de la fièvre avec un antipaludique) et la pratique en test (traitement de la fièvre avec l'AS-AQ si confirmation parasitologique) correspondant aux nouvelles directives OMS.

La mise à l'échelle de cette nouvelle approche a été suivie dans le cadre de ce programme. Cette stratégie a été adoptée officiellement par le PNLP en 2006 avec la mise à disposition des tests de diagnostic rapide en 2007 alors que le diagnostic parasitologique se faisait auparavant par frottis mince et goutte épaisse.

3.3 Considération éthiques

L'étude clinique présentée dans ce travail a été approuvée par le comité d'éthique du Sénégal. Les autres études ont été menées dans le cadre d'une surveillance épidémiologique. La collecte des données individuelles à partir des registres des centres de santé a été réalisée dans une base de données qui a été anonymisée.

3.4 Analyses statistiques

La méthodologie de chaque étude est décrite dans les publications présentées dans le cadre de ce travail.

4 Résultats

Ce chapitre présente un résumé des résultats de plusieurs travaux :

- La méta-analyse d'essais cliniques d'AS-AQ en Afrique,
- Une proposition de représentation graphique des études dans le cadre des méta-analyses, facilitant l'interprétation des résultats,
- Un essai pragmatique d'efficacité et de tolérance de l'AS-AQ à Mlomp,
- Une étude d'évaluation de la posologie en fonction de l'âge ou du poids,
- Une évaluation de la résistance à l'AS, l'AQ, la quinine et la chloroquine,
- Une proposition de méthode d'analyse pour l'analyse de la susceptibilité aux médicaments au cours du temps,
- L'évaluation de la gestion des fièvres et de l'application de la stratégie de traitement avec l'AS-AQ sur confirmation parasitologique,
- L'étude des changements dans l'épidémiologie du paludisme à Mlomp et les effets sur la distribution de l'âge des sujets à risque après l'introduction de la combinaison AS-AQ.

Ce chapitre se poursuit par une discussion de ces résultats et une conclusion générale. L'ensemble des articles a été placé en annexe.

4.1 Sélection du traitement adapté au contexte épidémiologique

Des combinaisons thérapeutiques efficace contenant de l'artémisinine (ACTs) sont recommandées par l'Organisation Mondiale de la Santé (OMS) pour le traitement du paludisme non compliqué aigu (accès simple) à *P. falciparum*. Actuellement, 73 des 81 pays où *P. falciparum* est endémique ont adopté les ACTs en traitement de première ligne (40 en Afrique). L'AS combiné avec l'AQ est actuellement adopté dans 22 pays, à savoir l'Indonésie, la Chine et 20 pays Africains (Burundi, Cameroun, Congo, Côte d'Ivoire, République Démocratique du Congo, Guinée Equatoriale, Gabon, Ghana, Guinée, Liberia, Madagascar, Malawi, Mauritanie, Sénégal, Sao Tome & Principe, Sierra Leone, Soudan (Sud), Zanzibar)[57].

AS-AQ est actuellement disponible en comprimés séparés ou sur un même blister, et une combinaison à dose fixe est maintenant enregistrée dans 27 pays d'Afrique endémiques pour le paludisme (Figure 6).

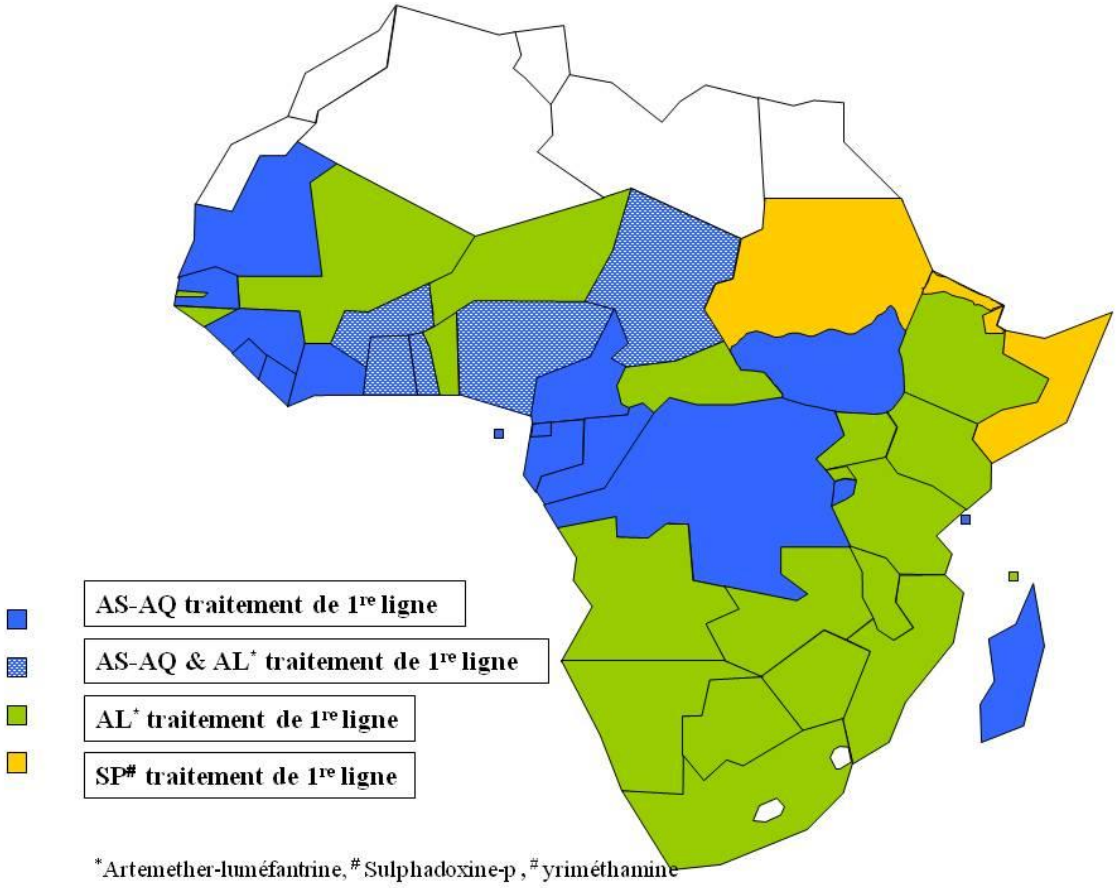

Figure 6. Répartition géographique de l'utilisation de la bithérapie AS-AQ [57] (*Artemether-luméfantrine, # Sulphadoxine-pyriméthamine)

Avec l'accumulation des données sur l'AS-AQ, une compilation des synthèses complètes de l'efficacité et de tolérance s'impose afin de mieux comprendre les avantages et les inconvénients de ce traitement. Ceci peut aider les décideurs en santé publique dans leurs décisions sur les conditions optimales d'utilisation de l'AS-AQ et mener à de nouvelles optiques de recherche.

4.1.1 Meta-analyse de l'efficacité de la bithérapie AS-AQ

Nous avons réalisé une revue systématique et méta-analyse de la littérature; une mise à jour est en cours et nous comptons la soumettre dans le courant du premier trimestre 2011.

Objectif :

L'objectif de cette méta-analyse était d'évaluer l'efficacité et la tolérance d'AS-AQ pour le traitement du paludisme à *Plasmodium falciparum* afin d'assister les décisions de santé publique.

Matériels et Méthodes :

Les essais cliniques publié et non publiés (littérature grise) conduits entre 1999-septembre 2008 ont été identifiés via des recherches électroniques et manuelles dans MEDLINE, EMBASE, LILACS et CENTRAL. Les essais non comparatifs, comparatifs randomisés et non randomisés d'AS-AQ ayant recrutés des patients de tout âges atteints de paludisme à *P. falciparum* ont été sélectionnés. Quatre investigateurs recherchèrent indépendamment et résumèrent les caractéristiques et les résultats. Trois différents investigateurs vérifièrent les données. Les critères principaux étaient les résultats parasitologiques brut et ajustés par PCR au jour 28 de la population per-protocole. Des modèles à effets aléatoires furent utilisés pour agréger les données des essais cliniques randomisés contrôlés.

Résultats :

Parmi 66 études potentielles, 42 essais comparatifs et non comparatifs remplissaient les critères d'inclusion. 40 études (35 essais comparatifs conduits dans 59 sites d'études, 5 essais non-comparatifs dans 6 sites) recrutant 18.808 patients (7.808 avec AS-AQ) dans 25 (22 en Afrique) pays contribuant à l'analyse d'efficacité au jour 28. 39 essais ont spécifiquement recruté des enfants africains. Les doses ciblées de médicament étaient généralement 12 mg/kg d'AS et 30 mg/kg d'AQ administrés sur 3 jours. Les taux d'échecs bruts à J28 de l'AS-AQ variaient considérablement (0%-80%) mais décroissaient en dessous de 26% après génotypage sur 37 des 59 sites d'études rapportant des résultats corrigés par PCR. Des différences entre les taux d'efficacité de l'AS-AQ étaient notées. Des 35 études comparant les taux à J28, AS-AQ était significativement plus efficace que l'amodiaquine(RR=0,41, 95%CI= [0,33; 0.49]), artésunate (RR=0,08, 95%CI= [-0,13; 0,28]), chloroquine (RR=0,11, 95%CI= [0,04; 0.19]), sulfadoxine/pyriméthamine (RR=0,46, 95%CI= [0,29; 0,63]), amodiaquine+sulfadoxine/pyriméthamine (RR=0,83 [95% CI: 0,71; 0,94]), chloroquine+sulfadoxine/pyriméthamine (RR=0,51 [95% CI: 0,26; 0,75]), et artésunate+sulfadoxine/pyriméthamine (RR=1.21 [95% CI: 0,94; 1,48]) mais ne différait pas significativement de artemether+lumefantrine 6 doses (RR=1,86 [95% CI: 1.51; 2,20] et dihydroartemisinin+piperaquine (RR=2,36 [95% CI: 0,54; 4,18]. Les comparaisons avec artemether+lumefantrine 4 doses et artésunate+méfloquine n'étaient pas valides car une seule étude était dans chaque groupe. Au total, AS-AQ était supérieure aux monothérapies combinées (RR=0,11 [95% CI: 0,01; 0,21]) et aux combinaisons sans artémisinine (RR=0,23 [95% CI: -0,06; 0,52]). Les analyses agrégées montraient que AS-AQ était plus efficace que les ACTs combinées (RR=0,16 [95% CI: 0,07; 0,26]) mais seulement avec la comparaison avec AS+SP significative dans ce groupe. L'ajustement à l'aide de la PCR ne modifiait pas ces résultats.

Conclusion :

Cette revue représente un inventaire de toutes les données disponibles jusque septembre 2008. Alors que de nouvelle données deviendront disponible, sa disponibilité dans la revue libre d'accès « Malaria Journal » permettra des recherches future d'étendre les connaissances sur l'efficacité et la tolérance de la combinaison AS-AQ. Cela permettra aussi de considérer la variabilité géographique de ces indicateurs dans le contexte actuel de révisions des recommandations. AS-AQ répond aux critères minimum recommandés par l'OMS d'une efficacité corrigée par PCR d'au moins 90% dans la majorité des pays, et devrait ainsi, être considérée pour le traitement du paludisme non compliqué à *falciparum* en Afrique. Bien que les évènements indésirables ne soient pas suffisamment documentés et ont été reportés de manière non standardisée, AS-AQ fut généralement bien tolérée et dotée d'un profil de tolérance similaire aux autres combinaisons. Alors que l'AS-AQ est le second antipaludique le plus utilisé, sa tolérance n'a pas encore été étudiée systématiquement. Cet article représente une avance significative dans notre connaissance de l'AS-AQ qui n'est pas reprise dans les revues Cochrane publiées. Cependant plus d'essais comparatifs et une surveillance continue de l'efficacité et de la tolérance d'AS-AQ sont nécessaires, incluant des études dans des conditions réelles et avec des expositions répétées. Ceci est crucial pour optimiser la diffusion des connaissances sur l'efficacité et la tolérance pour conduire les recommandations de traitement du paludisme promulguées par les agences réglementaires et les décideurs en santé publique comme l'OMS.

Article :

Vaillant M, Olliaro P, Mussano P, Phalkey R, Guthmann J, Dorsey G, Brasseur P, D'Alessandro U, Mårtensson A, Koram K, Faye B, Mugittu K, Sirima SB, Millet P, Sevcsik AM, Taylor WRJ. (2011) **A systematic review and meta-analysis of non-randomized and randomized controlled studies of artesunate and amodiaquine for the treatment of uncomplicated *falciparum* malaria.** *En preparation.*

Communications orales:

Efficacy and safety of artésunate+ amodiaquine (AS-AQ) in comparative trials in south-Saharan Africa: a systematic review and an individual patient meta-analysis. Piero L. Olliaro, Julien Zwang, Michel Vaillant, Walter R. Taylor. *57th annual meeting of the ASTMH, December 7-11, 2008, New Orleans, Louisiana, USA. American Journal of Tropical Medicine and Hygiene 2008 Dec; 79 (6): 1-390.*

Efficacy of Artesunate-amodiaquine (ASAQ) for the treatment of uncomplicated falciparum malaria in sub-saharan africa: an individual patient data meta analysis (IPDM) in 3,455 patients. Julien Zwang, Piero Luigi Olliaro, François Nosten, H Barennes, P. Brasseur, G. Dorsey, J.P. Guthmann, A Martensson, U D'Alessandro, M Vaillant. *56th annual meeting of the ASTMH, November 4-7, 2007, Philadelphia, Pennsylvania, USA. American Journal of Tropical Medicine and Hygiene 2007 Nov; 77 (5): 1-344.*

Artesunate+amodiaquine (AS-AQ) for the treatment of uncomplicated falciparum Malaria : an inventory and systematic review of safety and efficacy data. Piero L. Olliaro, Michel Vaillant, Revati Phalkey, Jean-Paul Guthmann, Grant Dorsey, Philippe Brasseur, Umberto D'alessandro, Pascal Millet, Walter (Bob) R. Taylor. *55th annual meet-*

ing of the ASTMH, November 12-16, 2006, Atlanta, Georgia, USA. American Journal of Tropical Medicine and Hygiene 2006 Nov; 75 (90050): 1-857

Artesunate + amodiaquine for the treatment of uncomplicated falciparum malaria: an inventory and systematic review of safety and efficacy data. Piero L. Olliaro, Michel Vaillant, Revati Phalkey, Jean-Paul Guthmann, Grant Dorsey, Philippe Brasseur, Umberto D'alessandro, Pascal Millet, Walter R. Taylor. *55th annual meeting of the ASTMH, November 12-16, 2006, Atlanta, Georgia, USA. American Journal of Tropical Medicine and Hygiene 2006 Nov; 75 (90050): 1-857.*

Revue systématique et méta-analyse d'études randomisées contrôlées comparant la combinaison artésunate + amodiaquine pour le traitement de l'accès palustre simple à Plasmodium falciparum. P. Olliaro, M. Vaillant, P. Mussano, R. Phalkey, M.O. Harhay, J-P. Guthmann, G. Dorsey, P. Brasseur, U. D'Alessandro, A.Mårtensson, K.Koram, B.Faye, K. Mugittu, S.B. Sirima, P. Millet, WRJ Taylor. *3ème Conférence Francophone d'Epidémiologie Clinique, Congrès thématique de l'ADELF, Fès, Maroc, 7 et 8 mai 2009. Epidemiuology and public health 2009 may, 57 (suppl1): S1-S66*

Poster :

Evaluation et réduction de l'hétérogénéité dans une méta-analyse d'essais clinique contrôlés, randomisés de l'artésunate combiné à l'amodiaquine pour le traitement de l'accès palustre simple. M. Vaillant, P. Olliaro, P. Mussano, R. Phalkey, M.O. Harhay, J-P. Guthmann, G. Dorsey, P. Brasseur, U. D'Alessandro, A.Mårtensson, K.Koram, B.Faye, K. Mugittu, S.B. Sirima, P. Millet, WRJ Taylor. *41èmes Journées de Statistique du 25 au 29 mai 2009 - Bordeaux, France*

Référence:

Efficacy of Artesunate-amodiaquine (ASAQ) for the treatment of uncomplicated falciparum malaria in Sub-Saharan Africa: an individual patient data meta-analysis in 3,455 patients. Julien Zwang, Piero Luigi Olliaro, François Nosten, H Barennes, P. Brasseur, G. Dorsey, J.P. Guthmann, A Martensson, U D'Alessandro, M Vaillant. *56th annual meeting of the ASTMH, November 4-7, 2007, Philadelphia, Pennsylvania, USA. American Journal of Tropical Medicine and Hygiene 2007 Nov; 77 (5): 1-344.*

Le résultat principal de cette étude est la supériorité de l'efficacité démontrée de l'AS-AQ sur les monothérapies et les combinaisons sans dérivés de l'artémisinine avec un taux d'efficacité d'au moins 90% en général. Dans la comparaison avec les autres ACT, la supériorité est démontrée par rapport à AQ+SP seulement. Le profile de tolérance de l'AS-AQ, insuffisamment décrit, nécessite des études supplémentaires.

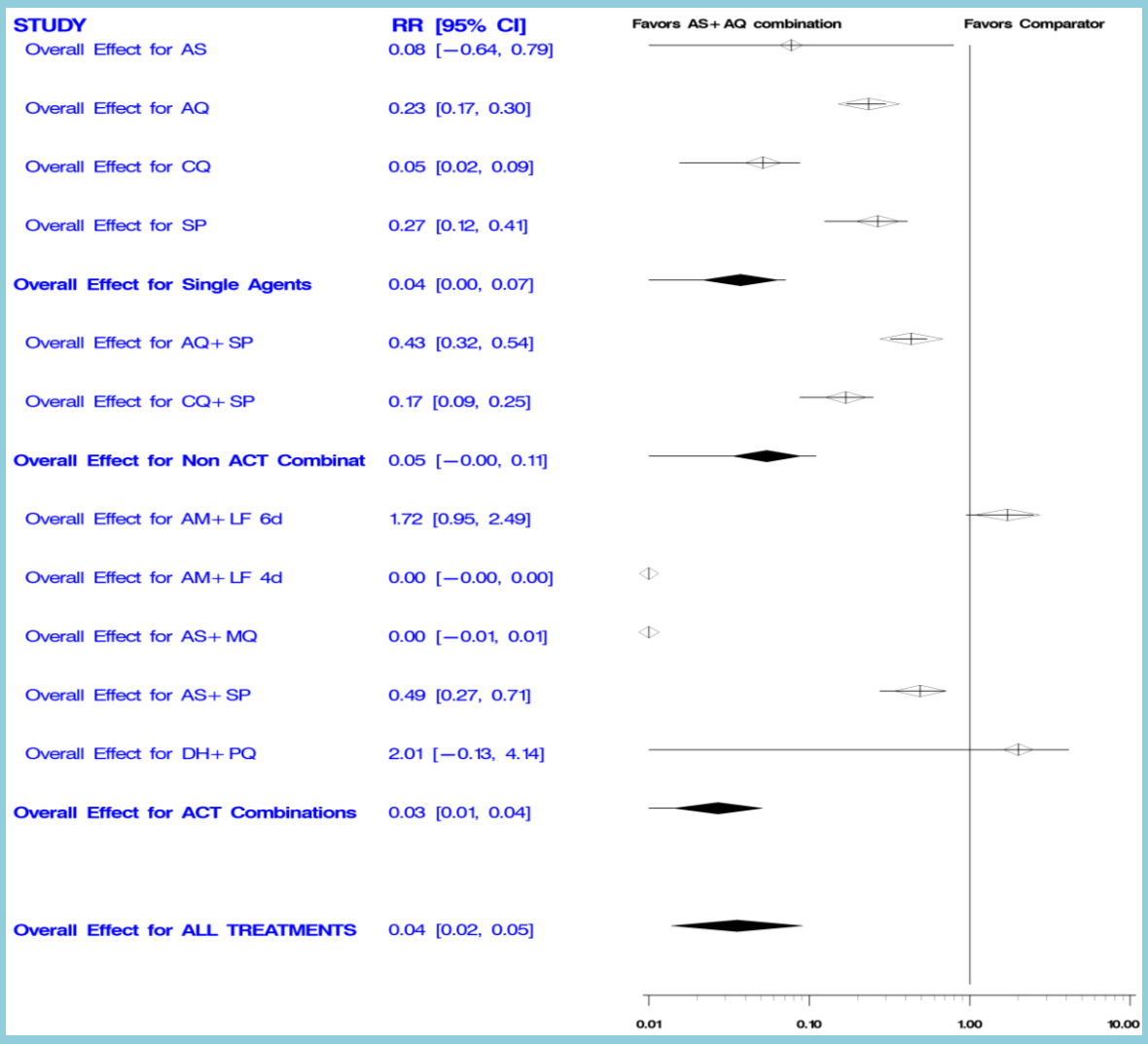

Figure 7. Graphique en forêt des taux d'échecs corrigés par PCR (génotypage) du traitement AS-AQ vs. les différents types de comparateurs, population Per Protocole.

Les réinfections déterminées par PCR et les données manquantes suite à la PCR ne sont pas considérées[58].

4.1.2 Contribution à l'interprétation des résultats et la prise de décision

Classiquement les méta-analyses montrent des effets relatifs et l'hétérogénéité résidant entre les études à agréger ; en revanche sont ignorés les effets absolus – un élément essentiel dans l'élaboration des politiques de santé. Des données obtenues grâce à une revue systématique d'essais cliniques sur les traitements antipaludiques et des essais virtuels ont été utilisées pour générer une représentation montrant et quantifiant les effets relatifs et absolus, ainsi que l'hétérogénéité des essais comparatifs. Un graphe des taux d'échecs (avec leurs intervalles de confiance à 95%) du médicament test sur l'axe des ordonnées et la différence de risque (RD) avec le médicament de référence sur l'axe des abscisses est réalisé ; la surface du graphique est divisée en 4 quadrants par une ligne verticale (pas de différence entre les risques) et une ligne horizontale (taux d'échec maximum toléré, e.g., 10% pour les antipaludique). Cela permet d'identifier où un médicament peut être utilisé (critères d'efficacité rempli) et de quantifier les différences (contre une autre option de traitement). L'aire du polygone connectant les points de coordonnées des études expriment l'hétérogénéité. Cette représentation graphique est simple à préparer et interpréter. Il combine en un graphe des mesures d'effet absolu du traitement et de différence de risque ainsi que d'hétérogénéité. Il devrait compléter les méthodes utilisées actuellement et apporter une information utile dans la réalisation des décisions de politique de santé.

Cet article actuellement est sous presse. La représentation graphique décrite a été utilisée dans une méta-analyse Cochrane des combinaisons à base d'artémisinine (ACTS). Ce travail avait été requis par l'OMS afin de fournir une évidence factuelle sur l'utilisation des différentes ACTs. Il a été utilisé pour l'établissement du dernier « World Malaria Report 2009 ».

Article :

Références:

Sinclair D, Zani B, Donegan S, Olliaro P, Garner P. (2009) **Artemisinin-based combination therapy for treating uncomplicated malaria.** Cochrane Database Syst Rev, Jul 8;(3):CD007483.

Sinclair D, Olliaro P, Garner P. (2010) **Guest Editorial: Artemisinin-based combination therapy for malaria, but which one?** *Clinical Evidence, 8th February: 1-4.*

Cet article propose une aide graphique à la décision :

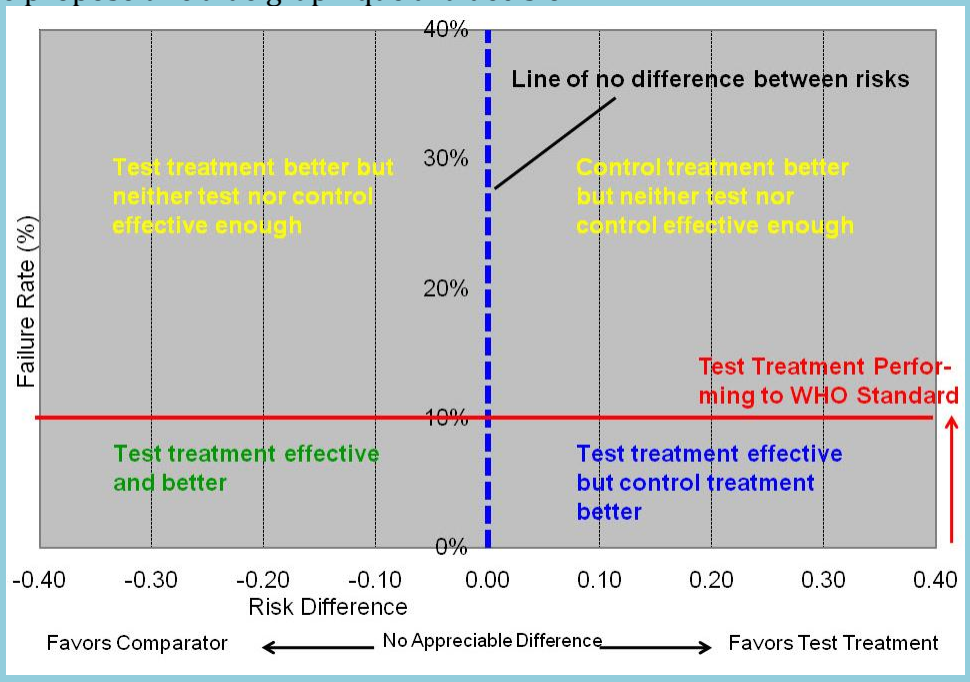

Figure 8. Aide à l'interprétation du graphique, exprimant les taux d'échecs en ordonnées et les différences de risques en abscisse.

La ligne rouge horizontale correspond à l'efficacité minimum requise par l'OMS des traitements du paludisme. La ligne bleue en pointillés correspond à une différence de risque nulle.

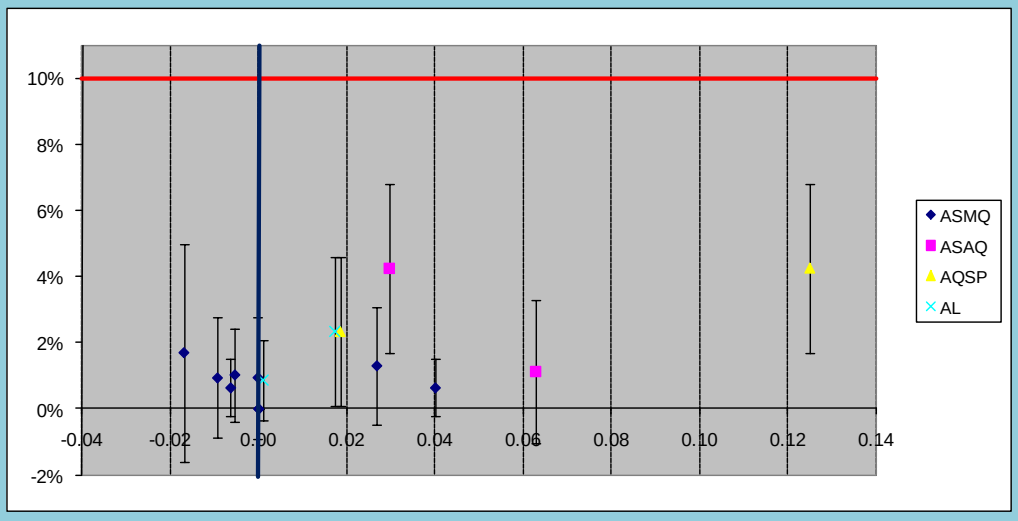

Figure 9. Olliaro-Vaillant plot. dihydroartemisinin/piperaquine (DP) vs comparateurs.

Les données obtenues à la dernière évaluation du suivi (J63, J42, J28) sont présentées vs. les comparateurs. Les points sous la ligne rouge représentent les essais où l'efficacité de DP correspondait au standard OMS. Les points à la droite de la ligne bleue représentent les essais où DP était supérieur aux comparateurs et ceux à gauche les essais où le comparateur était plus efficace que DP. Une différence de risque de 0,05 serait considérée significative[59].

4.2 Confirmation de l'efficacité in vivo de la combinaison non fixe en Casamance

Parce que le changement de politique de l'OMS est récent, il n'y a, jusqu'à présent, qu'une expérience toute relative de l'utilisation systématique de ces médicaments dans les pays endémiques pour le paludisme. Les données les plus fiables concernant l'utilisation systématique proviennent des zone de faible transmission de la frontière thailando-birmane où l'artésunate plus méfloquine a été utilisé en continu depuis le début des années 1990, bien avant les recommandations de l'OMS. Cette combinaison a invariablement produit des taux élevés de guérison, atteint une réduction de la transmission de *P. falciparum* alors que parallèlement la tendance à l'augmentation de la résistance *in vitro* à la méfloquine s'est inversée. Des résultats similaires ont été rapportés lors du déploiement de l'artemether/lumefantrine (Coartem®) dans une autre zone à faible transmission à la frontière Sud africano-mozambicaine, où l'importance du paludisme a chuté et où il y a eu une réduction de la morbidité et de la mortalité.

A l'inverse, il n'y a pas de données existantes sur l'utilisation à long terme des CTAs en provenance des zones de forte transmission du paludisme. Cette information est importante car les zones de transmission modérée à forte occupent globalement une plus grande place dans le fardeau que représente le paludisme, et les leçons apprises des zones de faible transmission pourraient ne pas s'appliquer dans le cadre de transmissions élevées.

Cet article décrit la tolérance et l'efficacité de l'AS-AQ dans le district chloroquino-résistant d'Oussouye, Casamance Sud, Sénégal, durant les années 2000 à 2005 pour le traitement de patients avec un paludisme à *falciparum* confirmé parasitologiquement.

Ce travail est la synthèse de l'évaluation de l'efficacité de l'AS-AQ utilisée pour traiter les patients de tous âges infectés par *P. falciparum* à Mlomp, sur une période de six ans. (2000 à 2005). L'efficacité a été déterminée à 28 jours en utilisant la méthode de survie de Kaplan-Meier (n=966) et la tolérance aussi (taux d'évènement indésirables n=752). Une posologie basée sur le poids fut utilisée pour l'association libre durant 2000-2003 (n=731) et un co-blister disponible dans le commerce fut utilisé en 2004-2005 (n=235). Les taux bruts annuels (non corrigés par PCR) restaient stable durant la période d'étude (94,6%, 95%CI=[92,9 ;95,9] ; range=88,5-96,7). Neuf patients traités avec le co-blister (0.9%) sont sortis de l'étude à cause d'évènements indésirables liés au traitement. Sept d'entre eux se plaignirent de douleur gastro-intestinales dont 2 furent hospitalisés pour vomissement. A J28 les valeurs moyennes de bilirubine totale (n=72), AST (n=94) et ALT (n=95) diminuèrent. 3 patients eurent des valeurs d'AST/ALT supérieure à 40 et

inférieure à 200 à jour 28. Les changements dans les globules blancs furent remarquables. AS-AQ en combinaison fut très efficace et bien tolérée dans cette zone and justifie la décision de l'utiliser en traitement de première ligne. Un suivi de long terme sur la tolérance et l'efficacité doit continuer.

Ce travail a fait l'objet d'un article publié dans le Malaria Journal en 2007. Ces résultats ont aussi été présentés lors du 55ième congrès de l'American Society of Tropical Medicine and Hygiene.

Article :

Brasseur P, Agnamey P, Gaye O, Vaillant M, Taylor WR, *et al.* (2007) **Efficacy and safety of artesunate plus amodiaquine in routine use for the treatment of uncomplicated malaria in Casamance, southern Senegal**. Malar J 6: 150.

Communication orale:

Artesunate (AS) plus amodiaquine (AQ) for treating falciparum malaria – Assessing its efficacy and tolerability during six years of deployment in southern Senegal. Philippe Brasseur, Patrice Agnamey, Moustafa Cisse, Philippe, Eldin De Pecoulas, Jean-François Faucher, Michel Vaillant, Oumar, Gaye, Walter (Bob) R. Taylor, Piero L. Olliaro**.** *55th annual meeting of the ASTMH, November 12-16, 2006, Atlanta, Georgia, USA. American Journal of Tropical Medicine and Hygiene 2006 Nov; 75 (90050) : 1-857.*

L'efficacité de l'AS-AQ était bien au-dessus de 90%, le niveau minimum requis par l'OMS, sans ajustement par génotypage (PCR) du taux de réinfections.

Tableau 2. Taux de succès estimés par la méthode de Kaplan Meier, distribution des échecs au traitement, et perdus de vue.

	2000	2001	2002	2003	2004	2005	2000-05
Success (%) (95%CI)	**96.7 (93.2-98.4)**	**94 (90.6-96.2)**	**95.7 (90.0-98.2)**	**94.9 (88.2-97.8)**	**88.5 (79.0-93.8)**	**95.9 (91.1-98.1)**	**94.6 (93.0-95.9)**
LTF	7	17	2	4	5	1	36
Withdrawn due to AE	0	0	0	0	4	5	9
Lost to follow up	2	1	3	2	1	23	32

Les échecs au traitement étaient les échecs parasitologiques Tardifs (LTF) et les sorties d'études dans l'analyse de Kaplan-Meier. Les perdus de vue étaient censurés.

Il n'y eu pas de toxicité observée dans les données biologiques. Aucune Hépatite ou leucémie sévère n'ont été détectée mais le monitorage des effets indésirables doit être intégré au déploiement à grande échelle des CTAs

Tableau 3. Type, fréquence et sévérité des signes et symptômes émergents sous traitement (TESS).

Symptom	Intensity				Total n(%)
	Mild	Moderate	Severe	Very severe	of TESS
Abdominal pain		5 (10.2%)			5 (5.9%)
Asthenia		2 (4.1%)	9 (53%)		11(13%)
Diarrhoea		3 (6.1%)			3 (3.5%)
Headache		1 (2%)	1 (5.9%)		2 (2.4%)
Nausea				1 (33.3%)	1 (1.2%)
Pruritus		4 (8.2%)	3 (17.6%)	1 (33.3%)	8 (9.4%)
Vertigo	4 (25.%)	13 (26.5%)	2 (11.8%)		19 (22.3%)
Vomiting	12 (75%)	21 (42.9%)	2 (11.8%)	1 (33.3%)	36 (42.3%)
Total	16 (18.9%)	49 (57.6%)	17 (20%)	3 (3.5%)	85 (100%)

4.3 Recherche opérationnelle en vue d'optimiser les conditions de mise en œuvre de la nouvelle stratégie de traitement

L'expérience à grande échelle, sur le long terme, de déploiement des CTAs est encore limitée aux zones de faible transmission frontalière de Thaïlande et Myanmar ou Kwazulu-Natal, où l'artésunate-méfloquine et l'artemether-lumefantrine, respectivement, ont contribué à une forte diminution du paludisme. Paradoxalement, il n'existe pas de telles données pour les zones de transmission modérée ou élevées.

Bien que l'efficacité de l'artésunate-amodiaquine (AS-AQ) varie selon les régions, en Casamance, l'AQ utilisée seule ou en association avec l'AS ont invariablement donné des taux élevés de guérison (>90%) récemment [27,60,61,62,63]. La combinaison est disponible en comprimés séparés dosés en fonction du poids nécessitant l'utilisation de fractions multiples de comprimés, ou ensemble en combinaison à dose fixe dans un blister et dosés en fonction de l'âge, avec des fractions de comprimés utilisés seulement pour les enfants de moins d'un an.

Peu d'attention a été donnée à la dose actuellement prise par les patients et comment cela pouvait affecter la tolérance, l'efficacité et la sensibilité du parasite. Ce sont des questions importantes car les ACTs sont utilisés sur une large échelle. Utiliser un nombre limité de catégories de dosage basées sur l'âge est plus aisé que le dosage basé sur le poids mais il peut en résulter des erreurs systématiques de dosages parce que certains patients recevront des doses inférieures ou supérieures aux intervalles thérapeutiques recommandés. Dans le cadre du développement d'une nouvelle combinaison à doses fixes (FDC) de comprimés d'AS-AQ de plusieurs concentrations, de nouveaux intervalles thérapeutiques de l'AS (2-10 mg) et de l'AQ (7.5-15 mg) ont été définis sur la base de données d'innocuité et d'efficacité publiées et non-publiées. La modélisation d'une grande base de données anthropométrique africaine a permis de prédire que 99.9% et 83.4% des patients recevraient des doses correctes d'AS et AQ, respectivement [64]. Il n'y pas de données disponible sur l'examen de l'exactitude des doses conventionnelles d'AS et AQ séparées (4 et 10 mg/kg/j, respectivement) ou des doses nouvellement définies et recommandées.

On rapporte ici l'exactitude d'un régime de doses basés sur l'âge et le poids des combinaisons AS-AQ actuellement disponible sous forme séparée ou en blister commun.

Les patients avec un paludisme non compliqué confirmé parasitologiquement ont été traités et suivis par des équipes de recherche ou par le personnel des centres locaux de santé en Casamance, Sénégal. L'AS-AQ fut donnée sous forme d'une association libre de

(AS 50 mg, AQ 200 mg) dosée sur la base du poids ; ou d'un co-blister (AS 50 mg, AQ 153 mg) dosé par poids ou par âge. Les doses cibles étaient (i) AS 4 (2-10) mg/kg/jour et (ii) AQ 10 (7.5-15) mg/kg/jour. L'exactitude des doses a été définie par les patients recevant les doses thérapeutiques. Les signes et symptômes émergeant sous traitements (TESS) ont été enregistrés. Un total de 3277 patients ont été traités avec l'association libre (n=1972, dosée par le poids) ou le co-blister (n=1305, 962 dosés par l'âge, 343 dosés par le poids), par l'équipe de recherche (n=966) ou par le personnel clinique (n=2311). l'AS fut dosée correctement dans plus de 99% des cas. La dose d'AQ de l'association libre dosée par le poids était à 98% adéquate. L'AQ du co-blister surdosait 18% des patients quand ils étaient dosés par âge et sous dosait 13% des patients dosés par le poids. Un faible poids était un facteur indépendant de risque de surdosage. Le co-blister avait significativement plus de TESS que l'association libre (117/1305 (9%) vs 41/1972 (2%), risque relatif = 4,3 (95%CI=3,0-6,1, p<0,0001). Le dosage basé sur l'âge était responsable de la différence. Les TESS survinrent principalement le premier jour de traitement (72%) et étaient faible ou modérée

En conclusion, l'AS est plus facile à doser que l'AQ. Le co-blister AS-AQ actuellement disponible avec une posologie basée sur l'âge tend à surdoser l'AQ et est moins bien toléré que les comprimés de l'association libre. Ce n'est pas la présentation optimale de l'AS-AQ.

Cet article fut publié dans la revue "Tropical Medicine and International Health" et une communication orale fut effectuée :

Article :

Brasseur P, Agnamey P, Gaye O, Cisse M, Badiane M, *et al.* (2009) **Dosing accuracy of artesunate and amodiaquine as treatment for *falciparum* malaria in Casamance, Senegal.** Trop Med Int Health 14: 79-87.

Communication orale:

Dosing accuracy of artesunate and amodiaquine as treatment for falciparum malaria in Casamance, Senegal. Philippe M. Brasseur, Patrice Agnamey, Oumar Gaye, Moustapha Cisse, Malick Badiane, Michel Vaillant, Walter R. Taylor, Piero L. Olliaro. *57th annual meeting of the ASTMH, December 7-11, 2008, New Orleans, Louisiana, USA. American Journal of Tropical Medicine and Hygiene 2008 Dec; 79 (6) : 1-390.*

Poster :

Efficacité, tolérance et adéquation du dosage de l' Artesunate plus amodiaquine (AS-AQ): 7 années d'utilisation en routine pour le traitement du paludisme non compliqué en Casamance, Sud-Senegal. M Vaillant, P Brasseur, P Olliaro, P. Millet. *9 ème Journée Scientifique de l'École Doctorale Sciences de la Vie et de la Santé, Mercredi 8 Avril 2009 au Palais des Congrès d'Arcachon*

Lorsque la posologie du co-blister est basée sur l'âge, on constate un surdosage alors qu'il y a sous dosage lorsque la posologie est dictée par le poids.

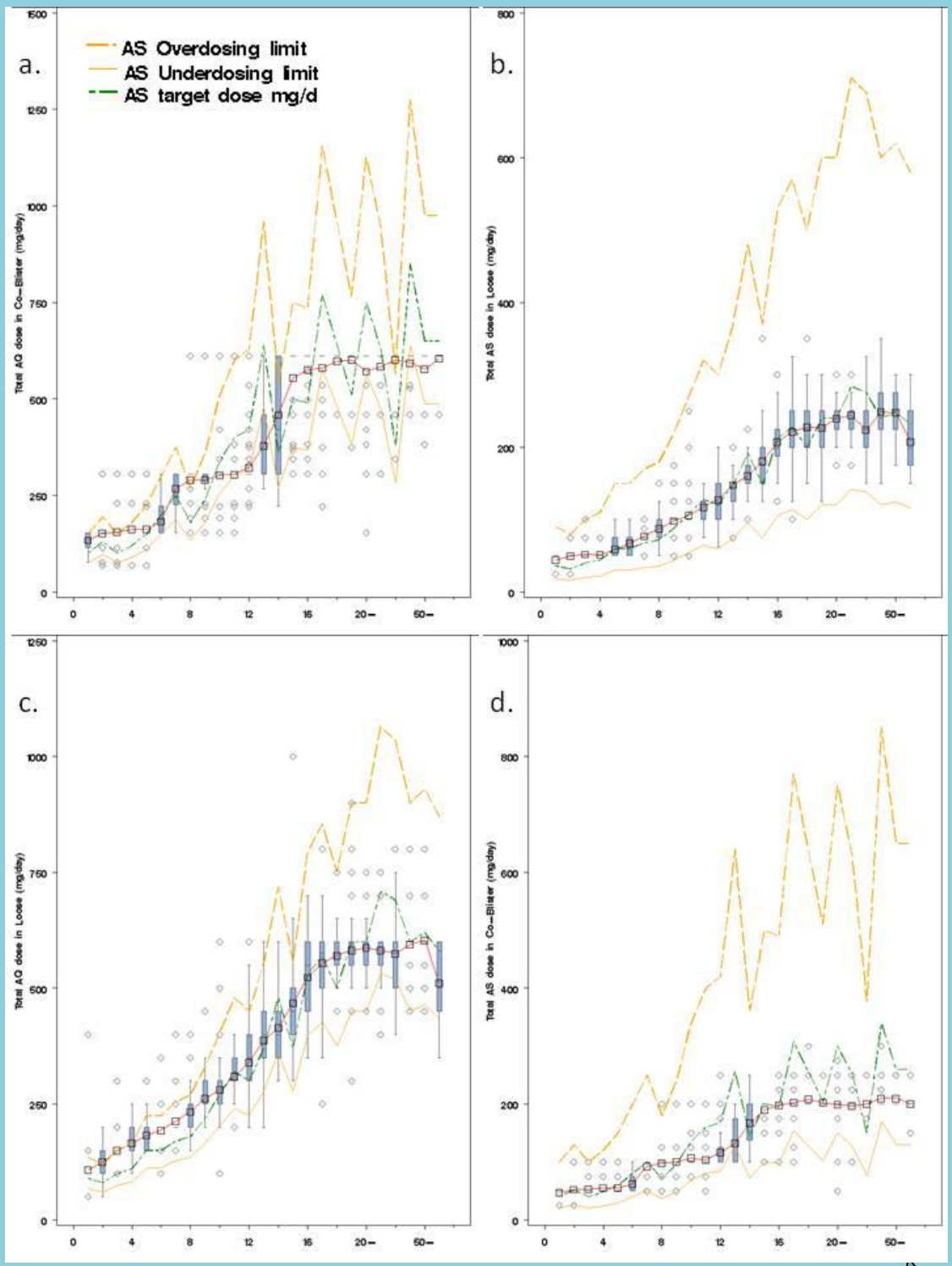

Figure 10. Boîtes à moustaches des doses (mg/j) de traitement AQ et AS avec l'intervalle thérapeutique.

4.4 Suivi de la performance des traitements en Casamance

4.4.1 Observation de la sensibilité *in vitro* des parasites aux médicaments utilisés en Casamance

La résistance des parasites à la chloroquine (CQ) s'est généralisée en Afrique subsaharienne. Les combinaisons à base d'artémisinine (CTAs) sont actuellement recommandées de préférence pour le traitement du paludisme non compliqué à *P. falciparum*. Dans le sud Sénégal la résistance à CQ *in vitro* et *in vivo* est établie. Nous avons comparé dans des essais cliniques CQ et AQ, et AS-AQ et AQ chez des enfants à Mlomp.

Par la suite nous avons déployé AS-AQ en 2000 pour les groupes d'âge, d'abord durant les saisons des pluies et puis durant toute l'année. AS-AQ a été systématiquement efficace et bien toléré. Avec plusieurs pays ayant déployé l'AS-AQ, il était important de savoir si la sensibilité du parasite était affectée par la généralisation de ces traitements. Pour cela nous avons suivi la susceptibilité *in vitro* d'isolats locaux à la CQ, la quinine (QN), l'artémisinine (ART) et au métabolite, la monodesethylamodiaquine (MdAQ) de l'AQ en utilisant les tests in vitro avant 1997 et après le déploiement 2000-2004 de l'AS-AQ.

Le niveau de résistance *in vitro* à CQ est stable avec des concentrations inhibitrices 50% (CI_{50}) de 50 à 60 nMoles, une résistance faible à QN, AQ et une bonne sensibilité à ART à l'exception de rares isolats présentant CI_{50} supérieures à la normale estimée. Les CI_{50} MdAQ restent stables ou ont tendances à diminuer au fil des années de surveillance et ART reste sensible. Il est encore trop tôt pour déterminer si ces changements ont une signification clinique. Ces données *in vitro* sont cohérentes avec les réponses in vivo. Ces données nous permettent de conclure que la susceptibilité parasitaire n'a pas été altérée par la phase initiale de déploiement de l'AS-AQ.

Article :

Agnamey P, Brasseur P, de Pecoulas PE, Vaillant M, Olliaro P (2006) ***P. falciparum in vitro* susceptibility to antimalarial drugs in Casamance (southwestern Senegal) during the first 5 years of routine use of artesunate-amodiaquine**. Antimicrob Agents Chemother 50: 1531-1534.

Entre 2000 et 2004, le niveau de résistance de *P. falciparum* à la chloroquine est resté stable (50 à 60%) et il y a peu de résistance *in vitro* à la quinine, la monodesethylamodiaquine et l'artémisinine.

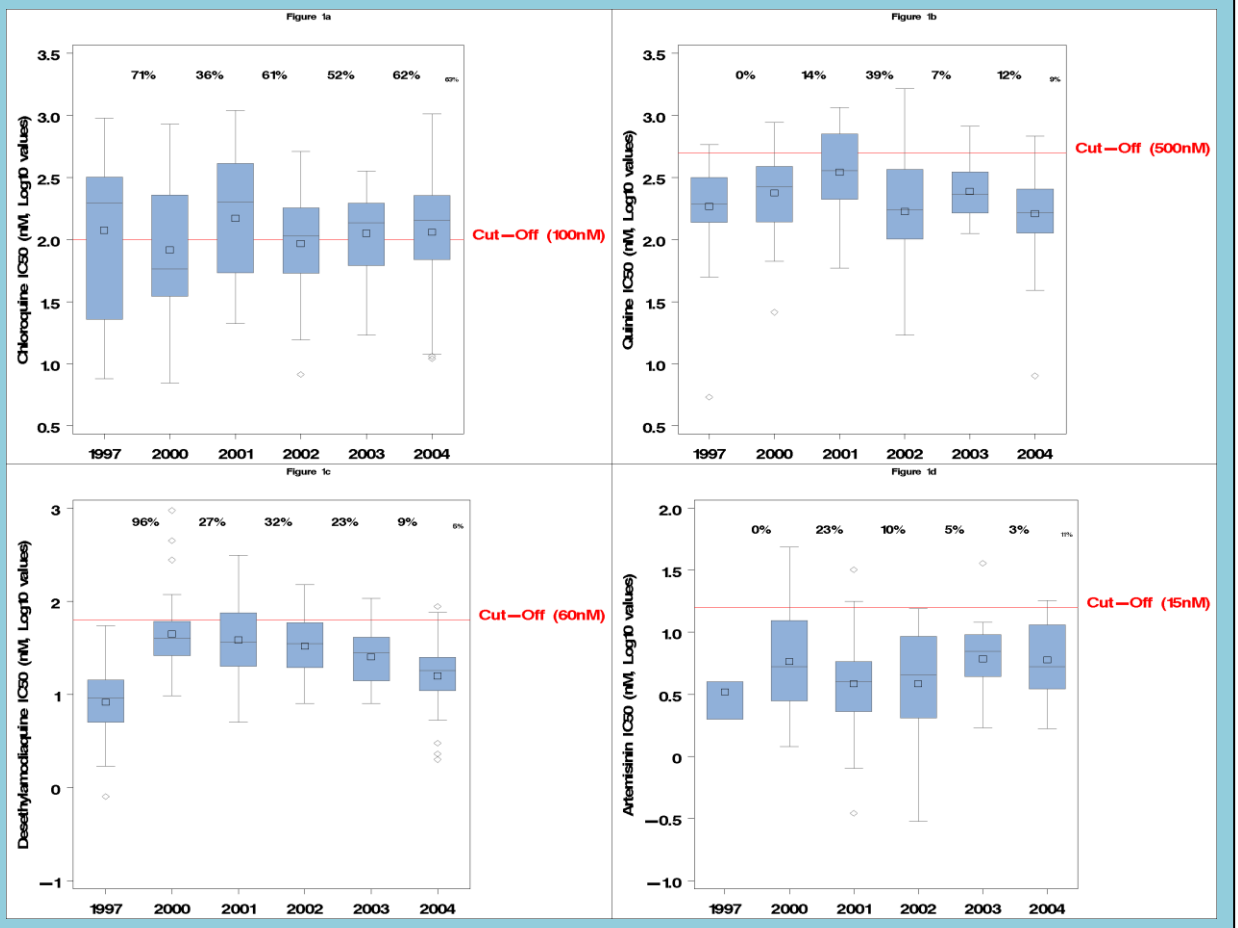

Figure 11. Boîte à moustaches des valeurs d'IC50 log-transformées (en nM) pour la chloroquine (figure 11a), la quinine (figure 11b), la monodesethylamodiaquine (figure 11c) et l'artémisinine (figure 11d) par années (1997 et 2000 à 2004).

Les pourcentages d'isolats au dessus du Cut-Off de résistance *in vitro* (ligne rouge horizontale) sont affichés pour chaque médicament. Les extrémités des moustaches correspondent valeurs extrêmes observées dans 1.5 x l'intervalle interquartile (IQ). La médiane est symbolisée par le trait dans la boîte ; moyenne = □ ; valeurs hors de 1.5xIQ = ◊

4.4.2 Comparaison interannuelle de la résistance aux traitements en Casamance

P. falciparum, l'espèce la plus responsable du paludisme dans le monde, peut être mise en culture, ce qui rend possible la mesure de la susceptibilité du parasite aux traitements *in vitro[65]*. Turnidge *et al* [66] ont présenté une nouvelle méthode pour évaluer les valeurs seuils afin de définir la résistance aux antibiotiques. Toutefois, alors que les méthodologies sont bien établies pour les traitements antimicrobiens [67], il n'est pas possible, pour une majorité de traitements, de classifier avec certitude une souche résistante ou sensible parce que des seuils validés ne sont pas disponibles, à l'inverse de la situation pour les antibiotiques [68].

Ce qui est particulièrement intéressant dans la sensibilité aux traitements de *P. falciparum* n'est pas le profil de sensibilité de l'isolat d'un patient donné mais plutôt l'étude des tendances d'évolution de la sensibilité aux traitements lorsqu'une pression médicamenteuse est exercée dans une zone donnée [69]. Cette information précoce de la résistance précède l'apparition de résistance in vivo et peut contribuer à modifier à temps les recommandations thérapeutiques. Toutefois l'évaluation de tendances temporelles pose des problèmes méthodologiques, principalement à cause (i) la grande variabilité des données fait que les méthodes statistiques conventionnelles comme l'analyse de variance (ANOVA) sont inadéquate, (ii) l'unité statistique n'est pas l'isolat du patient seul mais la population composite de parasite au sein d'un individu. A cause de cette variabilité, ces données sont usuellement présentées à l'aide de moyennes géométriques. Un élément clé dans l'analyse de ces données est la détection de tendances temporelles. Toutefois il n'existe pas encore d'approche statistique pour cela [70] sans perte d'information.
Les méthodes utilisées dans les études pharmacocinétiques traitent de problèmes similaires avec des données de l'état des traitements dans un individu ou une population d'individus [71,72,73]. La bioéquivalence entre deux formulations de traitements est typiquement évaluée en utilisant l'analyse de variance des valeurs log-transformées à partir d'un schéma en cross-over avec l'hypothèse nulle : H_0 : $\mu_T = \mu_R$ où μ_T et μ_R représentent les paramètres log-transformés attendus de biodisponibilité test/référence des formulations test et de référence respectivement [74,75]. Un intervalle de confiance (IC) à 90% du rapport pour les paramètres de biodisponibilité est construit en utilisant l'équation $\mu_T - \mu_R \pm S\sqrt{\frac{2}{nt_{0.05(1),\upsilon}}}$ où S est la racine carrée du carré moyen de l'erreur de l'ANOVA, n est le nombre de sujets par période, $t_{0.05(1)\nu}$ est la valeur critique de t à α=0.05 avec υ les degrés de liberté.

Dans le cas de tests de sensibilité temporelle, quand les sujets sont groupés par l'année d'échantillonnage, la variance dans l'ANOVA de la sensibilité parasitaire (exprimée en CI_{50}) peut être répartie entre les contributions du parasite, du sujet et le temps de mesure. L'ANOVA tient compte de l'année de mesure et test si la variabilité entre les années intervient aléatoirement ou pas. Le rapport de la somme des carrés moyens des paramètres (e.g. CI_{50}) et de la somme des carrés moyens des erreurs dans l'ANOVA donne une statistique de F testant l'hypothèse nulle H_0 : $\mu_{year} = \mu_{year0.}$ Cela fournit un test de la moyenne arithmétique de CI_{50} mesurée pour des années données est identique à la moyenne de l'CI_{50} obtenue lors de l'année de référence.

Une hypothèse sous jacente à l'utilisation de l'ANOVA est la normalité des résidus (la différence entre une valeur individuelle et la moyenne de l'échantillon duquel il est issus $x_i - \bar{x}$.). Toutefois vérifier l'hypothèse nulle d'égalité entre les moyennes peut ne pas être possible avec des distributions de résidus généralement non gaussienne ni log normale. En outre la taille d'échantillon est le plus souvent faible par rapport à la variance pour réaliser la comparaison des moyennes. La grande variabilité des données conduit à de grande variabilité des erreurs en termes de somme des carrés des erreurs. Ainsi la détection d'une différence peut être difficile à interpréter puisque elle sera fonction de la variabilité des données et de la taille d'échantillon pour chaque année. Les modèles mixtes peuvent être utilisées quand il y a différents niveaux de regroupement dans les observations. On peut supposer qu'il ya un regroupement par année et qu'une part aléatoire des mesures par année est due au sujet et aux souches parasitaires infectant ce sujet. Cela permet à l'utilisateur d'analyser des échantillons (ici : années) avec des tailles de groupes inégaux et de relâcher l'hypothèse de résidus indépendamment et également distribués tout en étant plus flexible dans la prise en compte de la structure des données [76].

Pour le paludisme, avec l'introduction de nouveaux traitements comme les combinaisons à base d'artémisinine (ACTs) [77], il est important d'évaluer si, avec des parasites étant exposés à la pression médicamenteuse, les quantités de traitements nécessaires pour inhiber la croissance du parasite s'éloignent de celles de l'année de référence, avant et durant le déploiement pour suivre l'évolution de la susceptibilité aux traitements. De plus des méthodes statistiques appropriées sont nécessaires pour tenir compte de la variabilité dans la détermination de la CI_{50} dans le but d'informer convenablement les décisions de politique de santé.

Plusieurs approches ont été explorées pour décrire les tendances temporelles de la susceptibilité *in vitro* des parasites aux traitements à Mlomp durant 200-2004 légèrement adapté pour l'objet à partir de l'article d'Agnamey *et al* [78].

Un modèle mixte a été généré sur les valeurs log transformées d'CI_{50} et les moyennes géométriques des moindres carrés (GLSM) avec leurs intervalles de confiance(IC) à 90% ont été calculées. Dans le but de comparer les CI_{50} entre les années, les rapports de GLSM (GLSMR) avec leurs IC90% ont été calculés et lorsque les limites des intervalles étaient au dessous ou au dessus de 100%, la différence a été considérée significative. Les résultats furent comparés avec ceux obtenus en utilisant un ANOVA ou un modèle linéaire généralisé. Les GLSMRs étaient plus conservateurs que l'ANOVA et ont résulté en des niveaux de significativité plus faibles. L'approche des GLSMRs a permis d'inclure un effet aléatoire et un ajustement pour les comparaisons multiples. Le modèle linéaire généralisé était limité dans le nombre de comparaison des années par paire du fait de la nécessité de définir une année de référence. Les trois types d'analyses aboutirent à des résultats cohérents.

Une méthode statistique robuste peut pallier aux limitations inhérentes au test de susceptibilité *in vitro*. Les GLSMRs à effets aléatoire avec un ajustement pour les comparaisons multiples et des intervalles de confiance à 90% requièrent uniquement des conditions d'applications liés aux modèles mixtes. Les résultats sont faciles à représenter graphiquement et à interpréter. Les GLSMRs pourraient être considérer comme une option pour la surveillance des changements dans la susceptibilité aux médicaments de *P. falciparum* et d'autres microbes.

Cet article a été publié dans le Malaria Journal. Deux posters ont été réalisés dans le cadre de congrès francophones d'épidémiologie et/ou de maladies tropicales :

Article :

Vaillant M, Olliaro P (2007) **Geometric least squares means ratios for the analysis of *P. falciparum in vitro* susceptibility to antimalarial drugs**. *Malar J 6: 156*.

Posters :

Rapports de moyennes géométriques pour évaluer la sensibilité *in vitro* de Plasmodium falciparum aux antipaludéens en Casamance (Sud-Ouest du Sénégal).Michel Vaillant, P. Agnamey, P.Brasseur, P. Eldin de Pecoulas, P. Olliaro. *CONGRES ADELF-EPITER du 30 août – 1er septembre 2006 Dijon*

Sensibilité *in vitro* de *Plasmodium falciparum* aux antipaludéens en Casamance (Sud-Ouest du Sénégal) : Analyse longitudinale des IC50. Michel Vaillant, P. Agnamey, P.Brasseur, P. Eldin de Pecoulas, P. Millet, P. Olliaro. *Congrès francophone d'Epidémiologie en milieu tropical, IRSP, Ouidah, Bénin du 23 au 25 janvier 2007*

Les rapports de moyenne géométriques des moindres carrés permettent de choisir l'année de référence (le dénominateur du rapport) et de lui comparer les autres années pour un estimateur avec une grande variabilité comme la CI_{50}. Les intervalles de confiance ne chevauchant pas la ligne d'identité (100%) montrent la présence d'une différence significative. L'ensemble de la représentation graphique permet de lire plusieurs mesures effectuée, ici les années 2000 à 2004 et d'appréhender l'évolution de la susceptibilité du parasite P. falciparum aux différents médicaments testés.

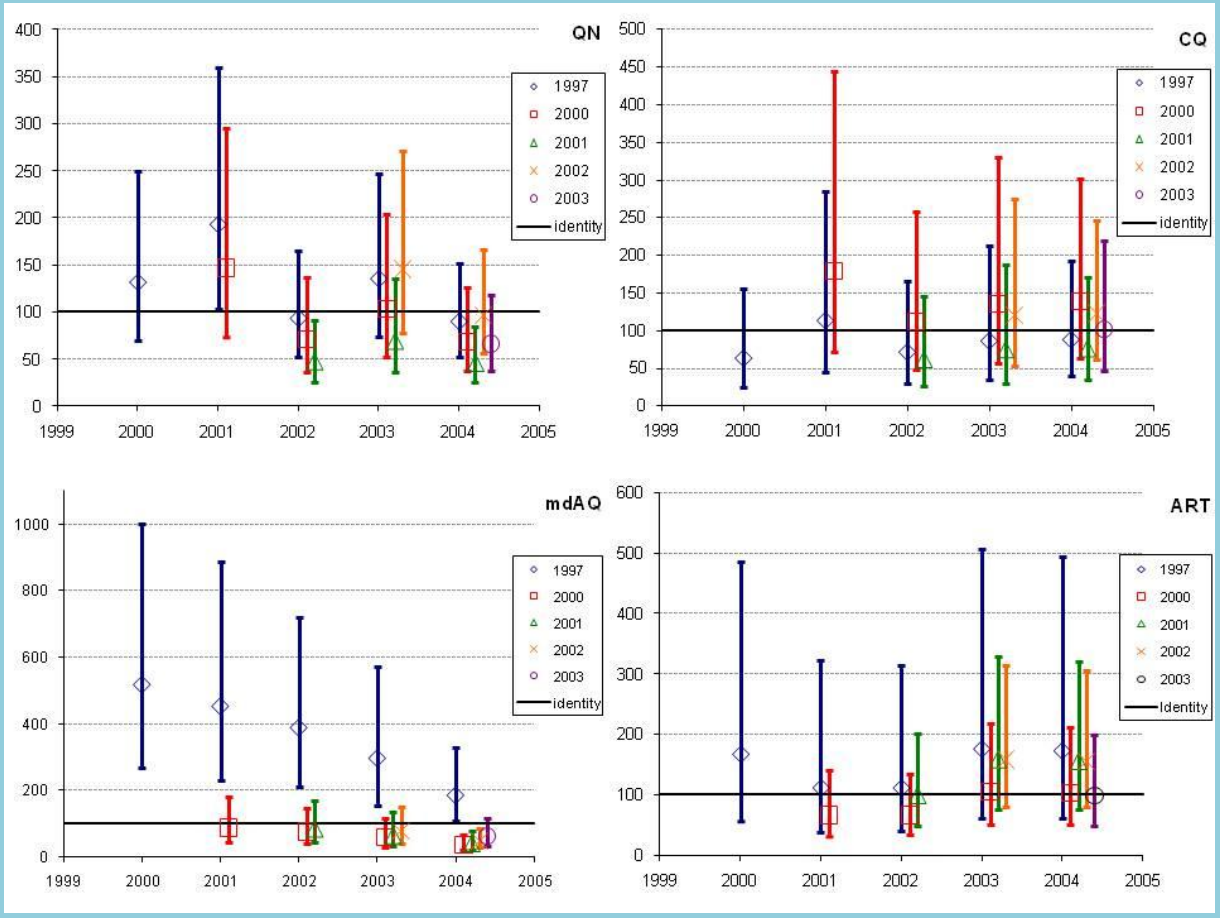

Figure 12. Rapports de moyennes géométriques des moindres carrés des IC_{50} de quinine (QN), chloroquine (CQ), monodesethylamodiaquine (mdAQ) et artémisinine (ART).

La légende indique l'année de référence utilisée dans le rapport. L'axe des abscisses indique l'année testée dans le rapport. Une échelle logarithmique a été utilisée pour QN.

4.5 Suivi des effets du traitement systématique par ASAQ

4.5.1 Gestion des fièvres à la présentation au dispensaire

Le traitement par combinaison à base d'artémisinine (ACT) est le traitement de première ligne recommandé par l'OMS pour le paludisme non compliqué [79] et est devenu une politique de santé dans les pays endémique [9], mais des problèmes apparaissent lors de l'implémentation locale de cette politique.

La politique de traitement du paludisme au Sénégal a changé en 2006 [80] partant de la chloroquine ou quinine donnée sur la base d'un diagnostic clinique à l'AS-AQ ou l'AM+LF après confirmation parasitologique pour les patients de tous âge, mais les tests de diagnostic rapide (TDR) ne devinrent disponible qu'en 2007. Avant le déploiement à grande échelle de la nouvelle politique aux ACT, une mesure intermédiaire avec AQ+SP fut recommandée de 2004 à 2005. Depuis 2006, seulement l'AS-AQ et QN sont mis à la disposition des infrastructures du secteur public. Les traitements et les TDR sont obtenus au travers d'appel d'offres par la pharmacie centrale nationale (Pharmacie d'Approvisionnement, PNA) et répartis vers les pharmacies régionales et de district, les hôpitaux, les centres de santé et les postes de santé des districts.

A l'occasion du changement de politique, le PNLP a organisé des cours pratiques pour toutes les catégories de fournisseurs de soins de santé (médecins, infirmières, agents de santé communautaires). Des cours de rappel sont aussi régulièrement organisés[81]. L'observance aux politiques est régulièrement vérifiée par l'inspection des registres cliniques par un superviseur du district.

Cet article décrit les pratiques de gestion de la fièvre dans les centres de santé de deux villages (Momp, Elinkinde) du sud Sénégal durant 2000-2008. Le paludisme y est mésoendémique avec une transmission faible durant la saison sèche (Janvier-Juin) et élevée durant la saison des pluies (Juillet-Décembre). La dernière enquête entomologique conduite avant le changement de politique a relevé 25 piqures infectantes par personnes et par an [82].

Les données ont été extraites des registres cliniques et catégorisées en cinq classes de gestion des cas. Les analyses ont été réalisées sur la base du semestre (correspondant aux saisons de faible et haute transmission du paludisme). Des modèles logistiques ont été utilisés pour calculer les odds ratios avec leurs intervalles de confiance à 95% pour le changement des pratiques de gestion des cas à travers le temps et pour la comparaison des deux dispensaires. En moyenne l'accès de la population aux structures sanitaires était similaire (0,8 consultations par personne/année ; stable à Mlomp alors qu'il a pratiquement triplé à Elinkinde entre 2004 et 2008) ; 13.509 et 6926 patients ont consulté pour une fièvre à Mlomp et Elinkinde respectivement. 8221 traitements ont été dispensé

à Mlomp (22% confirmés parasitologiquement) et 4085 à Elinkinde (2% confirmés). L'AS-AQ comptait pour 28% et la quinine pour 53% des traitements dispensé à Mlomp, comparé à 36% et 46% à Elinkinde. Les taux de prévalence des consultations totales, consultations pour fièvre, traitement antipaludique dispensé étaient significativement supérieurs à Mlomp jusqu'en 2006, similaire ou supérieurs à Elinkinde jusqu'à mi 2008. A Mlomp l'odd du traitement antipaludique donné sans test parasitologique fait ou en dépit d'un test négatif n'a pas décru sur la période d'étude.

La prévalence du paludisme et de traitements dispensés est en décroissance dans ce district rural mais des écarts aux recommandations nationales sont observés. Les traitements antipaludiques continuent à être donnés sans confirmation parasitologique en dépit de recommandations claires et de formations réitérées; L'utilisation de l'artémisinine en monothérapie, bien que faiblissant continue aussi. A Mlomp, initialement échelonnée, le déploiement soutenu par de la recherche a facilité l'observance à la politique de santé mais ce succès est en train de s'effriter. Des CTAions correctives sont nécessaires pour réduire les traitements superflus et leurs conséquences négatives (coût, toxicité potentielle, mauvaise gestion des cas).

Cet article a été proposé à la revue "Tropical Medicine and International Health" qui l'a refusé, le trouvant trop complexe. Un travail de simplification de l'expression des résultats statistiques est à mener en particulier sur les explications et interprétations des modèles statistiques impliquant des interactions entre les variables. Une communication orale avait été bien accueillie lors du 55ième congrès de l'American Society of Tropical Medicine and Hygiene.

Article :

Brasseur P, Badiane M, Cisse M, Sokhna C, Kindermans J, *et al.* (2010) **Management of fever in rural dispensaries in Southern Senegal following malaria treatment policy change.** In preparation.

Communication Orale:

Effects of applying new malaria treatment policies in a rural district of Casamance, Southern Senegal. Philippe M. Brasseur, Patrice Agnamey, Oumar Gaye, Moustapha Cisse, Malick Badiane, Michel Vaillant, Walter R. Taylor, Piero L. Olliaro. *57th annual meeting of the ASTMH, December 7-11, 2008, New Orleans, Louisiana, USA*

Ce travail vient alimenter la discussion sur l'opportunité de donner un traitement antipaludique sur la base d'une confirmation parasitologique [83,84].

L'analyse montre que, à Mlomp, le risque d'être traité avec un antipaludique en dépit d'un test parasitologique négatif, voire non réalisé, diminue drastiquement à partir du premier semestre 2006 comparé au risque de traitement à raison pour une autre cause. Toutefois le risque d'être traité sans test parasitologique sur la base de symptôme augmente à nouveau dans le premier semestre 2007.

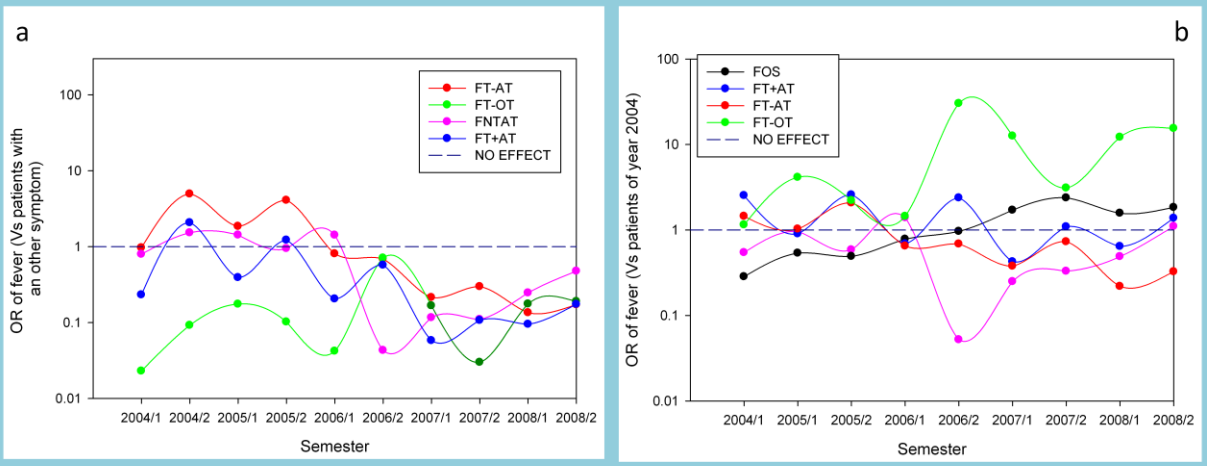

Figure 13. Gestion des fièvres au centre de santé de Mlomp au cours du temps (semestres entre 2004 et 2008).

Odds ratio de chaque type de gestion des fièvres (a) en référence aux fièvres d'une autre cause que le paludisme ; (b) en référence à la même catégorie en 2004. FT-AT =test parasitologique négatif et traitement antipaludique ; FT-OT = test parasitologique négatif et autre traitement ; FNTAT = traitement antipaludique sur symptômes cliniques ; FT+AT = test parasitologique positif et traitement antipaludique.

Des résultats encourageants après la mise en place de la stratégie de traitement antipaludique sur base d'une confirmation parasitologique à partir de l'année 2000 à Mlomp avec une augmentation relative des diagnostics autre que la paludisme sont observés. Toutefois des écarts aux directives nationales sont notés à partir de 2007 montrant une confiance toute relative dans le résultat du test parasitologique et une tendance à revenir à l'ancienne pratique de traitement antipaludique sur suspicion clinique.

4.5.2 Effet à moyen terme du traitement systématique par ACT sur l'épidémiologie, et l'âge des sujets atteints, du paludisme

Le risque de paludisme associé à l'âge et sa présentation clinique (non compliqué et/ou sévère) varie avec l'intensité de la transmission. Il y a des différences marquées dans le nombre de crises du paludisme à chaque âge dans des conditions épidémiologiques variées (revue de littérature par Lalloo et al, 2006 [85]). Indubitablement l'intensité de transmission et le nombre de stimulation durant le temps déterminent la susceptibilité au paludisme, en parallèle avec la vitesse à laquelle l'immunité se construit. Même dans les zones où le paludisme est stable, l'intensité de transmission dessine des profils d'âge très différents de cas de paludisme [86].

Alors que les tendances sont à la décroissance de la transmission du paludisme, la morbidité et la mortalité sont encore décrites [8,87,88], il y a peu d'information sur la façon dont ces changements affectent les différents âges. O'Meara *et al* [88] décrivent que bien que l'âge des fièvres non palustres vues dans un service pédiatrique à Kilifi, Kenya, est resté stable entre 1990 et 2007 (2,15 à 2,18 années), celui des patients avec des gouttes épaisses positives a augmenté de 2,3 à 3,59 ans.

La structure d'âge des cas de paludisme dans cette zone est restée stable pendant plusieurs années [89] et cohérente avec d'autres zones d'endémicité similaire au Sénégal [86]. Le risque de paludisme le plus élevé est retrouvé chez les enfants âgés et les jeunes adolescents (57% des patients sont âgés de 6-15 ans) et s'étend aussi plus tard dans la vie (8% sont âgés de 21-30 ans, 7% de 30 ans et plus).

Cet article analyse les changements intervenu dans le risque de paludisme en fonction de l'âge sur une période de 14 ans (1996-2009) à Mlomp. Entre janvier 1996 et Décembre 2009 un total de 99.758 consultations ont été effectuée pour lesquelles 45,801 traitements ont été délivrés (avec ou sans confirmation parasitologique). Parmi les patients bénéficiaires, une confirmation parasitologique a été obtenue pour 26.156 (28,9%) d'entre eux dont 36.7% (14.831) positifs pour *P. falciparum*. Sur l'ensemble de la période ces chiffres diminuaient progressivement. La proportion de cas de fièvre parasitologiquement positives chez les patients âgés de 0 à 5 ans diminuait progressivement sur l'ensemble de la période, plus faiblement chez 6-10 ans alors que elle restait stable chez les 11-15 ans et augmentait chez les patients âgés de 16 ans et plus. L'âge moyen des patients atteints de paludisme augmentait de 13,4 ans en 1996 à 20,1 ans en 2009. En utilisant un modèle binomial négatif pour l'analyse des comptages des différentes catégories de fièvres, il est montré une tendance générale de décroissance du risque test parasitologique positif entre 2002 et 2009. En considérant les classes d'âge, l'évolution

des 0-15 ans est relativement stable tout au long de la période alors qu'il est constaté une augmentation constante pour les patients âgés de 16 ans et plus qui en 1996 avait un risque plus faible d'avoir un test parasitologique positif.

En conclusion, le profil d'âge des patients impaludés a changé pendant la période d'étude et le risque de paludisme est devenu uniformément reparti sur l'ensemble de la vie, alors que dans le passé le paludisme concernait plus particulièrement les enfants. Cette tendance coïncide avec un risque de paludisme qui a diminué dans cette zone.

Article :

Brasseur P, Agnamey P, Cisse M, Badiane M, Vaillant M, Olliaro P *(*2010) **Changing patterns of malaria between 1996-2009 in an area of moderate transmission in Southern Senegal**. *in preparation.*

Poster:

Effets sur l'épidémiologie du paludisme non compliqué du déploiement de la combinaison d'artésunate et amodiaquine à Oussouye, district de Casamance, Sénégal (Recherche-Action). M.Vaillant, P.Brasseur, P.Agnamey, O.Gaye, , WRJ Taylor, P. Millet, P.Olliaro. *3ème Conférence Francophone d'Epidémiologie Clinique, Congrès thématique de l'ADELF, Fès, Maroc, 7 et 8 mai 2009*

Sur l'ensemble de la période de 1996 à 2009, les consultations, les prescriptions de traitement antipaludique et les cas de paludisme estimés décroissaient progressivement

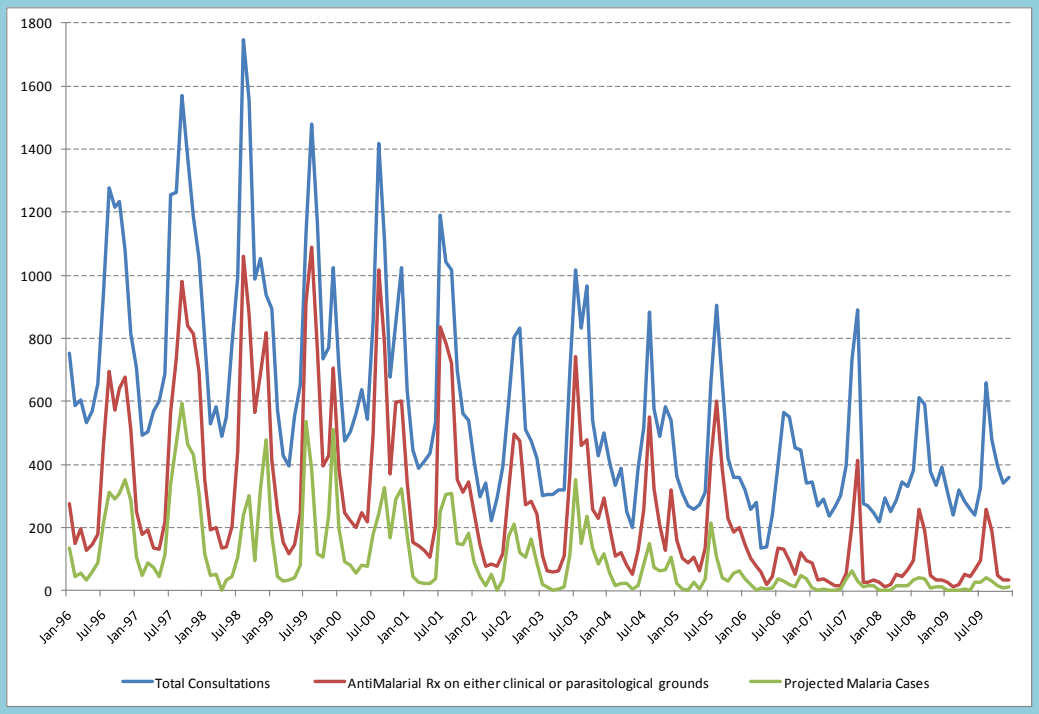

Figure 14 ; Consultations mensuelles pour fièvre, traitement antipaludique prescrits (sur base clinique ou parasitologique) et cas de paludisme extrapolé à Mlomp, 1996-2009

Le risque de test parasitologique positif des plus de 16 ans converge vers un risque identique aux 6-15 ans par rapport au risque de test positif des 0-5 ans : Alors qu'une tendance décroissante de la transmission du paludisme s'est mise en place, il ya une homogénéisation du risque d'attaque palustre sur toutes les catégories d'âge.

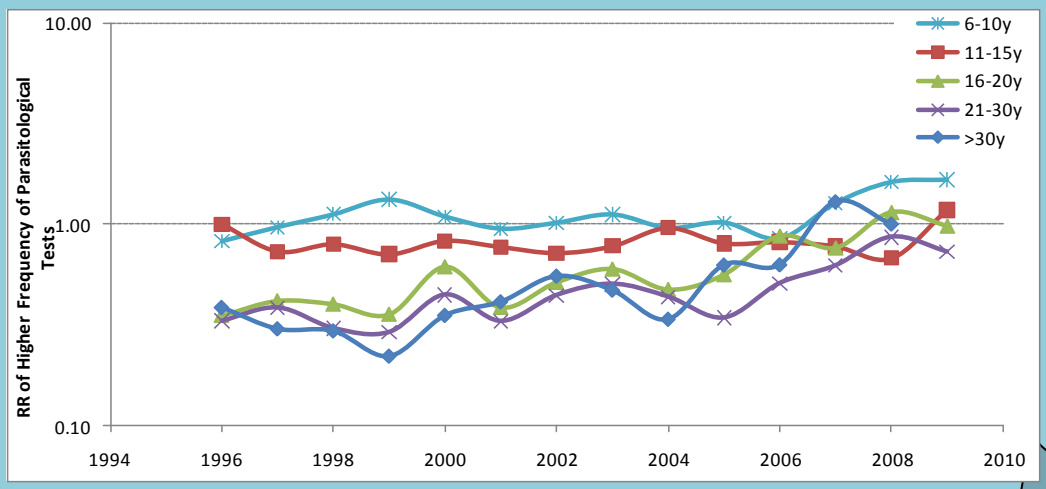

Figure 15. Risque de test parasitologique positif de chaque classe d'âge par rapport à la catégorie d'âge 0-5 ans

5 Discussion

La discussion reprend les travaux effectués à Mlomp en mettant en avant les résultats obtenus de la mise en place d'une nouvelle politique de traitement du paludisme avec l'AS-AQ et les outils d'aide à la décision de santé publique élaborés en parallèle. Un aperçu est donné de l'utilisation de ces travaux dans le développement d'une formulation bicouche de l'AS-AQ. Une conclusion vient ensuite terminer le chapitre par un résumé et les limites de ces études.

5.1 Mise en œuvre d'une nouvelle stratégie de traitement du paludisme

La revue systématique des études sur la combinaison à base d'artémisinine et d'amodiaquine publiées entre 1999 et 2008 a permis de montrer l'efficacité à 28 jours sur les monothérapies (chloroquine, l'amodiaquine, la sulfadoxine-pyrimethamine et l'artésunate) et les combinaisons ne contenant pas dérivé d'artémisinine (AQ+SP et CQ+SP), supérieure à la limite minimum de 90% fixée par l'OMS, mais sa supériorité par rapport aux autres ACT (AM+LF, AS+SP, AS+MQ, DH+PQ) n'a pas démontrée. Ces résultats donnent un tableau général de l'effet de l'AS-AQ mais leur valeur en termes de contrôle du paludisme peut varier à cause de considérations méthodologiques telles que la prise en compte de l'endémicité et le contexte de susceptibilité du parasite entre les sites d'études. Ce travail met aussi en lumière le danger de l'utilisation des monothérapies à la fois pour le contrôle global de la maladie mais aussi en termes d'efficacité puisque le taux de succès avec ces thérapies est inférieur à 90%. En 2008, Gardella et al montraient que la prévalence de trace de CQ dans les urines d'enfants âgés de 2 à 9 ans pouvait atteindre plus de 60% [90].

Paradoxalement les rapports sur l'innocuité de l'AS-AQ étaient très limités. Globalement, la combinaison fut considérée sans danger avec des évènements indésirables mineurs ou légers comme les vomissements mais avec des différences de traitements de ces évènements entre les études. Deux évènements sérieux qui susceptibles de survenir avec l'amodiaquine sont la neutropénie et l'hépatite. Leur survenue a déjà été décrite dans le cadre d'un traitement prophylactique [91,92,93]. Elles ont été décrites chez 6% et 8% des patients sous AS-AQ et AQ dans 2 études seulement [27,94] mais ces résultats n'ont pas été reproduits dans les autres essais inclus dans la méta-analyse.

Dans un contexte d'utilisation de routine, une étude pragmatique accompagnant le déploiement de cette association pour traiter le paludisme non compliqué causé par *P. falciparum* était nécessaire afin d'évaluer l'efficacité et la tolérance de l'association AS-AQ

hors des conditions contrôlées d'un essai thérapeutiques randomisé. Plusieurs études ont été menées dans différents contexte d'endémie de la maladie, de transmission et de résistance aux traitements du parasite [87,88,95,96]. Nous avons réalisé l'une d'entre elles dans le district d'Oussouye [60,62,89,97]. Les objectifs de cette étude étaient multiples et se proposaient d'évaluer l'efficacité, la tolérance et la précision du dosage de la combinaison en utilisation courante. L'effet de ce déploiement sur la sensibilité *in vitro* et ses répercussions in vivo étaient aussi des questions posées. L'épidémiologie globale du paludisme dans le district d'Oussouye durant les années de déploiement du nouveau traitement était également une des interrogations consécutive au changement de stratégie thérapeutique.

Après six années d'utilisation (2000-2005), l'AS-AQ était très efficace (>90%) et bien tolérée dans cette zone d'Afrique où le taux de résistance *in vitro* est supérieur à 60% [56,76]. L'AS-AQ fut d'une efficacité équivalente qu'elle soit dosée en fonction du poids dans le cas de la combinaison libre à deux comprimés distincts (Arsumax®+Camoquin®) ou de l'âge dans le cas de la combinaison co-blister (Arsucam®). Toutefois, bien qu'ayant respecté les recommandations du fabriquant, cette dernière a résulté en plus d'événements indésirables, et la raison des sorties d'études fut exclusivement l'intolérance du médicament, très probablement en raison de la toxicité attribuée à l'AQ. Un dosage plus équilibré basé sur une vaste base de données de poids par âge a été réalisé par Taylor *et al* [64] et a été utilisé pour le développement d'une combinaison fixe [98,99,100,101]. Cette combinaison fixe combinant l'artésunate avec l'amodiaquine dans un comprimé bi-couche (artesunate – amodiaquine Winthrop® ou co-Arsucam®, Sanofi Aventis) n'était pas introduite au Sénégal pendant notre étude. Nous n'avons donc pas pu comparer son efficacité et observance avec les combinaisons séparées (cf 4.3).

Le dosage de la combinaison AS-AQ a été étudié plus en détail sur la même période en étendant la population investiguée aux centres locaux de santé (N_{total}=3277). Les résultats montraient que l'AQ provenant du co-blister résultait en un surdosage dans 18% des sujets dont le traitement était dosé par rapport à l'âge et un sous dosage dans 13% des sujets dont le traitement était dosé par rapport au poids. Le nombre d'évènements indésirables, légers ou modérés, des patients sous co-blister était aussi plus élevé, confirmant la tendance observée dans le cadre de l'essai thérapeutique.

Un autre aspect du déploiement de l'AS-AQ en Casamance était son effet et son évolution sur la susceptibilité du parasite à l'AS, l'AQ ainsi que le suivi de la susceptibilité aux autres traitements utilisé avant l'AS-AQ, la CQ et la QN. Les rapports de moyennes des moindres carrés (GLSMR) montraient que le niveau de résistance *in vitro* à la CQ était stable (50-60%) entre 1997 et 2004 [78]. La résistance *in vitro* à QN a augmenté de

1997 à 2002 puis chuté ensuite tandis que la susceptibilité à la MdAQ, métabolite actif de l'amodiaquine, diminuait à partir de 2000. En revanche la susceptibilité à l'AS demeurait stable [102].

La stratégie de traitement du paludisme avec l'AS-AQ implique de réaliser un test parasitologique (goutte épaisse ou test rapide) à partir d'un prélèvement sanguin du sujet présentant une fièvre [79,103]. La précédente stratégie était de traiter toute fièvre à l'aide de quinine ou chloroquine. A Mlomp le déploiement de l'AS-AQ couplé à la nouvelle stratégie de traitement a permis de diminuer le nombre total de consultations et de traitements du paludisme depuis 2000. Jusqu'en 2006, les diagnostics de fièvres et traitements non paludiques ont relativement augmenté avec en parallèle un déclin des traitements antipaludiques prescrits. Toutefois cette tendance s'inversait après 2006, mettant en évidence de plus en plus d'écarts au programme national de traitement et une prédisposition à revenir à l'ancienne stratégie de traitement basée sur les symptômes cliniques. Concrètement, le diagnostic parasitologique a décru de 75% à 46% de 2004 à 2008 et seulement 22% des traitements antipaludiques sont donnés après un test parasitologique positif. La quinine est encore utilisée et l'AS-AQ n'est pas toujours donnée sur la base d'un test parasitologique. Plus alarmant sont les traitements antipaludiques donnés après un test parasitologique négatif : La confiance dans le test parasitologique reste très limitée. Les progrès effectués lors de le déploiement de l'AS-AQ, en particulier avec la mise en place de l'essai thérapeutique en 2000-2004 avec une équipe de recherche dédiée, supervisant le personnel soignant, sont en décroissance manifeste. En 2007 une étude transversale à Zanzibar portant sur des enfants de 0-6 ans a montré que 80% des patients étaient diagnostiqués cliniquement et recevait l'AS-AQ présomptivement [104]. Ces résultats mettent en lumière la question de l'observance aux programmes de traitement. Les pratiques peuvent varier dans le même district malgré les cours de soutien. La grande majorité des traitements antipaludiques sont donnés sur un jugement clinique du paludisme même en présence d'un test parasitologique négatif. Ce problème est aussi connu dans d'autres pays [105,106,107]. Or la présentation clinique est trompeuse, les maladies infectieuses autres que le paludisme sont fréquentes et dans certains cas, le diagnostic différentiel peut être difficile. A Mlomp, les infections du système respiratoire [108,109,110] suivent les mêmes tendances saisonnières que le paludisme. L'utilisation d'algorithmes cliniques seuls n'est pas suffisante, particulièrement dans les zones de faible endémie [111]. La combinaison d'un algorithme clinique et de la microscopie ont pu réduire d'un tiers les prescriptions d'antipaludiques à Zanzibar sans aucun effet sur l'utilisation des antibiotiques [112].

Toutefois la problématique est matière à débat entre le choix d'abandonner la prescription du traitement antipaludique sur présomption clinique (fièvre) [84] pour le diagnos-

tic parasitologique et le choix de conserver cette stratégie [83] en particulier s'agissant des enfants de moins de cinq ans. English *et al* et D'Acrémont *et al* sont en désaccord sur les performances des tests de diagnostics rapides en pratique de routine [113], la disponibilité de données épidémiologiques locales, les croyances et attitudes des agents de santé communautaire et l'innocuité de la stratégie de traitement basée sur le test parasitologique. A Mlomp, nous avons constaté que les croyances et attitudes des agents de santé jouent un rôle très important dans la réussite de la stratégie de déploiement du traitement. La présence d'une équipe de recherche et l'encadrement qu'elle a fourni a permis le succès de l'extension de l'usage du test parasitologique pour valider l'indication de traitement antipaludique. Mais l'observance à la nouvelle stratégie de traitement a chuté après le départ de l'équipe de recherche. A l'avenir Il sera donc nécessaire d'attacher un point important à la formation, l'encadrement et le suivi à long terme des agents de santé qui appliquent les stratégies de traitement.

La mise à disposition, dans chaque dispensaire, d'antibiotiques et de thérapies couvrant les maladies autres que le paludisme joue également un rôle important dans le respect de la réalisation des tests diagnostics et de leur résultat. En effet, avec un personnel soignant ne disposant pas d'un approvisionnement suffisant principalement aux antipaludiques, il aura tendance à donner aux patients le seul médicament qu'il possède afin de ne pas se discréditer à leurs yeux. Cette observation démontre qu'il est indispensable d'assurer au niveau des dispensaires des algorithmes décisionnels de prise en charge alternative de maladies autres que le paludisme en cas de négativité du test diagnostic

Le traitement avec l'AS-AQ participe à la diminution de la prévalence du paludisme (English *et al* et D'Acrémont *et al* sont en accord sur ce point [83,84]). A Mlomp, le profil d'âge des sujets atteints de paludisme a aussi changé avec un risque de paludisme plus uniformément distribué pendant la vie que dans le passé. Cette observation a aussi été réalisée par O'Meara *et al* [88] bien que le contexte Kenyan soit complètement différent. Dans le site Sénégalais, les catégories d'âge 16-20 ans, 21-30 ans et >30ans ont rejoint les 6-10 ans et les 11-15 ans en 2007 en terme de risque relatif d'un test parasitologique positif quand comparées aux 0-5 ans.

Ces observations ont un impact très important en termes de santé publique, car ils mettent potentiellement à risque des tranches de population qui étaient auparavant épargnées après avoir passé les premiers âges de la vie. Ces populations étant en âge de travailler, le poids du paludisme dans le PIB du pays devrait être plus lourd.

5.2 Aspects méthodologiques importants dans le prise de décision

Lors de l'évaluation statistique des données des différents aspects de cette thèse se sont imposées des questions méthodologiques qui avaient toutes en commun d'utiliser les méthodes les plus adaptées et de faire comprendre les analyses effectuées aux personnes non statisticiennes.

La méta-analyse des essais randomisés d'AS-AQ a été réalisée à partir des taux d'échecs car ils deviennent un standard dans l'évaluation de l'efficacité des traitements antipaludiques [114] eu égard aux taux de succès relativement élevés des traitements du paludisme actuels. Toutefois s'agissant d'une méta-analyse sur données agrégées et non individuelles, la méthode de Kaplan-Meier n'a pas été utilisée [114]. L'odds ratio, obtenu facilement à partir d'un modèle logistique, de la probabilité d'échec, est souvent interprété comme un risque relatif [115]. Cette pratique est souvent acceptée dans le cas d'évènement rare (<10%) [115,116]. Le risque initial de paludisme pour un patient donné, avant le recrutement dans un essai clinique, est susceptible de modifier l'approximation du risque relatif entre de groupe de traitements testés, par l'odds ratio [115,116]. Dans le cas du paludisme ce risque initial dépend de la situation épidémiologique de la zone d'étude. Par conséquent les différences d'endémicité et de susceptibilité du parasite entraîne des différences de ce risque initial entre les différentes études inclues dans cette revue de données. Le design prospectif par nature des essais cliniques a aussi orienté le choix de la mesure d'association vers le risque relatif.

Toutefois l'utilisation de mesures d'associations relatives, le rapport de deux risques ou de deux odds, a le désavantage de pouvoir prendre la même valeur pour des situations cliniques très différentes [117]. Néanmoins les méta-analyses sont souvent présentées avec des effets relatifs lorsque le caractère étudié est dichotomique ou binomial. Les représentations graphiques des résultats des méta-analyses, destinées à permettre au lecteur à mieux appréhender les résultats en termes de risque relatifs ou odds ratio, sont par conséquent difficile à comprendre. Les effets absolus ne sont pas présentés car ils sont considérés comme moins cohérent [118,119]. Pourtant certains auteurs recommandent l'utilisation d'une mesure relative et d'une mesure absolue afin que les utilisateurs disposent d'une image complète des résultats [117]. De plus les mesures absolues de l'efficacité d'un traitement sont d'un meilleur abord pour la prise de décision dans le cadre de recommandations de traitement.

Par ailleurs, l'estimation de l'hétérogénéité, inhérente à la méta-analyse qui agrège les données de plusieurs études [120], est estimée par le I^2 de Higgins [121] exprimé en pourcentage d'hétérogénéité mais qui ne permet pas de la visualiser. Le graphique proposé dans "Alternative visual displays of meta-analysis of malaria treatment trials to

facilitate translation of research into policy" représente chaque étude par la différence des risques et le taux d'échec du traitement test, une mesure d'effet relatif et une mesure d'effet absolu. Un guide d'interprétation expliquant les zones du graphe, est donné de façon à ce que toute étude puisse être placée sur le référentiel et qu'une décision de santé publique puisse être prise. Une estimation de l'hétérogénéité est visuellement représentée sur le graphique par le polygone que forment les points des études (la mesure d'effet absolu, le taux d'échec, et la mesure d'effet relatif, la différence de risque) et dont la surface est l'évaluation de l'hétérogénéité.

Un autre aspect dans la mise au point d'outil d'aide à la décision de cette thèse, est l'analyse de la susceptibilité *in vitro* mesuré par la CI_{50}, un paramètre qui exprime une variabilité trop importante pour des méthodes d'analyse dépendant de la loi normale. Nous avons adapté méthode utilisée en analyse comparative de biodisponibilité pour la description au cours du temps de la CI_{50}. La représentation graphique de cette analyse apporte visuellement l'explication et le sens de l'évolution de la susceptibilité des souches parasitaires aux différents médicaments utilisés.

5.3 Evolution de l'association artésunate et amodiaquine vers un comprimé bi-couche associant les deux produits.

L'ensemble du travail en Casamance a contribué à l'accumulation de données qui ont justifié et favorisé le développement du comprimé bi-couche par Drug for Neglected Diseases initiative (DNDi). Cette forme pharmaceutique n'a pas été utilisée dans le cadre des études que nous avons analysées, mais nous avons participé à l'étude de biodisponibilité comparative [98].

Le développement de cette nouvelle combinaison a été établi dans le but d'améliorer l'observance de la prise de traitement du patient (un comprimé unique en une prise par jour sur 3 jours), d'éviter les surdosages et surtout les sous dosages (en utilisant le travail de Taylor *et al* [64]), de maintenir la susceptibilité au traitement des parasites à un niveau faible [23,24] mais aussi avec un faible coût. Le produit a été développé en suivant un processus qui s'apparente à celui des génériques. Les deux molécules existent déjà sur le marché et sont déjà utilisées en association libre (ou en co-blister), mais il est nécessaire d'établir qu'aucune différence n'existe en terme de bioéquivalence, efficacité et tolérance. La pharmacocinétique de cette combinaison a été étudiée chez des volontaires sains malais et sa biodisponibilité a été comparée à celle de la combinaison libre [98]. Les deux traitements ont produit des profils cinétiques AS/DHA et AQ/DEAQ similaires mais la variabilité inter-sujet était importante. La combinaison fixe a montré une variabilité intra-sujet plus faible (~5%) pour les AUCs de la DHA totale et la DEAQ. Le

critère strict de bioéquivalence n'a pas été entièrement satisfait, la borne inférieure de l'intervalle de confiance du ratio des moyennes géométriques des AUCs étant sous la limite des 80% règlementaires. Toutefois la comparaison avec les données biologiques indiquent que ces différences n'altèreraient pas la réponse clinique des patients. Un AUC supérieur à la concentration inhibitrice de 50% de la croissance des parasites, est probablement le meilleur prédicteur de l'efficacité antipaludique pour les traitements à long temps de résidence (AQ/DEAQ) et les traitements à courte durée de vie (AS/DHA) alors qu'un Cmax supérieur à la CI_{50} est aussi pertinent pour l'AS/DHA. Le Cmax de DHA totale provenant du comprimé bi-couche était 170 fois supérieur à l'CI_{50} moyen [98].

Ces données sur des volontaires sains à qui on a donné une seule dose d'AS-AQ sont uniquement indicatrices de la disposition chez les patients impaludés traités sur 3 jours. Par conséquent il est utile de constater les effets des traitements *in vivo*. Un essai de non infériorité, contrôlé, randomisé, phase III, comparant la combinaison libre et la combinaison fixe a montré un taux d'efficacité de 92.1% dans les 2 bras dans le paludisme pédiatrique au Burkina Faso [122].

L'ASAQ Winthrop a à présent obtenu la qualification pour la liste des médicaments essentiels de l'OMS. Un partenariat entre le DNDi et Sanofi-Aventis a été signé pour réaliser la production industrielle et la distribution dans les pays d'Afrique.

6 Conclusion

Les travaux présentés dans cette thèse ont permis la compréhension des problèmes de santé publique relatifs aux changements de politique de traitement du paludisme en montrant :

- L'efficacité et l'innocuité de la combinaison thérapeutique AS-AQ,
- Les problèmes liés aux formes pharmaceutiques prescrites et par conséquent l'importance du choix de la présentation et du dosage des molécules actives dans l'exactitude de la posologie et l'observance au traitement,
- La stabilité dans le temps de la résistance à l'AS et l'AQ par une surveillance *in vitro*,
- Une baisse de la morbidité palustre dont les éléments d'explication sont très probablement multifactoriels avec une diminution de la pluviométrie ou l'utilisation de moustiquaires imprégnées.

Toutefois l'utilisation des moustiquaires n'a pas évolué depuis 2000, et il est plus vraisemblable que le changement de politique de traitement avec l'introduction des CTAs soit responsable de l'accélération de cette diminution de la morbidité palustre.

Ce travail apporte ainsi plus d'information sur l'utilisation de la combinaison AS-AQ en pratique courante et les données rapportées dans les différentes publications ont servi pour la prise de décision en santé publique, en particulier pour le PNLP. Il y est aussi fait des propositions d'outils d'aide à décision quant à l'exposé des résultats de recherche pour leur transposition en politique de santé.

Une des limites de cette recherche pourrait être la mise en place progressive de la nouvelle stratégie de traitement avec l'AS-AQ. En effet, le déploiement a débuté en 2000 avec le traitement des enfants de moins de cinq ans pendant la saison des pluies, puis toute l'année, et enfin toutes les classes d'âge toute l'année. Les pourcentages de cas traités avec l'AS-AQ ne correspondait pas à tous les cas de paludisme qu'il eut été possible de traiter avec la combinaison. De plus les vomissements observés chez les plus jeunes patients sont dus à des problèmes digestifs qui ont certainement été occasionnés par la prise répétée de plusieurs comprimés d'AS-AQ. Les agents de santé communautaire ont probablement été enclins à donner de la QN dans ces cas, expliquant ainsi en partie la forte résistance de la quinine au déploiement de la nouvelle stratégie. Cette observation vient encore étayer l'importance de la formulation utilisée en pratique courante et l'intérêt du développement d'une co-formulation.

Enfin, les études menées à Mlomp ont complètement intégré le système de santé en impliquant les agents de santé communautaire dans la mise en place de la stratégie de traitement grâce à des formations et un suivi local.

7 Prospective

Nous avons présenté dans cette thèse des travaux effectués à Mlomp, un village du district d'Oussouye. Toutefois les données épidémiologiques utilisées à Mlomp sont aussi disponible sur plusieurs postes de santé du district. Un objectif futur est de pouvoir comparer la situation décrite à Mlomp avec ces autres sites où les directives nationales de traitement ont été suivies sans activité de recherche.

L'aspect sociologique du traitement du paludisme est un autre sujet qu'il est prévu d'aborder avec l'étude des comportement face à la crise palustre et à la stratégie de traitement avec l'AS-AQ.

Il serait aussi nécessaire d'obtenir un complément d'information sur la tolérance de cette combinaison thérapeutique. Un des moyen d'y parvenir est l'utilisation de base de données contenant plus de 3000 sujets d'études cliniques réalisée en Afrique pour mieux comprendre l'évolution de l'hématologie lors du traitement (sur 28 jours) de l'attaque de paludisme non compliqué. Un autre aspect est aussi la tolérance de l'AS-AQ chez la femme enceinte au delà de quatre mois. Auparavant celle-ci sont traitées avec un traitement préventif intermittent.

Par ailleurs, le lien entre la résistance *in vitro* – in vivo et son évolution dans le temps reste encore mal connu et on se propose de l'étudier avec les données collectées sur le site de Mlomp.

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9 Articles:

A systematic review and meta-analysis of non-randomized and randomized controlled studies of artesunate and amodiaquine for the treatment of uncomplicated falciparum malaria.

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ABSTRACT

Background. Malaria remains a substantial burden worldwide, placing half the global population at risk and killing a million children every year. Artemisinin-containing combination therapies (ACTs) are recommended by the World Health Organization (WHO) for treating acute uncomplicated Plasmodium falciparum malaria. Artesunate combined with amodiaquine (AS-AQ) is currently used for the treatment of acute uncomplicated falciparum malaria in 19 countries. However, the accumulated data of AS-AQ have not been reviewed systematically against other antimalarial treatments.

Purpose. To assess the efficacy and safety of AS-AQ for uncomplicated falciparum malaria to assist policy decisions.

Data Sources. Published and unpublished trials conducted from 1999- September 2008 were identified via electronic and manual searches through MEDLINE, EMBASE, LILACS and CENTRAL.

Study Selection. Non-comparative, comparative randomised and quasi-randomised trials of AS-AQ enrolling patients of all ages with falciparum malaria

Data Extraction. Four investigators independently searched and abstracted trial characteristics and outcomes. Three different investigators verified data.

Data Synthesis. Primary endpoints were crude and PCR adjusted parasitological outcomes by Day 28 using the per-protocol dataset. Random effects models were used to aggregate estimates from randomised controlled trials. Of 66 potential studies identified, 42 comparative and non-comparative trials met our inclusion criteria. 40 studies (35 comparative trials conducted at 59 study sites, 5 non-comparative trials at 6 sites) enrolling 18,808 patients (7,808 on AS-AQ) in 25 (22 African) countries contributed to the Day 28 efficacy analysis. 39 trials specifically recruited African children. The target drug doses were generally AS 12 mg/kg + AQ 30 mg/kg administered over three days. Crude Day 28 failure rates for AS-AQ varied widely (0%-80%) but decreased to ≤ 26% after genotyping in 37/59 study sites reporting PCR-adjusted results. Differences between efficacy rates for AS-AQ was seen.

Of the 35 studies comparing Day 28 rates, AS-AQ was significantly more effective than amodiaquine (RR=0.41, 95%CI= [0.33; 0.49]), Artesunate (RR=0.08, 95%CI= [-0.13; 0.28]), chloroquine (RR=0.11, 95%CI= [0.04; 0.19]), sulfadoxine/pyrimethamine (RR=0.46, 95%CI= [0.29; 0.63]), amodiaquine+sulfadoxine/pyrimethamine (RR=0.83 [95% CI: 0.71; 0.94]), chloroquine+sulfadoxine/pyrimethamine (RR=0.51 [95% CI: 0.26;

0.75]), and artesunate+sulfadoxine/pyrimethamine (RR=1.21 [95% CI: 0.94; 0.1.48]) but not significantly different from artemether+lumefantrine 6 doses (RR=1.86 [95% CI: 1.51; 2.20] and dihydroartemisinin+piperaquine (RR=2.36 [95% CI: 0.54; 4.18]. Comparison with artemether+lumefantrine 4 doses and artesunate+mefloquine were not valid because of only one little study in each. On aggregate, AS-AQ was superior to all single-agents combined (RR=0.11 [95% CI: 0.01; 0.21]) and non-artemisinin combinations (RR=0.23[95% CI: -0.06; 0.52]). Aggregated analyses showed that it was more efficacious than ACTs combined (RR=0.16 [95% CI: 0.07; 0.26]) but with only the AS+SP treatment as a significant comparison. PCR-adjustment did not modify these results. AS-AQ was reportedly well tolerated but safety information was inadequate.

Conclusions. This review represents an inventory of all available AS-AQ data through September 2008. While new data has become available since this time, its availability in the open access Malrai Journal will allow future researches to readily expand efficacy and safety knowledge as newer data becomes available. It also allows consideration of geographical variability within the current policy making context. AS-AQ met the WHO recommended minimum PCR adjusted efficacy of $\geq$90% in most countries, and may, therefore, be considered to treat uncomplicated falciparum malaria in Africa. Though adverse events were insufficiently documented, and not reported in a standardized fashion across studies, AS-AQ was generally reported as, "well-tolerated" and with a similar safety profile to other ACTs. While AS-AQ is the second most widely used anti-malarial, its safety has yet to be studied systematically. This papers represents a signifcant advancement in our understand of AS-AQ, that is not picked up in other published Cochrane Reviews. Nonetheless, more comparative trials and continued surveillance of AS-AQ efficacy and safety are needed, including studies in real life conditions and with repeat exposure. This is crucial to optimize the uptake of safety and efficacy knowledge to drive Malaria Treatment Guidelines promulgated by regulators and policy makers, such as the World Health Organization.

BACKGROUND

Effective artemisinin-containing combination therapies (ACTs) (1) are recommended by the World Health Organization (WHO) to treat acute uncomplicated Plasmodium falciparum malaria. Currently, 77 of the 81 countries endemic for *P. falciparum* have adopted ACTs worldwide as first-line treatment (40 in Africa) (2). Artesunate (AS) combined with amodiaquine (AQ) is presently adopted in 19 countries, namely, Indonesia and 18 African countries (Burundi, Cameroon, Congo, Côte d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Liberia, Madagascar, Malawi, Mauritania, Senegal, Sao Tome & Principe, Sierra Leone, Sudan (South), Zanzibar).

AS-AQ is currently available as either separate or blister-packaged tablets, and a fixed-dose combination has now been registered in 21 malaria-endemic countries in Africa (3). With AS-AQ data accumulating, compiling comprehensive summaries of efficacy and safety is important to better understand the advantages and disadvantages of this treatment. This can aid policymakers' decisions on the optimal conditions for the use of AS-AQ and lead to new avenues of further research.

PURPOSE

A systematic review was conducted by compiling an inventory of published and unpublished clinical trials to assess the efficacy and safety of AS-AQ for uncomplicated falciparum malaria and to form a basis for the development of optimal malaria control strategies by the national control programmes of endemic countries.

For comparative trials, we conducted individual study and aggregate comparative analyses of efficacy. The search ended in September 2008 to allow for an appropriate time to harmonize published data, obtain additional reported data and conduct the analyses. The intent of the authors is to periodically update this database to best inform decisions.

METHODS

Data Sources and Searches

Criteria for considering studies for this review.

Both non-comparative and comparative randomised and quasi-randomised controlled trials that recruited patients of all ages with parasitologically confirmed acute uncomplicated falciparum malaria were considered. The intervention was AS plus AQ (any products) with or without one or more comparators (single agent or combination treat-

ments). The efficacy outcome measure sought was conversion from a pre-treatment positive blood smear for *P. falciparum* to a negative one at the end of a 28-day follow-up.

Search strategy

All relevant studies were identified regardless of language or publication status (published, unpublished, in press, reports). Published studies were identified through two, independent electronic searches up to September 2008 of MEDLINE, EMBASE, LILACS, the Cochrane Infectious Diseases Group's trials register and the Cochrane Central Register of Controlled Trials (CENTRAL) using the search terms: malaria, amodiaquine, artesunate, artemisinins.

Unpublished studies were identified by manually searching the reference lists of studies identified by the above methods, contacting individual researchers working in the field, and examining WHO records. Consequently, for the selected studies meeting inclusion criteria (see below) investigators were contacted to provide additional data. Attrition was summarised following the QUORUM guidelines (4).

Study Selection

PMu and PO independently screened the identified studies and assessed their eligibility for inclusion. We took care to ensure that each trial was only included once (e.g. studies for which we had both a confidential report and a publication were linked). PMu, RP and PO assessed the quality of studies, according to Juni et al (5), looking at the methods described for generating the drug allocation sequence and for drug allocation concealment.

For the comparative trials, we assessed: (i) the generation of allocation sequence (described as adequate if the method used indicated that the resulting sequences were unpredictable, unclear if the trial was randomised but the method was not described, inadequate if sequences could be predicted, or were not described); (ii) drug allocation concealment (described as adequate if the methods used prevented prior knowledge of investigators enrolling participants and participants of treatment assignment, inadequate if participants and investigators enrolling participants could foresee upcoming assignment, or was not described); we verified who were blinded to the interventions, such as the participants, study physicians, or outcome assessors.

For all trials, compliance with the Consort statement (6) (study profile and patient attrition clearly displayed) was assessed; the inclusion of all participants in the main analysis was considered to be adequate if > 90% were included in the analysis, inadequate if < 90%, or if the percentage was unclear.

MH, PMu, PO and RP extracted data of trial characteristics and outcomes; MV, PMi, and PO verified data.

Data Extraction and Quality Assessment

Data were extracted independently by four persons (MH, PMu, RP, MV), then compared and reconciled by PO. In case of inconsistencies, the paper was checked back by the two persons to agree on a resolution. Study investigators were asked to double check the information and provide additional data as needed. Data were entered using Microsoft™ Access®.

Data Synthesis and Analysis

Definition of populations and endpoints for the analyses of efficacy

The primary efficacy parameters were the Day (D) 28 crude (unadjusted) and adjusted (by polymerase chain reaction (PCR) to distinguish new from recrudescent infections) failure rates. To compare efficacy rates, failure was used rather than cure because comparing low event rates (in this case failure) is statistically more appropriate in meta analyses. D14 failure rates, which are no longer recommended by the WHO, and D42 were not analysed.

Patient attrition was reconstructed from the study reports so that there were comparable populations across studies, defined as: (i) Modified Intent to Treat (MITT, not presented here) includes all patients enrolled except protocol violators (e.g. wrongly randomised, took other antimalarial drugs during follow-up); and (ii) Per Protocol (PP) excludes all protocol violators and all non parasitological study withdrawals including those due to adverse events. Patients who failed to clear their parasites or who had recurrent parasitaemia after initial clearance were defined as treatment failures.

Definition of population and endpoints for the Analysis of Safety

Papers/reports were assessed for the quality and quantity of safety information, and these data were tabulated. A study and patient attrition was reconstructed from the study reports.

2 persons reviewed each paper systematically for safety data. The papers were assessed for quality by retrieving if safety information was given, defined as outcome measure and if AEs were defined and discussed. The adverse events were extracted and were declared adequately described if reported per groups of treatments with the total number of AEs, the total number of patients with al least an AE and a description of each AE.

The primary safety endpoint was adverse event (AE) reporting. We also sought information on deaths, other serious AEs (SAEs), and laboratory assessments (haematology, biochemistry).

PCR adjustment of treatment outcomes

Patients with recurrent parasitaemia were classified as recrudescence (treatment failure) or new infections (reinfections). The D28 PCR-adjusted failure rates are herein reported only for the PP population. Patients who were withdrawn, with missing or ambiguous PCR, or with a new infections were excluded, as recommended by the WHO 2003 guidelines (WHO/HTM/RBM/2003.50). The studies used for PCR adjustment either the merozoite surface protein (msp) 1 or 2 alone, msp1 + msp2, or msp1 + msp2 + glutamate rich protein (glurp).

Populations for analysis

The following efficacy analyses are presented in the PP population: (i) D28 crude (PCR-unadjusted) outcomes; and (ii) D28 PCR-adjusted outcomes. For each treatment arm in all comparative and non-comparative studies, the crude and PCR-adjusted D28 failure rates with 95% confidence intervals (CIs) were calculated. The safety analysis was performed on all studies (comparative and non comparative) retrieved for detailed evaluation.

Publication bias

Publication bias was examined through the use of a funnel plot (7) of the log-transformed relative risk [log(RR)] of individual studies against the precision (1/SE, standard error). Funnel plot asymmetry was further tested by using Egger's method (8). These methods are particularly useful when a high number of large studies are included in the meta-analysis (9).

Heterogeneity assessment

The Cochran's Q (10) and I^2 (11) test of heterogeneity were performed to detect non homogeneity between RRs of individual studies. Galbraith (or radial) plots were also used to identify the source of heterogeneity (12). Comparisons are also presented as L'Abbé scatter plots, whereby the proportions of failed patients on AS-AQ are plotted on the Y axis against the failure rate of comparator drugs (X axis). These plots were adapted so that the bubble size was proportional to study size. In addition to the line of equality, we plotted also the overall RR line with 95%CIs to identify outliers and sources of heterogeneity. (13)

A meta-regression of the individual study-site log(RR) was done using the following categorical covariates: age, dose of AS-AQ, geographical location, WHO protocol used, year of beginning and year of end of the study, study type, randomisation type, allocation concealment, supervised doses or not, genotyping strategy for PCR adjustment, number of sites and the use of the CONSORT statement). A mixed model was used to account for multiple sites per study (see explanation in the statistical methods paragraph). Studies were classified by age into 3 groups: (i) <59 months, (ii) =>60 months to 15 years or (iii) all ages. Drug dose was categorised as either AS 4mg/kg/d+AQ10 mg/kg/d x 3days (12MK+30MK) or other dosages (i.e, 12MK+25MK (n=7), 8MK+30MK (n=1), age dependent (n=2) or weight dependent (n=1). For geographical location, studies were classified as Eastern, Central and Western Africa. The WHO protocol was either the 2003 (WHO/HTM/RBM/2003.50) or older versions. The year of study was also considered categorical and accounted for in the analyses only when there were patients treated in a specific year and for a specific comparison. Randomization was classified as either by block or age-based, computer generated and not specified. Allocation concealment was not specified or staff blinded and double blinded.

Statistical methods

Efficacy of AS-AQ is presented as failure rate with 95% CIs for all (both non-comparative and comparative) studies. For comparative studies, the failure rates are compared as RRs with 95% CIs and displayed as forest plots for individual and aggregate comparisons(14). The pooled RR is calculated as a weighted average of the treatment effects estimated in the individual studies, using a random effects model to account for heterogeneity between countries, sites, comparators, and year of study conduct (15, 16). Since some studies had multiple comparisons and several sites, data were arranged in a longitudinal manner in order to avoid multiple counts of the same AS-AQ arm when calculating the pooled estimate. A logit model with fixed effects was used for each individual study. To estimate the pooled RRs, a multilevel random effect model was used with a random intercept for each studies or sites in muticentric studies to account for the between sites / studies heterogeneity (17, 18) not captured by fixed effects. RRs and 95% CIs were fitted by grouping comparators as single agents, non ACT combinations, ACT combinations and the global treatment effect.

For ease of reading, RR estimates and their 95%CI lower and upper limits less than 0.01 or higher than 100 were limited to these values. A 95%CI = [0.01, 100] was meaning a convergence problem of the model due a low study sample size(18).

Statistical software

Statistical analyses were all performed with the SAS System version 9.1.3 (SAS Institute, Cary, NC, USA). Proc gplot was used for all graphics and proc nlmixed was used for statistical analysis(19). Forest plots were done by using the macro from Foster & Goldsmith(20) and Galbraith plots by adapting the macro from Kuss and Koch(21).

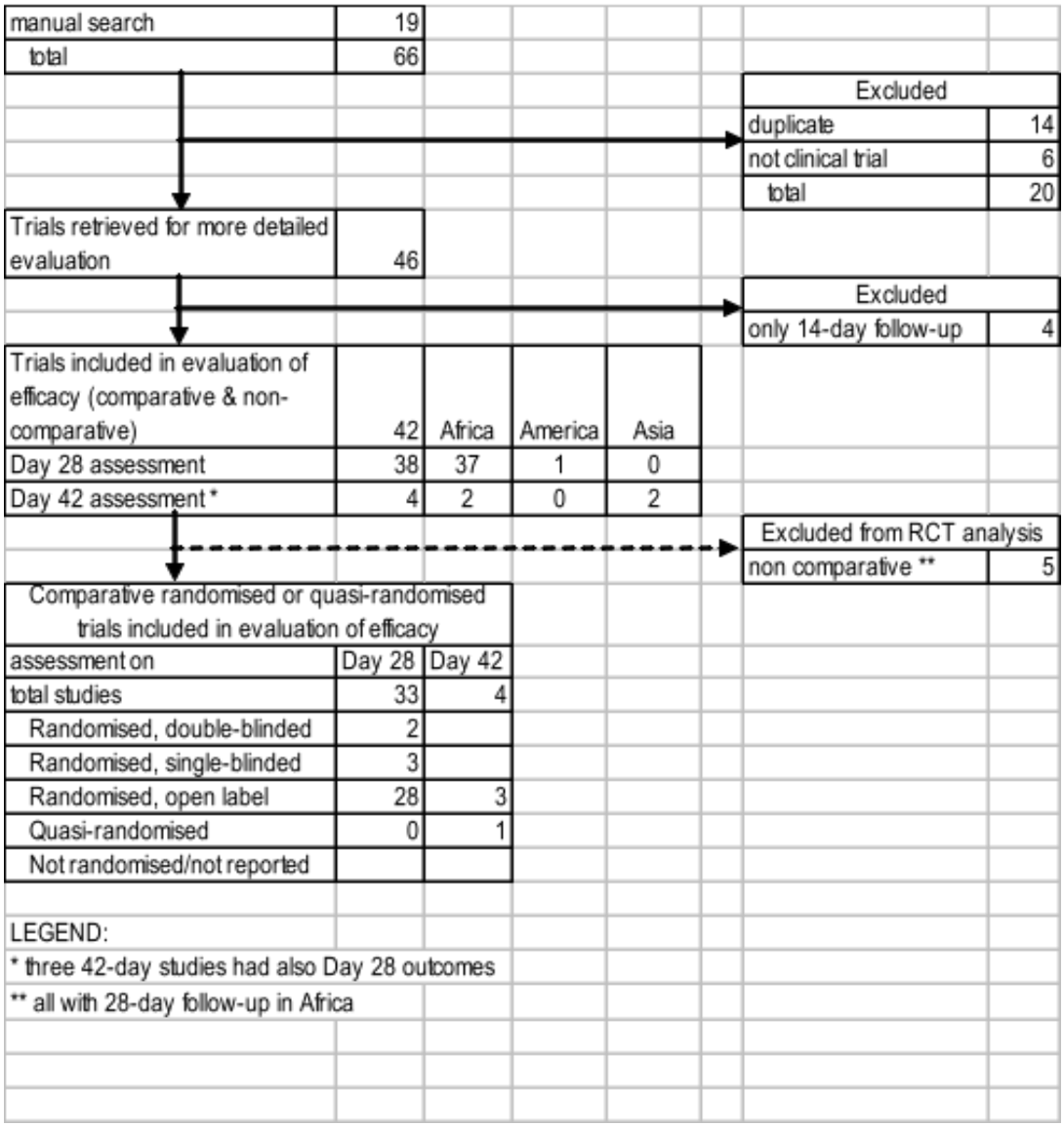
RESULTS

Study characteristics

A total of 66 potential studies were identified through either electronic (n=478) or manual search/contacts (n=19), of which 14 were duplicates and 6 were not clinical trials, leaving 46 eligible trials (Figure 1): (i) 16 only published, (ii) 25 published with additional data and (iii) 5 unpublished (additional and unpublished data were obtained from the authors or unpublished reports from Epicentre, WHO/TDR and DNDi). Four trials were excluded from the efficacy analysis because the follow-up duration was limited to 14 days, leaving 42 trials with either 28 (n=41) or 42 day (n=4) assessment (apparent discrepancy due to three trials having both 28 and 42 day results); of these, 37 were comparative and 5 non-comparative. The studies reported the following different versions of the WHO protocol for in vivo assessment of antimalarial drug activity: 29 used the current WHO protocol (WHO/HTM/RBM/2003.50), while 17 referenced a previous WHO document.

Considering the controlled trials, two were randomized double-blinded and three single-blinded, 31 were randomized open-label, one quasi-randomized, and for one, information was not available. In these studies, generation of allocation sequences and drug allocation concealment was considered adequate for 16 (44%) and 15 (41%), respectively.

Figure 1. Search flow diagram



As seen in Table 1, all 46 studies were conducted in 25 countries, 22 from sub-Saharan Africa, between the period from 1999 to 2008. Five countries – Uganda, Senegal, Tanzania, Madagascar and Rwanda – contributed 55% of all patients, whereas the contribution of Rwanda and Senegal to comparative trials was only 6% and 7 %, respectively.

Table 1.

Ref#	identified thru search	ID (Author, year)	Source of data	Country	Number of sites	Year of study	Age of patients	Control arm(s)
Studies with analysable Day 28 outcome								
Comparative randomised or pseudo-randomised								
Africa								
(28)	electronic	Adjei, 2008	Published article + additional data	Ghana	1	2004-2006	6m-14y	AM+LF
(34)	electrronic + manual	Adjuik, 2002	Published article + unpublished WHO/TDR reports.	Gabon, Kenya, Senegal	4	1999-2000	6m-10y	AQ
(35)	electronic	Barennes, 2004	Published article. No additional data	Burkina-Faso	1	2001	1-15y	AQ, AS
(36)	manual	Bonnet, 2004	Unpublished Epicentre report	RDC	1	2003-2004	6-59m	AS+SP
(37, 38)	electrronic + manual	Bonnet, 2007	Published article + unpublished Epicentre report	Republic of Guinea	1	2004	6-59m	AS+SP
(39)	electrronic + manual	Bukirwa, 2006	Published article + additional data	Uganda	1	2005	1-<10y	AM+LF
(40)	manual	Cohuet, 2004	Unpublished Epicentre report	RDC	1	2003-2004	6-59m	AS+SP
(41)	electronic	Dorsey, 2007	Published article + additional data	Uganda	1	2004-06	1-10y	AS+SP AM+LF
(42)	electronic	Djimde, 2008	Published article. No additional data	Mali	1	2002-2004	3m-38y	AS+SP AS
(43)	electronic	Falade, 2008	Published article. No additional data	Nigeria	1	2004-2005	6m-10y	AM+LF
(44)	electronic	Faye, 2007	Published article + additional data	Senegal	5	2002-03	all ages	AQ+SP DH+PQ AM+LF4 AM+LF
(45)	manual	Grandesso, 2004	Unpublished Epicentre report	Uganda	1	2003	6-59m	CQ+SP, AS+SP
(46)	electronic	Guthmann, 2005	Published article + unpublished Epicentre report	Angola	1	2003	6-59m	AS+SP

(47)	electrronic + manual	Guthmann, 2006	Published article + unpublished Epicentre report	Angola	1	2004	6-59m	AM+LF
(48)	electrronic + manual	Hamour, 2005	Published article + unpublished Epicentre report	Sudan	1	2003	6-59m	AS+SP
(49)	electronic	Ibrahium, 2007	Published article. No additional data	Sudan	1	2005	all ages	
(50)	electronic	Karema, 2006	Published + additional data	Rwanda	3	2003-2004	12-59m	DH+PQ, AQ+SP
(51)	electronic	Koram, 2005	Published article + additional data	Ghana	2	2003	6-59m	CQ, SP, AM+LF
(52)	electrronic + manual	Martensson, 2005	Published article + additional data	Zanzibar	2	2002-2003	6-59m	AM+LF
(53)	electronic	Ménard, 2007	Published article. No additional data	Madagascar	1	2004	6m-15y	CQ, AQ, SP, AQ+SP
(54)	electronic	Ménard, 2008	Published article + additional data	Madagascar	8	2004-2006	6m-15y	CQ, AQ, SP, AQ+SP
(55)	electronic	Mutabingwa, 2005	Published article. No additional data	Tanzania	1	2002-2004	4-59m	AQ, AQ+SP, AM+LF
(56)	electronic	Owusu-Agyei, 2008	Published article + additional data	Ghana	1	2005-2006	6m-10y	AL, AS+CD
(57)	electronic	Rwagacondo, 2004	Published article + additional data	Rwanda	3	2002	6-59m	AQ
(58)	electronic	Sowunmi, 2005	Published article. No additional data	Nigeria	1	2004	<12y	CQ+SP
(59)	electronic	Sowunmi, 2007	Published article. No additional data	Nigeria	1	2005-2006	6-59m	AQ, AS
(60)	electronic	Staedke, 2004	Published article + additional data	Uganda	1	2002-2003	6m-10y	CQ+SP, AQ+SP
(61)	electronic	Swarthout, 2006	Published article + unpublished Epicentre report	RDC	1	2004	6-59m	AS+SP
(62)	electronic	Tall, 2007	Published article. No additional data	Comoros Union	3	2003	all ages	AS+SP, CQ+SP
(63)	electronic	van den Broek, 2005a	Published article + unpublished Epicentre report	Sudan	1	2004	6m-<10a	AS+SP
(64)	manual	van den Broek, 2005b	unpublished Epicentre report	RDC	1	2004-2005	6-59m	AS+SP, SP
(65)	electrronic + manual	Van den Broek, 2006	Published article + unpublished Epicentre report	Republic of Congo	1	2004	6-59m	AS+SP, AM+LF

(66)	electronic	Yeka, 2005	Published article + additional data	Uganda	4	2002-2004	>6m	CQ+SP, AQ+SP
(67)	electronic	Zoungrana, 2008	Published article + additional data	Burkina Faso	1	2006	6m-10y	AS-MB, AQ-MB

Other continents

(68)	electronic	Hasugian, 2007	Published article	Papua	1	2005	all ages	DH+PQ
(69)	electrronic + manual	Osorio, 2007	Published article + additional data	Colombia	1	2000-2004	all ages	AQ

Non comparative

(29)	manual	Brasseur, 2006	Published abstract + unpublished TDR report	Senegal	4	2004	6-59m	none
(70, 71)	electrronic + manual	Grandesso, 2006	Published article + unpublished Epicentre report	Sierra Leone	1	2002-2005	all ages	none
(72)	electronic	Kabanywanyi, 2007	Published article. No additional data	Tanzania	1	2004	6-59m	none
(73)	electronic	Oyakhirome, 2007	Published article. No additional data	Gabon	1	2004-2005	6-59m	unsupervised
(22)	manual	Sirima, 2007	Unpublished DNDi report	Burkina-Faso	1	2002-2006	6-59m	AQ+AQ loose

Studies with only Day 42 outcome

(74)	electronic	Durrani, 2005	Published article. No additional data	Afghanistan	1	2002-2003	>3y	CQ, AQ, SP

Excluded from efficacy analysis: studies with only Day 14 outcome

(75)	electronic	Abacassamo, 2004	Published article. No additional data	Mozambique	1	2002	6-59m	AS+SP, AQ+SP
(76)	electronic	Kimbi, 2007	Published article. No additional data	Cameroon	1	2004-2005	all ages	AS
(77)	electronic	Meremikwu, 2006	Published article. No additional data	Nigeria	1	2004-2006	6-59m	AM+LF
(78)	electronic	Ndayiragije, 2004	Published article. No additional data	Burundi	1	2001-2002	6-59m	AM+LF

Legend: AQ=amodiaquine; AS=artesunate; AM+LF=artemether+lumefantrine; CQ =chloroquine; SP=sulfadoxine/pyrimethamine; DH+PQ=dihydroartemisinin+ piperaquine

The 46 studies included enrolled a total of 19,209 patients of whom 8,296 received AS-AQ (7,039 in comparative studies) and 10,913 received a comparator drug (Figure 2) as follows: (i) monotherapies: AQ (n=1866), AS (n=484), chloroquine (CQ, n=552), and sulfadoxine/pyrimethamine (SP, n=816); (ii) Non ACT combinations: AQ+SP (n=2053), CQ+SP (n=1053); and (iii) ACTs: non-fixed AS+mefloquine (AS+MQ n=145), non-fixed AS+SP (n=1351); fixed artemether/lumefantrine 6 doses (AM+LF, Coartem®, n=2008), AM+LF 4 doses (n=140), fixed dihydroartemisinin/piperaquine (DH+PQ, Artekin™, n=346).

AS and AQ were given together as individually packaged products in 27 studies, 4 studies used the co-blistered product Arsucam®, one the coformulated Coarsucam® while the product was not specified in 9 studies. The target dose was 4 mg/kg/d for AS and 10 mg/kg/d for AQ for three days (total doses: AS 12 and AQ 30 mg/kg) in 32 studies; five studies used a lower total dose of AQ (25 mg/kg), one a lower dose of AS (8 mg/kg), one study used an aged-dependent dosage and one study did not provide details. The product brand was specified in 27 studies for AS and 25 for AQ.

Treatment doses were fully supervised in 42 studies, partially supervised (first dose only) in two study, unsupervised in one, not reported in one and one study compared supervised with unsupervised AS-AQ intake. One non-comparative study evaluated a fixed and a non-fixed combination of AS-AQ.

Databases for the Day 28 efficacy analyses

Of the 46 eligible studies, 40 reported D28 crude failure rates (n=18,008) and 37 PCR-adjusted outcomes (n=16,289). Of these, 35 and 33 were comparative studies that enrolled a total of 16,652 and 15,899 patients, respectively.

Sample size losses in controlled trials were small for crude outcomes (MITT [-3%]; PP [- 6%]), but increased to 26% for the PCR-PP data set (Figure 2).

Because of possible within-country heterogeneity in terms of parasite susceptibility and infection transmission, the results were sorted by individual study site. There was a total of 64 sites testing 120 treatment arms (including AS-AQ and non-comparative studies), or 173 treatment arm-sites (6 non-comparative, 59 AS-AQ and 108 comparators).

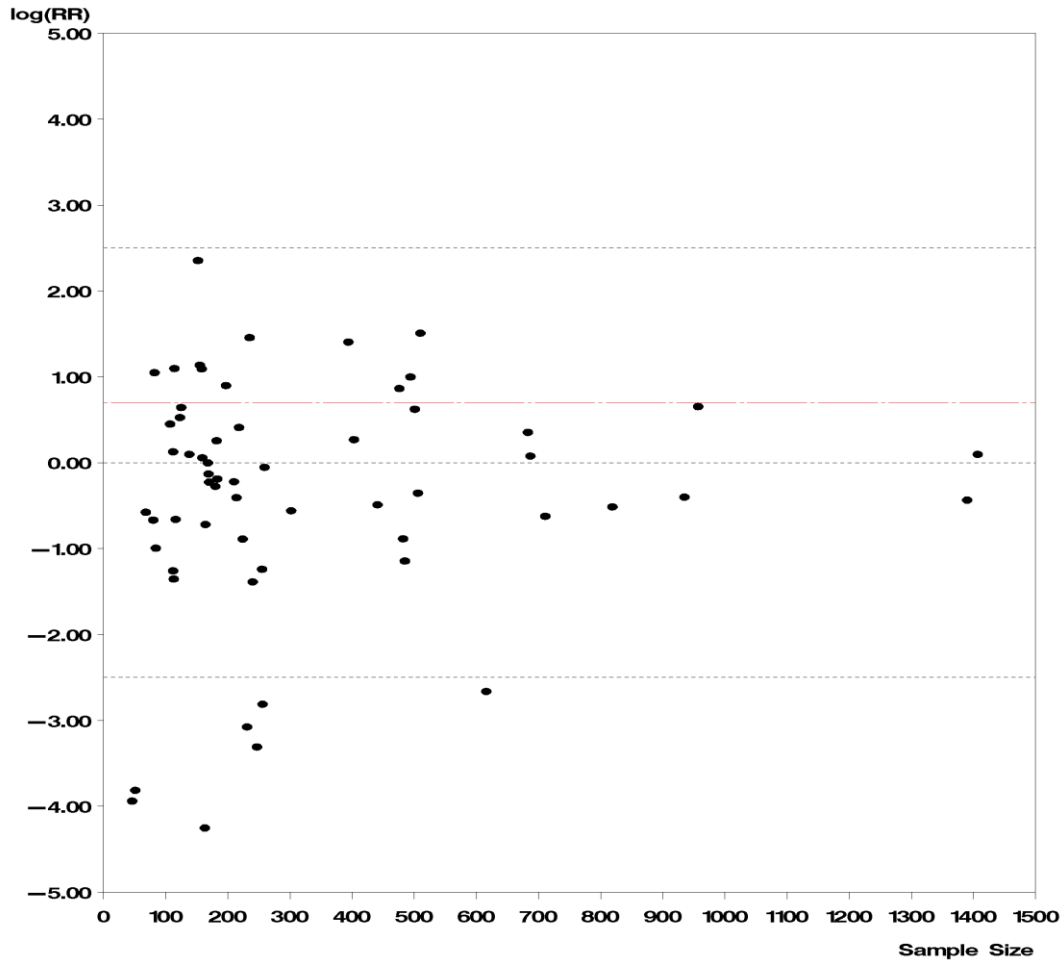
Publication bias

There was a moderate asymmetry between individual studies as detected by the funnel plot (Figure 4); the majority of studies were aggregated around the null log(RR) value whereas the fixed effects pooled estimate showed a slight decay. 7 studies with negative estimates (Menard 2008 (CQ), Guthman 2005 (AQ), Sowunmi 2007 (AS), Guthman 2005 (SP) Barennes 2004 (AS), Barennes 2004 (AQ) and Guthman 2005 (CQ)) were located outside the -2.5 lower limit . All other studies clustered around the fixed effect value.

Figure 2. Study and patient attrition

	Number. of studies		AS-AQ	comparator	total		
Enrolled in all studies	**5**	non-comparative	1257	99	1356		
	41	RCTs	7039	10814	17853		
	46	All	8296	10913	19209		
Enrolled in studies with D28 outcome	**40**	with crude outcome	7808	10200	18008		
	37	with PCR-corrected outcome	6590	9699	16289		
of which						% lost of enrolled	
RCTs with D28 outcome	**35**	enrolled	6551	10101	16652	in 28D RCTs	
crude (uncorrected)	analysis on	modified Intent-to-Treat	6286	9878	16164	3%	
		Per-Protocol	6099	9509	15608	6%	
						% lost of enrolled	
PCR-corrected	**33**	Enrolled	6299	9600	15899	in 28D RCTs with PCR	
(PP dataset)	**33**	modified Intent-to-Treat	6034	9378	15412	3%	
	32	missing and reinfections excluded (WHO, 2003)	4649	7154	11803	26%	23%
	33	missing excluded reinfections included	5703	8825	14528	9%	
	33	missing and reinfections included	6099	9509	15608	2%	
Studies with D28 outcome	**5**	non-comparative	1257	99	1356		
	35	RCTs	6551	10101	16652		
	40	All	7808	10101	18008		

Figure 4. Funnel plot of all comparative trials



This was confirmed by a significant Egger test on the log(RR) scale (142, 95%CI=[93; 190]), with a negative slope (-17.8, 95%CI=[-17.8;-17.7]). The deviation of the intercept from zero indicates a publication bias suggesting a more pronounced beneficial effect of larger than small studies.

Investigation of heterogeneity

The comparators in Sowunmi 2005 (CQ+SP) and Faye 2007 (AM+LF) did not contribute to the funnel plots because of outlying values in the standard errors of the effect estimate. These studies were clearly contributing to heterogeneity as they had a large variance and a small sample size (the size of the control group was half of the experimental group.)

The Cochran's Q was not significant for the pooled estimate over all trials and the I^2 showed that 1.9% of the overall variability observed was due to heterogeneity (although the H statistic had higher variability than expected) (Table 2).

Table 2. Heterogeneity search using Cochran's Q and I^2

Analysis	Cochran's Q			Cochran's H				I squared 1		
	Q	DF	p-value	H	SE H	Upper limit	Lower limit	I2	Upper limit	Lower limit
ALL TRIALS	19.9	58	1	1.707	5.338	59749	0	1.915	1	0
SINGLE AGENTS	19.18	22	0.634	1.071	3.239	612.4	0.002	0.147	1	0
vs. AQ TRIALS	0.496	9	1	4.259	1.995	212.5	0.085	17.14	1	0
vs. CQ TRIALS	16.32	3	0.001	0.429	0.092	0.514	0.358	0.816	-2.79	0
vs. SP TRIALS	1.482	5	0.915	1.837	1.399	28.53	0.118	2.374	0.999	0
vs. AS TRIALS	0.881	2	0.644	1.507	0.577	4.672	0.486	1.271	0.954	0
NON ACT COMBINATIONS	0.287	9	1	5.597	1.995	279.2	0.112	30.32	1	0
vs. CQ+SP TRIALS	0.215	2	0.898	3.053	0.577	9.465	0.984	8.318	0.989	0
vs. AQ+SP TRIALS	0.073	6	1	9.085	1.571	197.3	0.418	81.53	1	0
ACT COMBINATIONS	0.432	25	1	7.607	3.463	6747	0.009	56.87	1	0
vs. AM+LF TRIALS	0.223	10	1	6.698	2.117	424.5	0.106	43.86	1	0
vs. AS+SP TRIALS	0.187	11	1	7.665	2.232	609.2	0.096	57.76	1	0
vs. DH+PQ TRIALS	0.019	1	0.89	7.199	.	.	.	50.83	.	0
vs. AS+MQ TRIALS	0.003	0	.	.	.	.	.	.	.	0

Heterogeneity was further investigated after grouping studies by type of comparator (single agent, non ACT and ACT): while not significant with the Cochran's Q, heterogeneity was high in the non-ACT and ACT groups and negligible in the single agent group. Results by product pointed to comparisons of AS-AQ with CQ and CQ+SP as one source of heterogeneity. The inclusion of the Sowunmi 2005 (CQ+SP) trial in the non ACT and CQ+SP investigation of heterogeneity gave a significant Cochran's Q and higher H and I^2 (data not shown).

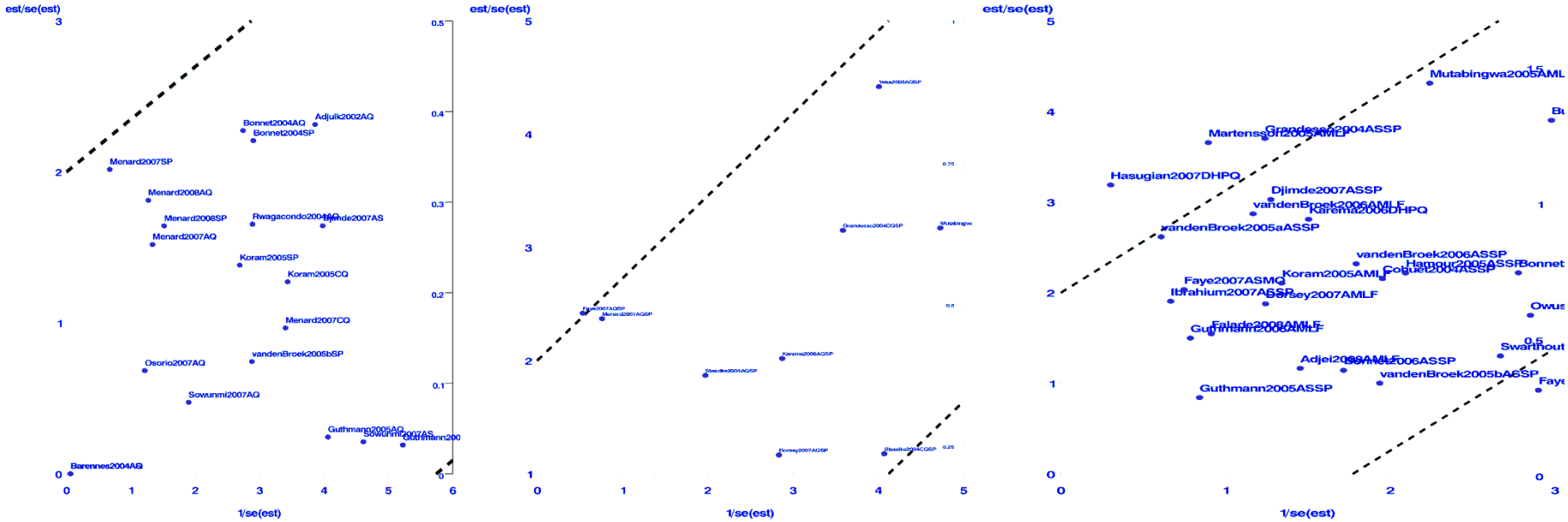
This was confirmed by the Galbraith plot displaying all studies around the overall pooled estimate with some exceptions: regarding the single agents: the studies of Guthmann 2005 (CQ), Mutabinggwa 2005 (AQ) and Menard 2008 (CQ) were not on the plot (Figure 5) but were between the lines of homogeneity and had high precisions (Precision = 1/StandardError(RR)). The study of Sowunmi and Yeka in 2005 with CQ+SP in the non ACT group and the Faye study in 2007 (AM+LF) in the ACT group had also a high precision, contributing to the homogeneity of the studies.

Figure 5. Galbraith plot of clinical trials comparing AS-AQ to Comparators

5a. Single agents (AS, AQ, CQ, SP)

5b. Non ACT combinations (AQ+SP, CQ+SP)

5c. ACT combinations (AM+LF 6 doses, AM+LF 4 doses, AS+MQ)



A meta-regression was performed on the PP population in order to investigate a potential source of heterogeneity among covariates (see the heterogeneity part of the methods paragraph). This model did not show a relationship between the ln(RR) of individual studies and each of the covariates assumed as potentially responsible for heterogeneity. Therefore it was not necessary to make an adjustment for heterogeneity based on these factors in the meta-analyses.

Based on the above results and the statement of parasite susceptibility and transmission heterogeneity, analyses are presented both by individual comparator and grouped as single agent, non-ACT and ACT comparators.

Day 28 efficacy results for all studies

The crude and PCR-adjusted D28 failure rates are summarised by site for AS-AQ in non-comparative and comparative studies in Table 3a and for comparators in Table 3b. Figure 6 presents the crude and PCR-adjusted D28 failure rates with AS-AQ in controlled and uncontrolled trials conducted in sub-Saharan Africa. These results are based on 33 studies with crude failure rates from 6,591 AS-AQ patients, and 28 studies with PCR-adjusted failure rates from 5,416 AS-AQ patients (not shown in Figure 6). The PP analysis population included 6,044 and 4,804 patients, respectively (not shown in Figure 6).

Crude failure rates varied widely from 0% (Burkina-Faso, Senegal, Madagascar, Nigeria) to 80% (Ghana). After genotyping, AS-AQ was ≤ 10% failures in 46 of the 59 trial sites reporting PCR-adjusted data.

Day 28 efficacy results for comparative studies

The 37 comparative studies with D28 assessment enrolled a total of 16,652 patients, of whom 6,551 received AS-AQ and 10,101 received a comparator drug in 65 AS-AQ study arms and 119 comparator study arms. The PP analysis population numbered 4,427 and 7,009, respectively.

Crude failure rates

AS-AQ was significantly more efficacious than all single-agents both individually and combined (Figure 7), for an aggregate failure rate RR (95% CI) of 0.15 [0.07;0.24]. Considering the comparisons by treatments, AS-AQ was significantly better than AS, AQ, CQ and SP. Several studies in this category were small and thus generated wide CIs.

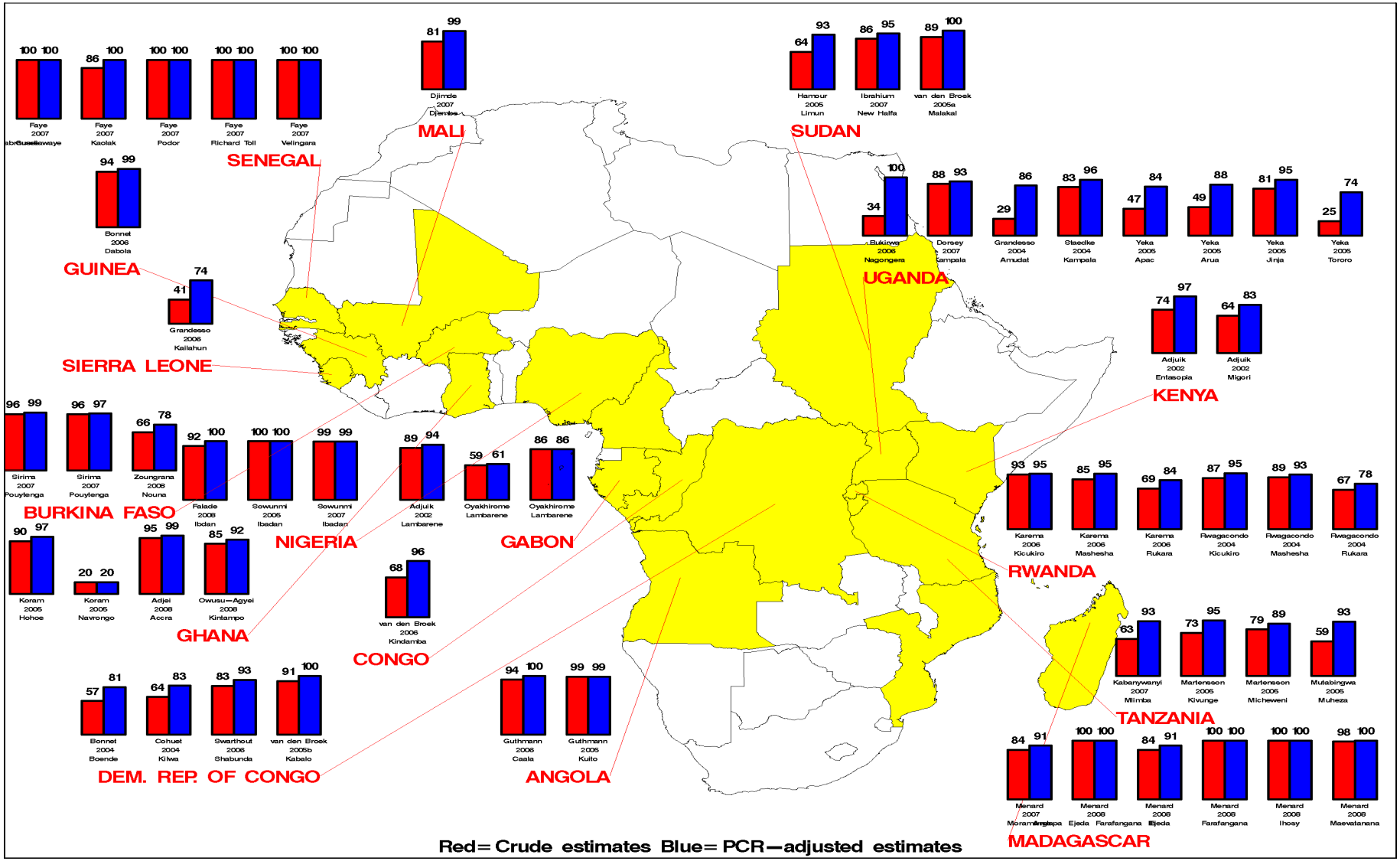


Figure 6. Unadjusted and PCR-adjusted success rates with AS-AQ in comparative and non comparative trials of uncomplicated falciparum malaria in Africa (PP dataset). PCR adjusted new infections were considered treatment successes (i.e. non-failures).

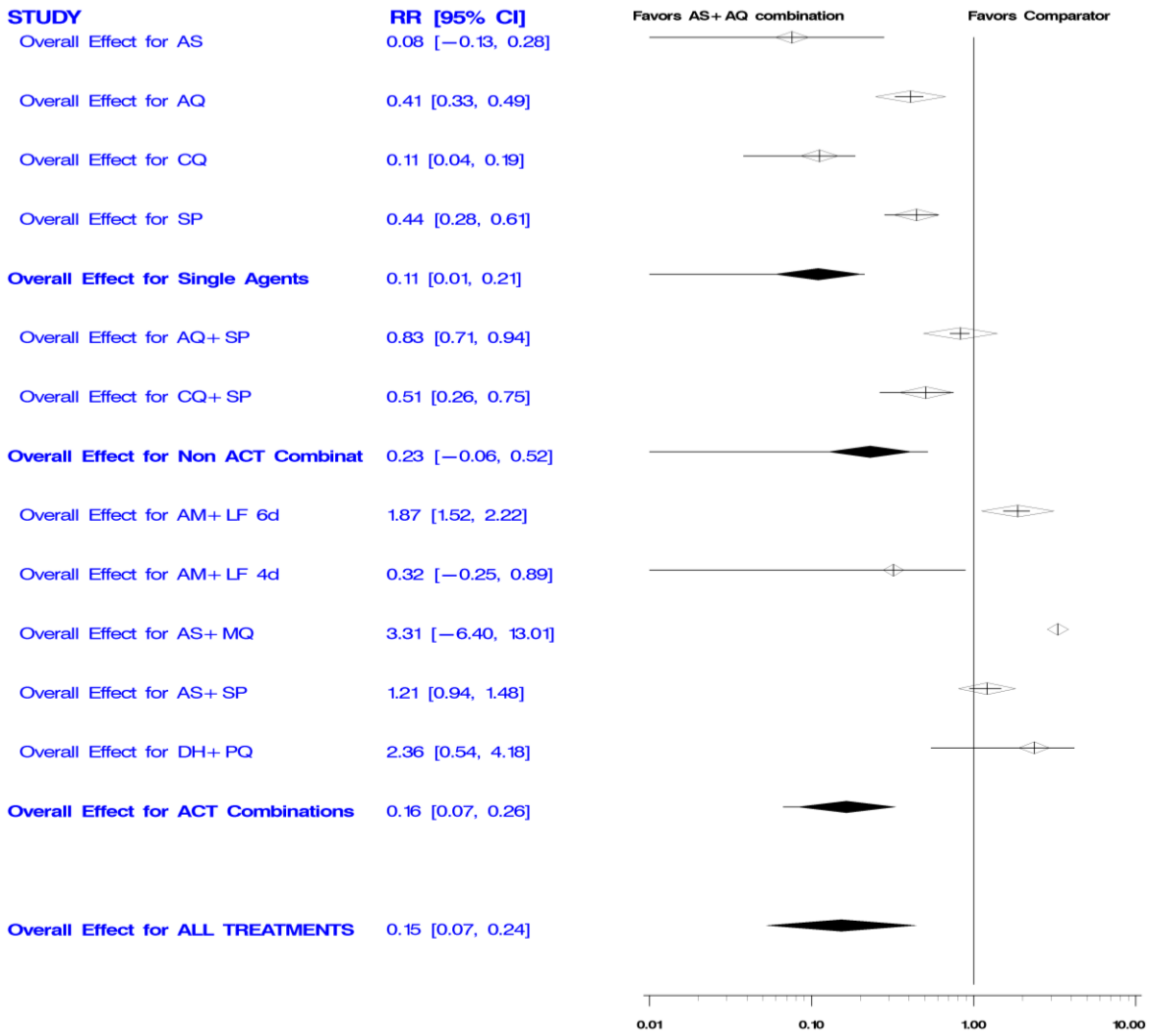


Figure 7. Forest plot of PCR-unadjusted Day 28 failure rates for 'treatment' AS-AQ vs. 'control' (PP dataset).

The overall pooled effect of AS-AQ versus non-ACT comparators was RR=0.23 [-0.06;0.52]. AS-AQ was significantly better than AQ+SP (RR=0.83, 95% CI =[0.71;0.94]) and CQ+SP (RR=0.51, 95% CI =[0.26;0.75].)

AS-AQ was significantly more effective than all ACTs combined (RR=0.16, 95% CI=[0.07;0.26]. Looking by comparators, AS-AQ was more effective than AM+LF 4 doses only (RR=0.32, 95% CI =[-0.25;0.89]) which refer to the study of Faye *et al.* with 3 sites. There was no difference with other individual comparators: AM+LF 6 doses (RR=1.86, 95% CI =[1.51;2.20]), AS+MQ (RR=3.31, 95% CI =[-6.40;13.01]), AS+SP (RR=1.21, 95% CI =[0.94;1.48]) and DH+PQ (RR=2.36, 95% CI =[0.54;4.18]). It should be noted that, even though not statistically significant, the RR were in favour of the comparator regimen.

Taking AS-AQ as the reference treatment, a multivariate model was performed with the treatment type as covariate. Treatment comparators CQ, AS+MQ, AS+LF 4 doses and DH+PQ were not significant in the model but the following regimens were significant: SP (RR=1.7, 95%CI=[1.2; 2.3]), AQ (RR=2.7, 95%CI=[2.2; 3.2]), AS (RR=7.1, 95%CI=[4.5; 9.7]), non ACT combinations AQ+SP (RR=1.8, 95%CI=[1.5; 2.2]), CQ+SP (RR=5.1, 95%CI=[3.8; 6.4]) and the ACT combinations AS+LF 6 doses (RR=0.5, 95%CI=[0.4; 0.6]) and AS+SP (RR=7.8, 95%CI=[5.9; 9.7]).

PCR adjusted failure rates

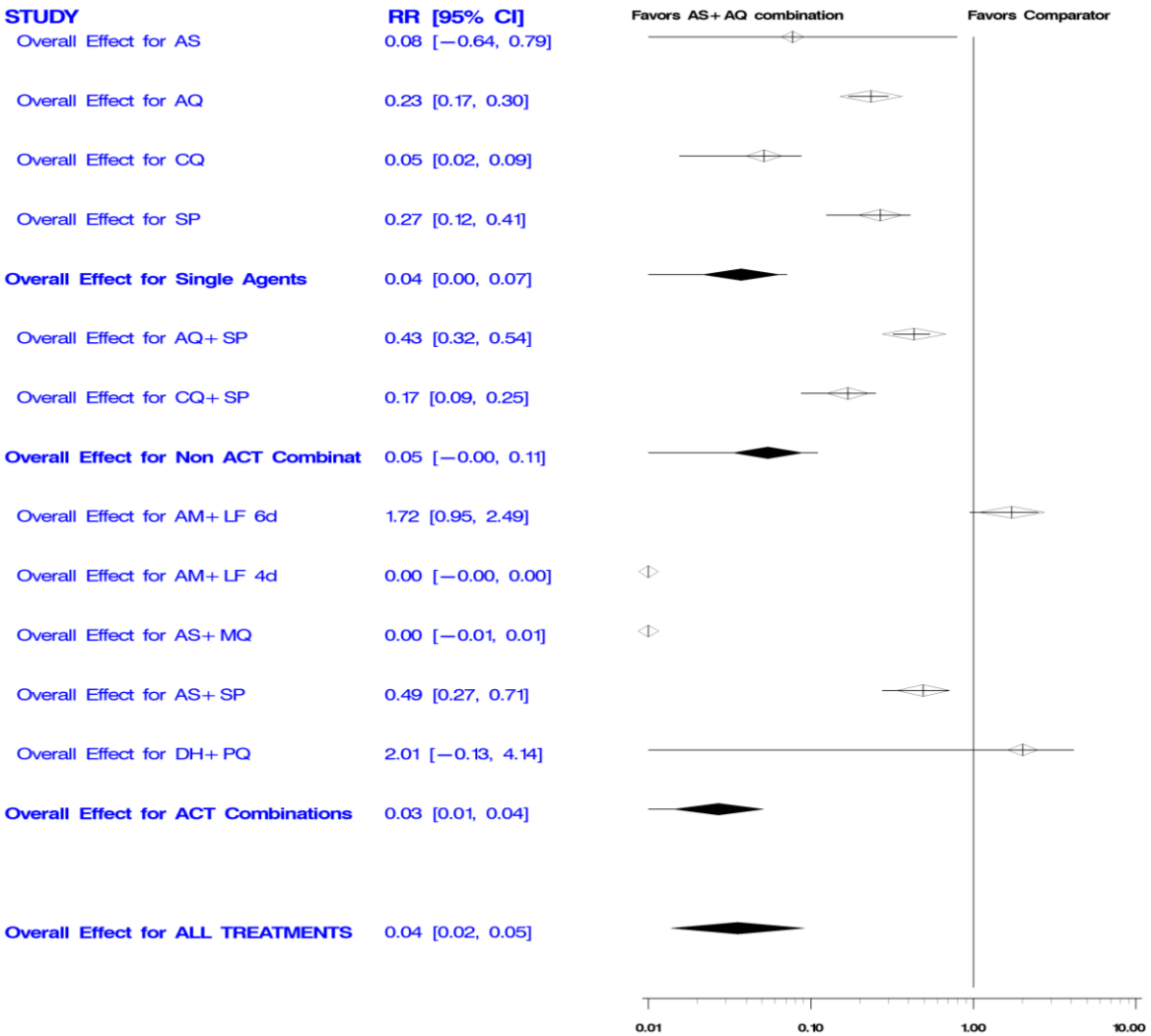


Figure 8. Forest plot of PCR-adjusted Day 28 failure rates for 'treatment' AS-AQ vs. 'control' (PP dataset). PCR-adjusted new infections and missing PCR were not considered following WHO 2003 guidelines.

AS-AQ was significantly better (Figure 8) than single agents (RR=0.04, 95% CI =[0.01;0.06]) on aggregate though also statistically better than AS (RR=0.08, 95% CI =[-0.64;0.79]), AQ (RR=0.23, 95% CI =[0.17;0.30]), CQ (RR=0.07, 95% CI =[0.02;0.13]) or SP

(RR=0.32, 95% CI =[0.16;0.48]) but AS showed large CI especially with regards to the lower limit.

There was a significant difference with non-ACT comparators both on aggregate (RR=0.05, 95% CI =[-0.00;0.11]) and individually for CQ+SP (RR=0.17, 95% CI =[0.09;0.25]) and AQ+SP (RR=0.43, 95% CI =[0.32;0.54]). AS-AQ was statistically superior to all ACTs combined (RR=0.03, 95% CI =[0.01;0.05] but not on the individual comparisons: AM+LF 6 doses (RR=1.66, 95% CI =[0.96 ; 2.36]) and DH+PQ (RR=2.01, 95% CI = [-0.13;4.14]) except AS+SP (RR=0.49, 95% CI =[0.27;0.71]) AM+LF 4 doses and AS+MQ were not valid due to a a low number of failures in the single study of these comparisons (Faye et al). There were no failures in the treatment arm and in the comparator arm only one of the 3 sites of had failures.

Comparators as independent predictors of AS-AQ failure (Crude rates)

The multivariate model gave similar values as with the crude values. The comparators AS+MQ, AM+LF 4doses and DH+PQ were not significant explanatory factors but the following were: CQ (RR=1.5, 95%CI=[1.0; 1.9]), AQ (RR=7.1, 95%CI=[5.1; 9.0]), SP (RR=2.8, 95%CI=[1.5; 4.2]), AS (RR=18.0, 95%CI=[9.5; 26.5]), non-ACT combinations AQ+SP (RR=3.2, 95%CI=[2.2; 4.1]), CQ+SP (RR=13.3, 95%CI=[8.4; 18.2]) and ACT combinations AM+LF 6 days (RR=0.3, 95%CI=[0.2; 0.5]) and AS+SP (RR=17.0, 95%CI=[12.5; 21.6]).

L'Abbé plots

L'Abbé plots of crude and PCR adjusted failure rates (Figure 9) show that crude rates of both AS-AQ and comparator regimens varied broadly but became ≤10% following PCR adjustment in most studies. In both cases the overall RR line is in the upper triangle (above the equal line). Outliers can be identified by the distance between the study and the RR line.

Database for safety analyses

Of the 46 eligible studies for analysis, adverse events (AE's) or serious adverse events (SAE's) were mentioned in 39 papers, 24 studies provided empirical data that could be quantified (n= 22 studies, 4,527 patients) or explicit remarks (n= 2 studies, 166 patients) on adverse events with AS-AQ (total n = 4,693, 56.57%). Only 16 studies formally defined outcome measures for safety; of these, only 10 clearly defined AE's or SAE's. In total, only 8 papers (n=1,409, 16.89%) made quantitative remarks regarding AE's as "minor", "mild" or showing unremarkable distribution between study groups (Figure 3).

Figure 9. L'Abbé plot of the Day 28 efficacy rates in comparative trials for (a) PCR-unadjusted and (b) PCR-adjusted outcomes. The failure rates with AS-AQ is on the Y axis, that of the comparator arm on the X axis. The diagonal divides the graph into an upper-left area (bubles in thisarea favour the comparator drug) and a bottom-right area (favouring AS-AQ). The grayed lines correspond to a 10% failure rate (the 90% efficacy rate recommended by the WHO).

Figure 9a

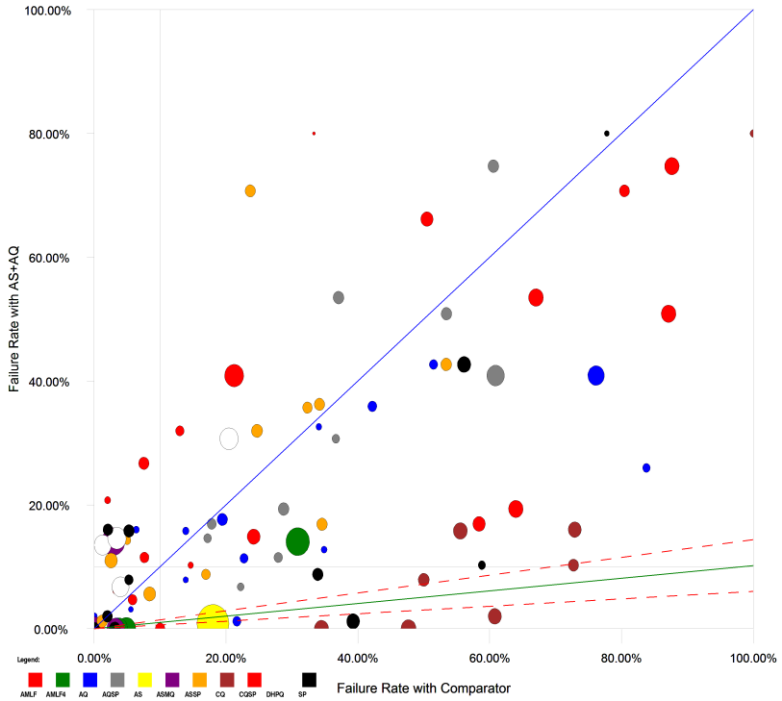
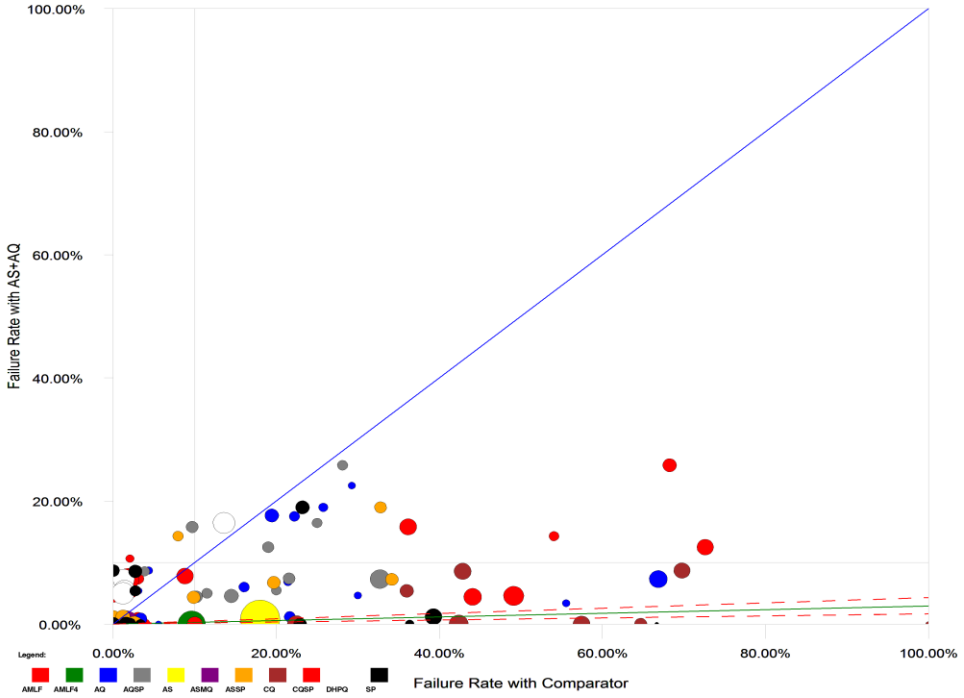
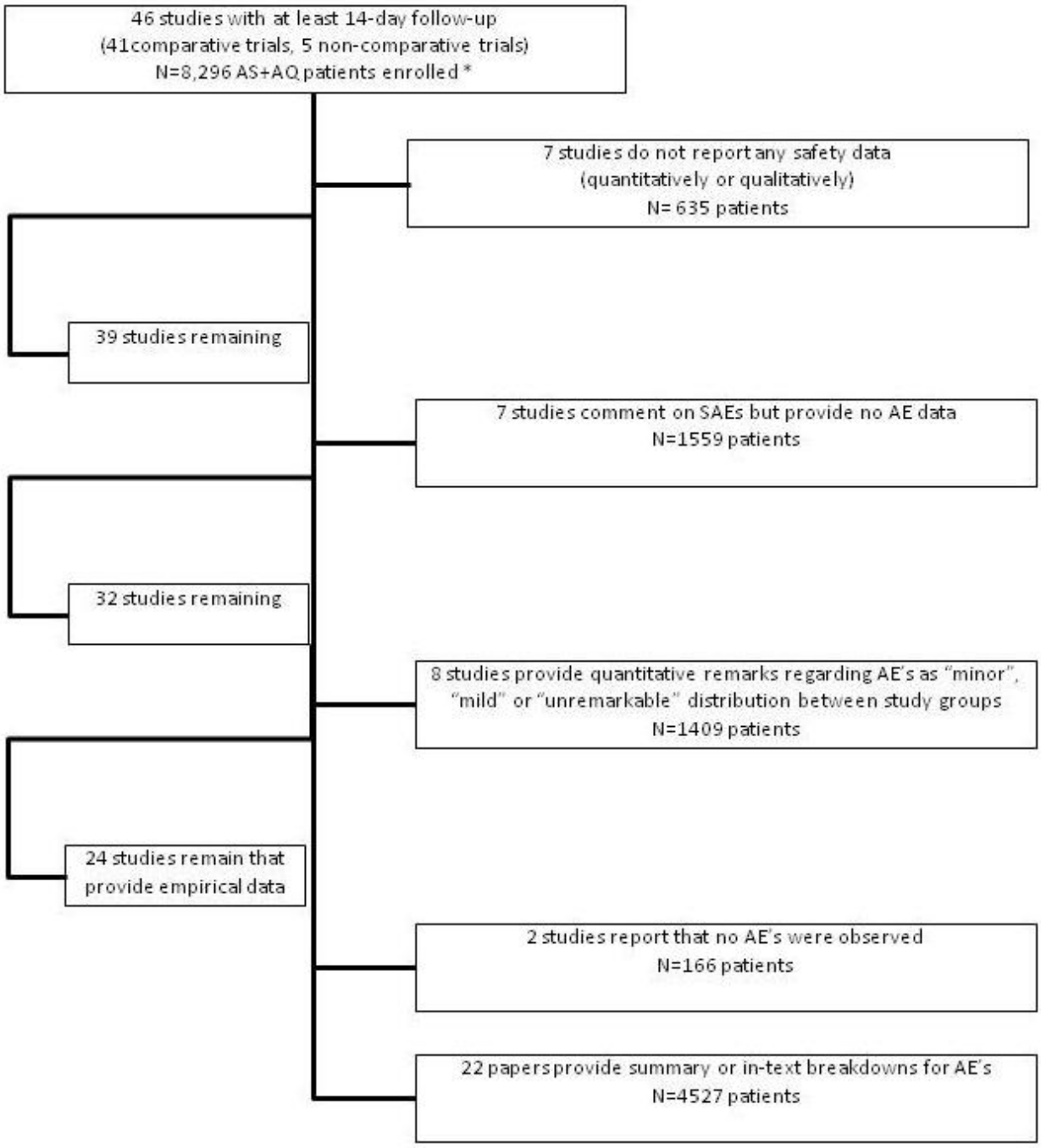


Figure 9b



Legend: AQ=amodiaquine; AS=artesunate; AM+LF=artemether+lumefantrine; CQ =chloroquine; SP=sulfadoxine/pyrimethamine; DH=dihydroartemisinin; PQ=piperaquine

Figure 3. Safety flow diagram



Combined there exists quantitative or qualitative data regarding safety (AE's) in 32 studies representing a total of 6,102 (73.55%) enrolled patients.

Adverse events reported varied between studies but included, vomiting, fever, anorexia, nausea, abdominal pain, diarrhoea, cough, skin rash, pruritus, headache, weakness, leukopaenia and neutropenia. AE's were reported as number of events and not by patients. The denominator (n=4,693) we used was the total number of patients enrolled in the 24 studies that provided safety data that could be quantified. Vomiting was most

commonly observed event (223, 4.75%), followed by cough (123, 2.62%), abdominal pain (110, 2 .34%), anorexia (103, 2.13%), fever (67, 1.43%), pruritus (53, 1.13%) and diarrhea (51, 1.08%). All the remaining were AE's events reported were n<40 (1%) events. A total of 1538 AE's were reported for all studies but 204 were only reported as "events" and not specified. Additionally, the number of patients who suffered AE's was not provided or calculable. The number of patients suffering AE's would likely be significantly lower than the event rates based on the data. For example, one study with 66 patients reported 149 events and another with 178 patients reported 256 events. Vomiting was the most discussed AE in all the studies but the details, such as severity and day of drug consumption, varied.

SAE reporting differed widely across papers, often without providing clear documentation of which SAE's were associated with which treatment. 11/46 studies (3,369 patients) reported SAE's that were attributed to AS-AQ, accounting for a total of 41 SAEs. However, in one paper with the most significant count of SAE related to AS-AQ (n=15) the SAE's breakdown was not provided (38). Thus, after reducing these 15 SAE's from the count the denominator becomes 26 SAEs that were documented to have occurred due to AS-AQ treatment: Of these 8 were severe vomiting, 4 convulsions, 3 severe anaemia, 3 deaths (1 due to renal failure, 2 were not specified), 2 respiratory distress, 1 pneumonia, 1 dehydration, 1 gastroenteritis, 1 severe prostration, 1 bilateral cerebellar signs, 1 respiratory illness. A verbal autopy led the investigators to conclude that the renal failure death was unrelated to AS-AQ and that the patient had been in poor health for three months prior.

Clinical laboratory assessments were seldom reported. Haemoglobin, a 'soft' efficacy end point, was the only laboratory investigation uniformly monitored. AS-AQ resulted in an increase in the mean haemoglobin by Day 28 which, on aggregate, was not significantly different to the comparator regimens. Only 8 studies reported white blood cell counts and 9 liver function tests. AS-AQ did not appear to have more haematological or liver toxicity than its comparators. No AS-AQ related liver toxicity was in 2672 patients. Severe neutropaenia (neutrophil count <1000/μL) occurred in nine (3 AS-AQ and 6 AQ) of 153 patients (6%) with normal baseline neutrophil counts in combined data from Kenya and Gabon (Adjuik2002) and six (4 AS-AQ and 2 AQ) of 71 patients (8%) in Colombia (Osorio2007). The latter study also reported a 10% incidence of abnormal liver enzymes (ALT > 48 U/L) by Day 28. Both neutrophil and liver abnormalities were asymp-

tomatic. Karema et al 2006 report 35/247 (14.2%) patients with neutropenia at D14 but did not provide D28 data.

Sirima et al (22) provide the most detailed laboratory breakdown (n=750) of all studies. The total number of children with D0 and D28 neutropaenia, and D0 and D28 leukopaenia were: (i) 32 of 657 (4.9%) and 87 of 569 (15.3%), (ii) 22 of 674 (3.3%) and 34 of 569 (6.0%), respectively. All children with reported neutropaenia were well; 4 were mildly febrile and only required symptomatic treatment. Compared to D0, the mean D28 AST (70 vs. 60 IU per L, p=0.02) and D28 total bilirubin (25.85 vs. 13.1 μmol per L, p<0.0001) values fell significantly. Mean serum ALT (32 vs. 29 IU/L) and creatinine (37 vs. 38 μmol per L) values changed little over time. Six of 529 (1.1%) patients with D28 ALT results had CTC grade 2 raised (>2.5 to 5 x ULN) ALT concentrations (range 104 to 196 IU per L) and one 2-year-old child (0.19%) in the AS-AQ group had asymptomatic, CTC grade 4 hepatitis [AST 1052, ALT 936 IU per L]). All 7 children had normal ALT values at baseline, were clinically well and aparasitaemic with normal D28 total bilirubin values, but all had raised D28 AST (range 73 to 1052 IU per L).

DISCUSSION

This paper reports the analytical findings of a large database of AS-AQ trials that were conducted between 1999 and September 2008 and represents a comprehensive summary of the efficacy of AS-AQ. Most studies were comparative, conducted in African children, diligent in following the CONSORT statement for presenting patient attrition and report standard WHO efficacy outcomes over 28 days. The use of common endpoints made data collation and analyses easier for efficacy. Safety reporting was very limited and needs to be improved. In the past, research on AQ was severely curtailed because of its serious toxicity when used as prophylaxis in travellers(23) so this systematic review represents a substantial increase in our knowledge of AQ efficacy and safety.

Although D14 outcomes were reported by many studies, we chose not to present these data because they have been shown to significantly underestimate treatment failure rates (24) Four studies only reported Day14 outcomes postdating the WHO recommendation of 28 days. Only four studies followed patients up to 42 days so little can currently inferred from these studies. Given the nature of the data, we could not analyse efficacy by the Kaplan-Meier survival estimates, as currently recommended by the WHO.

Day 28 efficacy varied substantially across studies when crude failure rates were taken into account. After removing PCR-proven new infections, variations decreased and treatment efficacy rates rose substantially. 42/59 study sites (78%) reported failure rates ≤ 10% which are considered acceptable by the 2006 WHO treatment guidelines (1).

Although the assembled database was large, several studies were very small resulting in wide confidence intervals and were underpowered to show a difference between regimens. Most comparative studies (AQ, SP, AQ+SP, CQ+SP AM+LF 6 doses and AS+SP) were adequately sized and powered. In these studies the 95% CIs were tight and a difference in favour of AS-AQ was seen over AQ, SP, AQ+SP, CQ+SP and AS+SP. No differences were detected between AS-AQ and all other treatments.

PCR genotyping, differentiating new from recrudescent infections, was performed in 37 studies with D28 outcomes. Without PCR correction, all cases of recurrent parasitaemia would be considered 'treatment failures', thus overestimating the true failure rate. Nevertheless, PCR correction did not change the overall direction of results in comparative trials despite the substantial loss of data because of the stringent WHO definition of a PP analysis population.

Failure rates were preferred over cure rates for the comparisons because they occurred at lower frequency. Several studies were underpowered to study cure rates whereas analysing failure rates allowed a gain in power in the analyses. Effect modifiers, such as underlying differences in endemicity and background parasite susceptibility across the study sites, contribute to the discordance between Odd Ratios (OR) and Relative Risks (RRs) (25). RRs were preferred also because of prospective study design nature of the clinical trials (26) and because it is easier to interpret without error (27). These results give a broad picture of the relative effect of AS-AQ, but their value to malaria control may vary because of some methodological considerations.

Although aggregating results in a meta-analysis increases statistical power and confidence to interpret the data, caution should be exercised because of potential heterogeneity and the effects of multiple comparisons. In particular, splitting results by site, while increasing geographical relevance within a country, may increases heterogeneity by reducing the sample size and broadening the confidence intervals of some comparisons. However, heterogeneity in these data was minimal and confined to CQ studies and we didn't identify significant covariate for D28 failure. Statistical models were used to account for the weight of each study in the analysis in order to avoid the multiple counting of the same dataset in studies with more than one comparator arm, which would have artificially inflate the sample size of the study treatment group (AS-AQ) and thus influence the level of significance of the comparison. Results were reported by study site rather than by country because efficacy rates can vary considerably within the same country and such geographical information is more useful for determining treatment policies.

While aggregate comparisons from meta-analyses are useful, the absolute failure rates are still essential because these data are of prime importance for malaria control programmes (MCPs), especially now that the WHO has set efficacy limits for drug policy change. Hence, both absolute crude and PCR-corrected failure rates for all studies were presented using the L'Abbé plots.

Currently, the number of trials comparing AS-AQ to other ACTs is relatively small. More comparative trials are certainly needed. Such studies are timely because a number of ACTs are in development and several may be options for MCPs. Deciding which ACT to use will need research evidence and MCPs must assess the local applicability of

aggregated results. A thorough assessment of safety is also important if ACTs may have similar efficacies.

In general, the reported safety data were very limited and made summarising information difficult. There was no standardised reporting or analytical pattern across studies. What may have been considered an AE in one study was not in others; this may reflect a lack of understanding of what constitutes an AE. Overall, AS-AQ was reportedly "safe" with “minor” or “mild” side effects across the studies and when reported in comparison, AS-AQ treated patients were observed with a safety profile comparable to comparators. Nonetheless, this safety analysis aligns with more recent safety studies (28-31).

The incidence of vomiting, the most commonly reported AE, was low and similar to other treatments. Data from these studies were insufficient to evaluate the risk of rare but clinically significant neutropenia and hepatitis, the two most serious complications of AQ therapy. Neutropenia (<1000/μL) was detected in 6% and 8% of AS-AQ and AQ patients in two double blind studies but these figures were not repeated in a small number of other studies. More prospective data are needed in large numbers to determine the risk of severe neutropaenia. No serious, clinically manifest, drug-induced reactions (e.g. acute hepatitis) were seen in >8,000 AS-AQ recipients, however liver function was tested only in few patients. Given that the risk of serious hepatotoxicity with AQ as prophylaxis in European travellers was estimated at 1 in 15,650 (32), then the sample size here was almost certainly insufficient to detect this SAE. Additional data are needed and should be collected through pharmacovigilance and in clinical trials. Spontaneous reports of AEs may be a simpler but less accurate way to detect hepatotoxicity or neutropenia because these will only be detected clinically. Only more monitoring of total and differential white cell counts and liver enzymes can answer these questions. Although individual clinical trials often lack the statistical power to accurately estimate the prevalence of rare and serious adverse drug reactions, it is still important for studies to monitor for and report such events; this will be useful for systematic reviews.

It is clear that the reporting of safety data has generally been inadequate to date, and we strongly encourage journals to make a greater effort in ensuring that this reporting is improved. The development of CONSORT-like statement for safety could help to ensure that such reporting is improved and should be developed. In addition, safety data from pharmaceutical company registration trials would provide a substantial contribution to our knowledge and should be made publicly available through either reports or

peer-reviewed publications. The recently announced commitment by Glaxo Smith Kline (GSK) is a promising development which other companies should follow.

Another important issue is access to safety and efficacy information. This meta-analysis has been put together by a number of investigators. Its usefulness will increase only if new studies are added and results made available to policy makers at international (e.g. WHO, other international agencies) and national levels (e.g. Ministries of Health). Such availability is crucial for the development of optimal malaria treatment guidelines. However, all data are not always made available. Several sponsor/authors of unpublished studies did not agree to share their data for this meta-analysis and it was, perhaps surprisingly, not possible to access the 13 in vivo AS-AQ efficacy studies from Africa in the WHO database (33). Furthermore, there are some inconsistencies between the findings of this meta-analysis and the drug policies of countries that have adopted AS-AQ. Greater coordination and data sharing between researchers and policy makers is key to defining optimal drug policies.

In summary, this systematic review and meta-analysis confirms that AS-AQ may be considered for the treatment of falciparum malaria in Africa. It presents an expended analysis , especially of safety than that is available in a recent Conchrane Review on ACTs (79). This analysis, unlike the Cochrane review includes the comparisons of ASAQ against non ACT's and mono-therapies. Drug resistance is a major concern with uncomplicated malaria, and while policy guidelines are to use ACT's, their use in reality still occurs, and herein we present epirical data to highlight that in addition to being dangerous to global malaria control, they are also inferior in regard to efficacy, especially in Africa. This is important to inform African Ministries of Health and this is not illustrated in the Cochrane Review. Furthermore, data on single-agent treatments provide information on the performance of the companion drug in the ACT, and important element of decision (according to the WHO its efficacy should be at least 85%).

Noting this, it is suggestive that AS-AQ usefulness may be limited in areas of AQ resistance; furthermore, more research is needed to compare the safety and efficacy of AS-AQ with other ACTs, and safety and efficacy monitoring should be continued in countries where AS-AQ and also other ACTs are already deployed.

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Roles of authors:

- P.Olliaro conceived and organised the project; wrote the protocol; contributed to the collection, assessment and analysis of the data; designed analyses; prepared the manuscript.
- M.Vaillant designed and conducted analyses; participated in data extraction and verification; prepared the manuscript.
- M.Harhay participated in data extraction and verification; and in preparing the manuscript.
- P.Mussano and R. Phalkey collected, assessed, keyed and extracted data, specifically for efficacy (PM) and safety (RP).
- J-P. Guthmann, G. Dorsey, P. Brasseur, U. D'Alessandro, A. Mårtensson, K. Koram, B. Faye, K. Mugittu, B. Sirima and WRJ. Taylor contributed unpublished data, participated intellectually in this project and in the writing of the manuscript.
- A. Sevcsik provided input through the analysis and in developing the manuscript.
- WRJ Taylor wrote the safety analysis plan and Pascal Millet contributed to the safety analyses.

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Supplemental Tables and Figures

Supplemental Table 1. Unadjusted and PCR-adjusted failure rates of AS-AQ and Comparators

Table 3a. AS-AQ failure rates

Table 3b. Comparators failure rates

Supplemental Figure 1. Detailed Forest plot of PCR-unadjusted Day 28 failure rates for 'treatment' AS-AQ vs. 'control' (PP dataset).

Supplemental Figure 2. Detailed Forest plot of PCR-adjusted Day 28 failure rates for 'treatment' AS-AQ vs. 'control' (PP dataset). PCR-adjusted new infections and missing PCR were not considered following WHO 2003 guidelines.

Supplemental Table 1.

Supplemental Table 1a.

				Not PCR adjusted			PCR Adjusted		
Study	Country	Site	Comparator	N	failure	rate	N	failure	rate
Adjuik, 2002	Gabon	Lambarene	AQ	88	10	11.0%	83	5	6.0%
Adjuik, 2002	Kenya	Entasopia	AQ	77	20	26.0%	59	2	3.0%
Adjuik, 2002	Kenya	Migori	AQ	103	37	36.0%	80	14	18.0%
Adjuik, 2002	Senegal	Mlomp	AQ	136	24	18.0%	136	24	18.0%
Barennes, 2004	Burkina-Faso	Bobo-Dioulasso	AQ, AS	24	0	0.0%	24	0	0.0%
Bonnet, 2004	RDC	Boende	AS+SP, SP, AQ	82	35	43.0%	58	11	19.0%
Bonnet, 2006	Rép de Guinée	Dabola	AS+SP	107	6	6.0%	102	1	1.0%
Bukirwa, 2006	Uganda	Nagongera	AM+LF	201	133	66.0%	68	0	0.0%
Cohuet, 2004	RDC	Kilwa	AS+SP	70	25	36.0%	54	9	17.0%
Djimde, 2007	Mali	Djembe		235	44	19.0%	193	2	1.0%
Dorsey, 2007	Uganda	Kampala	AQ+SP, AM+LF	113	13	12.0%	108	8	7.0%
Falade, 2008	Nigeria	Ibdan		61	5	8.0%	56	0	0.0%
Faye, 2007	Senegal	Guediawaye		98	0	0.0%	98	0	0.0%
Faye, 2007	Senegal	Kaolak	AS+MQ, AM+LF 4d, AQ+SP	64	9	14.0%	55	0	0.0%
Faye, 2007	Senegal	Podor		91	0	0.0%	91	0	0.0%
Faye, 2007	Senegal	Richard Toll	AS+MQ, AM+LF 4d, AM+LF, AQ+SP	44	0	0.0%	44	0	0.0%
Faye, 2007	Senegal	Velingara	AS+MQ, AM+LF 4d, AM+LF, AQ+SP	52	0	0.0%	52	0	0.0%
Grandesso, 2004	Uganda	Amudat	CQ+SP, AS+SP	82	58	71.0%	28	4	14.0%
Guthmann, 2006	Angola	Caala	AM+LF	64	4	6.0%	60	0	0.0%
Guthmann, 2005	Angola	Kuito	AS+SP, AQ, SP	84	1	1.0%	84	1	1.0%
Hamour, 2005	Sudan	Limun	AS+SP	80	29	36.0%	55	4	7.0%
Hasugian, 2007	Indonesia	Timika	DH+PQ	74	10	14.0%	69	5	7.0%
Ibrahium, 2007	Sudan	New Halfa	AS+SP	42	6	14.0%	38	2	5.0%
Karema, 2006	Rwanda	Kicukiro	AQ+SP, DH+PQ	74	5	7.0%	73	4	5.0%
Karema, 2006	Rwanda	Mashesha	AQ+SP, DH+PQ	89	13	15.0%	80	4	5.0%
Karema, 2006	Rwanda	Rukara	AQ+SP, DH+PQ	88	27	31.0%	73	12	16.0%
Koram, 2005	Ghana	Hohoe	CQ, SP, AM+LF	39	4	10.0%	35	0	0.0%
Koram, 2005	Ghana	Navrongo	CQ, SP, AM+LF	15	12	80.0%	3	0	0.0%
Martensson, 2005	Zanzibar	Kivunge	AM+LF	146	39	27.0%	113	6	5.0%
Martensson, 2005	Zanzibar	Micheweni	AM+LF	53	11	21.0%	47	5	11.0%
Menard, 2007	Madagascar	Moramanga	CQ, AQ, SP, AQ+SP	76	12	16.0%	70	6	9.0%
Menard, 2008	Madagascar	Andapa Ejeda Farafangana Ih		41	0	0.0%	41	0	0.0%
Menard, 2008	Madagascar	Ejeda	AQ, CQ, SP	50	8	16.0%	46	4	9.0%

Menard, 2008	Madagascar	Farafangana	AQ, SP	29	0	0.0%	29	0	0.0%
Menard, 2008	Madagascar	Ihosy	AQ, CQ, SP	57	0	0.0%	57	0	0.0%
Menard, 2008	Madagascar	Maevatanana	AQ, CQ, SP	50	1	2.0%	49	0	0.0%
Menard, 2008	Madagascar	Miandrivazo	AQ, CQ, SP	65	0	0.0%	65	0	0.0%
Menard, 2008	Madagascar	Moramanga	AQ, CQ, SP	38	3	8.0%	37	2	5.0%
Mutabingwa, 2005	Tanzania	Muheza	AQ, AQ+SP, AM+LF	472	193	41.0%	301	22	7.0%
Osorio, 2007	Colombia	Quibdo	AQ	32	1	3.0%	31	0	0.0%
Rwagacondo, 2004	Rwanda	Kicukiro	AQ	47	6	13.0%	43	2	5.0%
Rwagacondo, 2004	Rwanda	Mashesha	AQ	61	7	11.0%	58	4	7.0%
Rwagacondo, 2004	Rwanda	Rukara	AQ	46	15	33.0%	40	9	23.0%
Sirima, 2007	Burkina Faso	Pouytenga		326	13	4.0%	317	4	1.0%
Sirima, 2007	Burkina Faso	Pouytenga		329	14	4.0%	324	9	3.0%
Sowunmi, 2005	Nigeria	Ibadan	CQ+SP	103	0	0.0%	103	0	0.0%
Sowunmi, 2007	Nigeria	Ibadan	AQ, AS	120	1	0.8%	120	1	0.8%
Staedke, 2004	Uganda	Kampala	AQ+SP, CQ+SP	130	22	17.0%	113	5	4.0%
Swarthout, 2006	RDC	Shabunda	AS+SP	83	14	17.0%	74	5	7.0%
van den Broek, 2005a	Sudan	Malakal	AS+SP	118	13	11.0%	105	0	0.0%
van den Broek, 2005b	RDC	Kabalo	SP, AS+SP	57	5	9.0%	52	0	0.0%
van den Broek, 2006	Rep.Congo	Kindamba	AS+SP, AM+LF	97	31	32.0%	69	3	4.0%
Yeka, 2005	Uganda	Apac	AQ+SP, CQ+SP	172	92	53.0%	95	15	16.0%
Yeka, 2005	Uganda	Arua	AQ+SP, CQ+SP	171	87	51.0%	96	12	13.0%
Yeka, 2005	Uganda	Jinja	AQ+SP, CQ+SP	181	35	19.0%	153	7	5.0%
Yeka, 2005	Uganda	Tororo	AQ+SP, CQ+SP	182	136	75.0%	62	16	26.0%
Adjei, 2008	Ghana	Accra	AM+LF	107	5	5.0%	103	1	1.0%
Owusu-Agyei, 2008	Ghana	Kintampo	AL	222	33	15.0%	205	16	8.0%
Zoungrana, 2008	Burkina Faso	Nouna		61	21	34.0%	51	11	22.0%

Supplemental Table 1b.

				Not PCR adjusted			PCR Adjusted		
Study	Country	Site	Comparator	N	failure	rate	N	failure	rate
Adjuik, 2002	Gabon	Lambarene	AQ	88	20	23.0%	81	13	16.0%
Adjuik, 2002	Kenya	Entasopia	AQ	74	62	84.0%	27	15	56.0%
Adjuik, 2002	Kenya	Migori	AQ	109	46	42.0%	81	18	22.0%
Adjuik, 2002	Senegal	Mlomp	AQ	144	28	19.0%	144	28	19.0%
Barennes, 2004	Burkina-Faso	Bobo-Dioulasso	AQ	22	0	0.0%	22	0	0.0%

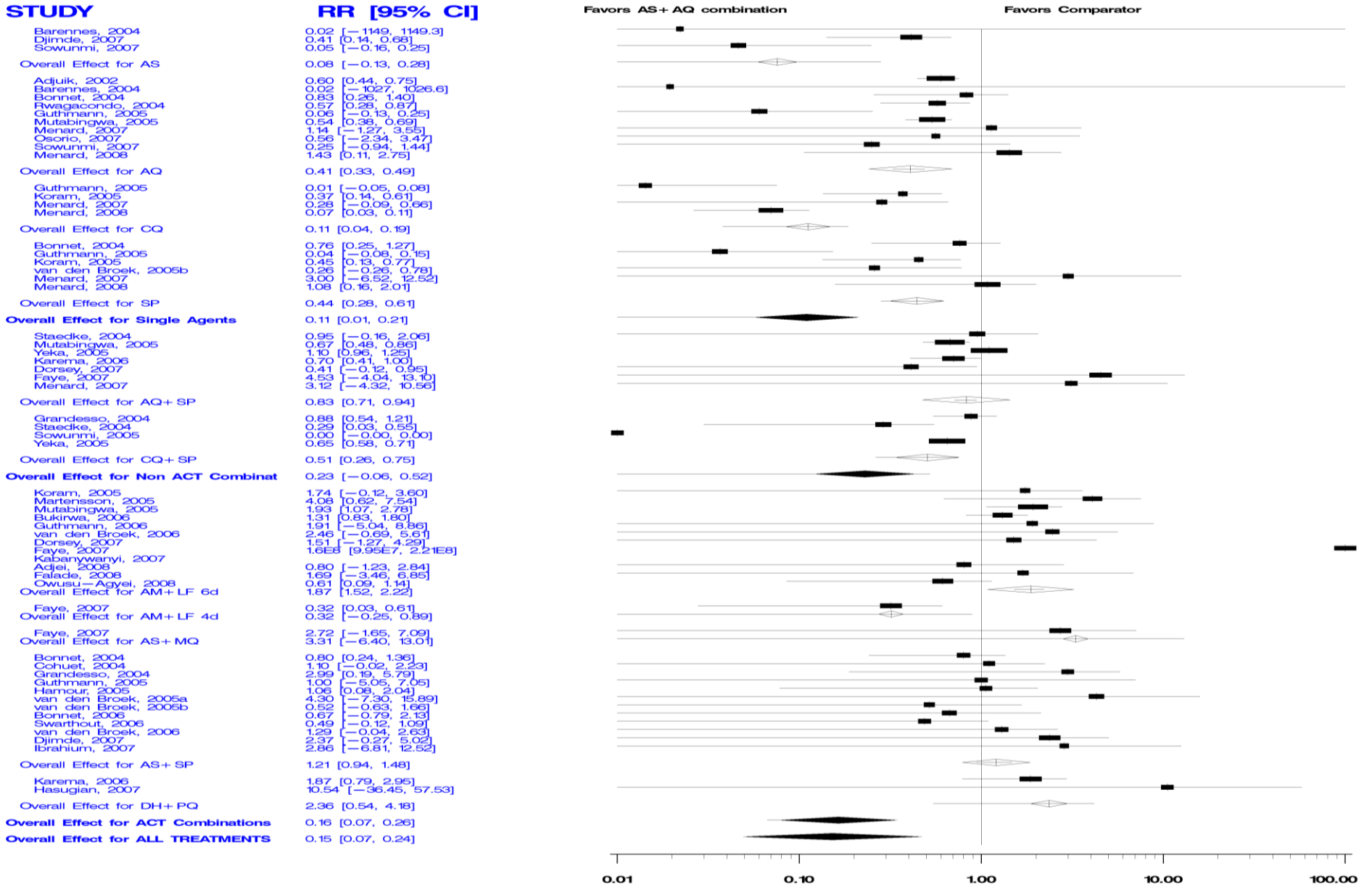
Barennes, 2004	Burkina-Faso	Bobo-Dioulasso	AS	27	0	0.0%	27	0	0.0%
Bonnet, 2004	RDC	Boende	AS+SP	88	47	53.0%	61	20	33.0%
Bonnet, 2004	RDC	Boende	SP	98	55	56.0%	56	13	23.0%
Bonnet, 2004	RDC	Boende	AQ	101	52	51.0%	66	17	26.0%
Bonnet, 2006	Rép de Guinée	Dabola	AS+SP	107	9	8.0%	98	0	0.0%
Bukirwa, 2006	Uganda	Nagongera	AM+LF	202	102	50.0%	104	4	4.0%
Cohuet, 2004	RDC	Kilwa	AS+SP	68	22	32.0%	53	7	13.0%
Djimde, 2007	Mali	Bougoula-Hameau	AS+SP	241	19	8.0%	230	8	3.0%
Djimde, 2007	Mali	Bougoula-Hameau	AS	247	112	45.0%	156	21	13.0%
Dorsey, 2007	Uganda	Kampala	AQ+SP	111	31	28.0%	102	22	22.0%
Dorsey, 2007	Uganda	Kampala	AM+LF	105	8	8.0%	100	3	3.0%
Falade, 2008	Nigeria	Ibadan	AM+LF	62	3	5.0%	59	0	0.0%
Faye, 2007	Senegal	Kaolak	AS+MQ	69	2	3.0%	68	1	1.0%
Faye, 2007	Senegal	Kaolak	AM+LF 4d	68	21	31.0%	52	5	10.0%
Faye, 2007	Senegal	Kaolak	AQ+SP	70	2	3.0%	68	0	0.0%
Faye, 2007	Senegal	Richard Toll	AS+MQ	31	1	3.0%	30	0	0.0%
Faye, 2007	Senegal	Richard Toll	AM+LF 4d	41	2	5.0%	39	0	0.0%
Faye, 2007	Senegal	Richard Toll	AM+LF	22	0	0.0%	22	0	0.0%
Faye, 2007	Senegal	Richard Toll	AQ+SP	42	0	0.0%	42	0	0.0%
Faye, 2007	Senegal	Velingara	AS+MQ	45	0	0.0%	45	0	0.0%
Faye, 2007	Senegal	Velingara	AM+LF 4d	27	1	4.0%	26	0	0.0%
Faye, 2007	Senegal	Velingara	AM+LF	127	0	0.0%	127	0	0.0%
Faye, 2007	Senegal	Velingara	AQ+SP	49	0	0.0%	49	0	0.0%
Grandesso, 2004	Uganda	Amudat	CQ+SP	87	70	80.0%	37	20	54.0%
Grandesso, 2004	Uganda	Amudat	AS+SP	76	18	24.0%	63	5	8.0%
Guthmann, 2006	Angola	Caala	AM+LF	61	2	3.0%	59	0	0.0%
Guthmann, 2005	Angola	Caala	CQ	79	66	84.0%	79	66	84.0%
Guthmann, 2005	Angola	Caala	AQ	75	13	17.0%	75	13	17.0%
Guthmann, 2005	Angola	Caala	SP	79	20	25.0%	79	20	25.0%
Guthmann, 2005	Angola	Kuito	AS+SP	84	1	1.0%	84	1	1.0%

Guthmann, 2005	Angola	Kuito	AQ	97	21	22.0%	97	21	22.0%
Guthmann, 2005	Angola	Kuito	SP	84	33	39.0%	84	33	39.0%
Hamour, 2005	Sudan	Limun	AS+SP	79	27	34.0%	79	27	34.0%
Hasugian, 2007	Indonesia	Timika	DH+PQ	78	1	1.0%	78	1	1.0%
Ibrahium, 2007	Sudan	New Halfa	AS+SP	40	2	5.0%	39	1	3.0%
Karema, 2006	Rwanda	Kicukiro	AQ+SP	72	16	22.0%	70	14	20.0%
Karema, 2006	Rwanda	Kicukiro	DH+PQ	75	3	4.0%	73	1	1.0%
Karema, 2006	Rwanda	Mashesha	AQ+SP	93	16	17.0%	87	10	11.0%
Karema, 2006	Rwanda	Mashesha	DH+PQ	87	3	3.0%	85	1	1.0%
Karema, 2006	Rwanda	Rukara	AQ+SP	90	33	37.0%	76	19	25.0%
Karema, 2006	Rwanda	Rukara	DH+PQ	88	18	20.0%	81	11	14.0%
Koram, 2005	Ghana	Hohoe	CQ	22	16	73.0%	17	11	65.0%
Koram, 2005	Ghana	Hohoe	SP	17	10	59.0%	11	4	36.0%
Koram, 2005	Ghana	Hohoe	AM+LF	41	6	15.0%	36	1	3.0%
Koram, 2005	Ghana	Navrongo	CQ	8	8	100%	6	6	100%
Koram, 2005	Ghana	Navrongo	SP	9	7	78.0%	6	4	67.0%
Koram, 2005	Ghana	Navrongo	AM+LF	6	2	33.0%	4	0	0.0%
Martensson, 2005	Zanzibar	Kivunge	AM+LF	146	11	8.0%	138	3	2.0%
Martensson, 2005	Zanzibar	Micheweni	AM+LF	49	1	2.0%	49	1	2.0%
Menard, 2007	Madagascar	Moramanga	CQ	36	20	56.0%	28	12	43.0%
Menard, 2007	Madagascar	Moramanga	AQ	36	5	14.0%	32	1	3.0%
Menard, 2007	Madagascar	Moramanga	SP	38	2	5.0%	37	1	3.0%
Menard, 2007	Madagascar	Moramanga	AQ+SP	79	4	5.0%	78	3	4.0%
Menard, 2008	Madagascar	Andapa	AQ	32	0	0.0%	32	0	0.0%
Menard, 2008	Madagascar	Andapa	SP	29	0	0.0%	29	0	0.0%
Menard, 2008	Madagascar	Ejeda	AQ	47	3	6.0%	46	2	4.0%
Menard, 2008	Madagascar	Ejeda	CQ	48	35	73.0%	43	30	70.0%
Menard, 2008	Madagascar	Ejeda	SP	48	1	2.0%	47	0	0.0%
Menard, 2008	Madagascar	Farafangana	AQ	28	0	0.0%	28	0	0.0%
Menard, 2008	Madagascar	Farafangana	SP	30	1	3.0%	30	1	3.0%
Menard, 2008	Madagascar	Ihosy	AQ	53	0	0.0%	53	0	0.0%

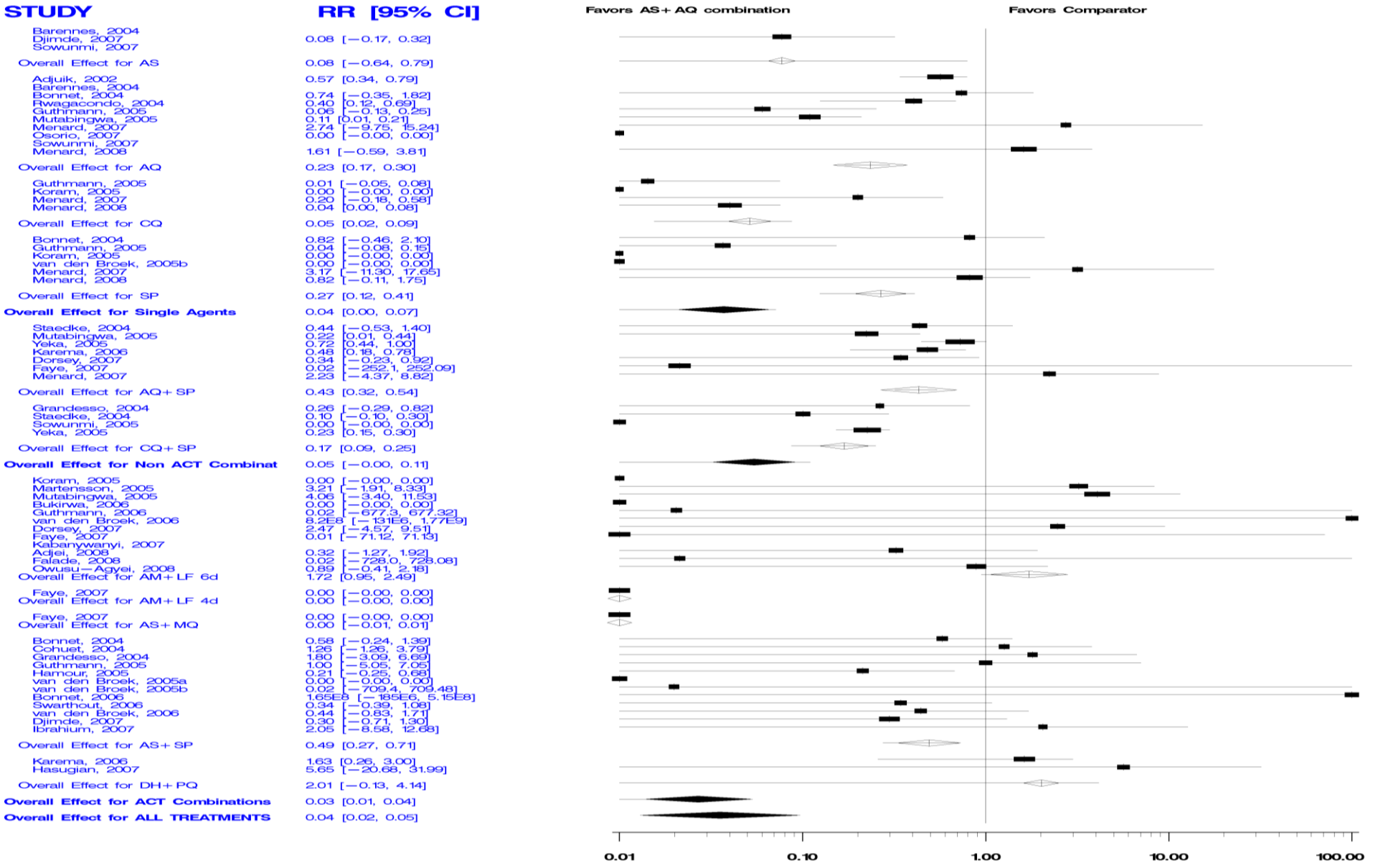
Menard, 2008	Madagascar	Ihosy	CQ	58	20	34.0%	49	11	22.0%
Menard, 2008	Madagascar	Ihosy	SP	59	0	0.0%	59	0	0.0%
Menard, 2008	Madagascar	Maevatanana	AQ	52	0	0.0%	52	0	0.0%
Menard, 2008	Madagascar	Maevatanana	CQ	51	31	61.0%	47	27	57.0%
Menard, 2008	Madagascar	Maevatanana	SP	50	1	2.0%	50	1	2.0%
Menard, 2008	Madagascar	Miandrivazo	AQ	66	0	0.0%	66	0	0.0%
Menard, 2008	Madagascar	Miandrivazo	CQ	65	31	48.0%	59	25	42.0%
Menard, 2008	Madagascar	Miandrivazo	SP	64	2	3.0%	63	1	2.0%
Menard, 2008	Madagascar	Moramanga	AQ	36	5	14.0%	32	1	3.0%
Menard, 2008	Madagascar	Moramanga	CQ	32	16	50.0%	25	9	36.0%
Menard, 2008	Madagascar	Moramanga	SP	38	2	5.0%	37	1	3.0%
Menard, 2008	Madagascar	TDD	AQ	39	1	3.0%	39	1	3.0%
Menard, 2008	Madagascar	TDD	CQ	32	16	50.0%	32	16	50.0%
Menard, 2008	Madagascar	TDD	SP	39	5	13.0%	38	4	11.0%
Mutabingwa, 2005	Tanzania	Muheza	AQ	239	182	76.0%	172	115	67.0%
Mutabingwa, 2005	Tanzania	Muheza	AQ+SP	463	282	61.0%	269	88	33.0%
Mutabingwa, 2005	Tanzania	Muheza	AM+LF	485	103	21.0%	389	7	2.0%
Osorio, 2007	Colombia	Quibdo	AQ	36	2	6.0%	36	2	6.0%
Rwagacondo, 2004	Rwanda	Kicukiro	AQ	43	15	35.0%	40	12	30.0%
Rwagacondo, 2004	Rwanda	Mashesha	AQ	61	17	28.0%	56	12	21.0%
Rwagacondo, 2004	Rwanda	Rukara	AQ	44	15	34.0%	41	12	29.0%
Sowunmi, 2005	Nigeria	Ibadan	CQ+SP	50	5	10.0%	50	5	10.0%
Sowunmi, 2007	Nigeria	Ibadan	AQ	120	4	3.0%	120	4	3.0%
Sowunmi, 2007	Nigeria	Ibadan	AS	111	20	18.0%	111	20	18.0%
Staedke, 2004	Uganda	Kampala	AQ+SP	129	23	18.0%	118	12	10.0%
Staedke, 2004	Uganda	Kampala	CQ+SP	125	73	58.0%	93	41	44.0%
Swarthout, 2006	RDC	Shabunda	AS+SP	81	28	35.0%	66	13	20.0%
van den Broek, 2005a	Sudan	Malakal	AS+SP	117	3	3.0%	117	3	3.0%
van den Broek, 2005b	RDC	Kabalo	SP	56	19	34.0%	48	11	23.0%
van den Broek, 2005b	RDC	Kabalo	AS+SP	59	10	17.0%	49	0	0.0%
van den Broek, 2006	Rep.Congo	Kindamba	AS+SP	85	21	25.0%	71	7	10.0%

van den Broek, 2006	Rep.Congo	Kindamba	AM+LF	100	13	13.0%	87	0	0.0%
Yeka, 2005	Uganda	Apac	AQ+SP	178	66	37.0%	124	12	10.0%
Yeka, 2005	Uganda	Apac	CQ+SP	182	122	67.0%	94	34	36.0%
Yeka, 2005	Uganda	Arua	AQ+SP	174	93	53.0%	100	19	19.0%
Yeka, 2005	Uganda	Arua	CQ+SP	179	156	87.0%	84	61	73.0%
Yeka, 2005	Uganda	Jinja	AQ+SP	174	50	29.0%	145	21	14.0%
Yeka, 2005	Uganda	Jinja	CQ+SP	161	103	64.0%	114	56	49.0%
Yeka, 2005	Uganda	Tororo	AQ+SP	175	106	61.0%	96	27	28.0%
Yeka, 2005	Uganda	Tororo	CQ+SP	162	142	88.0%	63	43	68.0%
Adjei, 2008	Ghana	Accra	AM+LF	103	6	6.0%	100	3	3.0%
Owusu-Agyei, 2008	Ghana	Kintampo	AM+LF	219	53	24.0%	182	16	9.0%

Supplemental Figure 1.



Supplemental Figure 2.



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Alternative visual displays of meta-analysis of malaria treatment trials to facilitate translation of research into policy

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Abstract Typically, meta-analyses show relative effects and heterogeneity, but not absolute effects - an essential element in policy decision.

Data obtained through a systematic review of antimalarial treatment trials and virtual trials were used to generate a display that shows and quantifies absolute and relative effects as well as het-erogeneity for comparative trials results.

A plot of failure rates (with 95% confidence intervals) of the test drug on the Y axis against the risk difference (RD) versus the comparator drug on the x axis; the area is divided into four quadrants by a vertical line (no RD) and a horizontal line (maximum tolerated failures, e.g. 10% for antimalarials). This allows identifying where a drug can be used (meeting efficacy requirements) and quantifying differences (vs. another treatment option). The area of the polygon connecting the study points expresses he-terogeneity.

This graphical display is simple to prepare and interpret, and combines in one graph both measures of absolute treatment effect and difference, as well as heterogeneity. It may complement cur-rent methods and provide useful information in policy decision making.

Keywords: Meta-analysis; Malaria; Meta-analysis

INTRODUCTION

The Question

Systematic reviews of available evidence on benefits and risks of medical interventions are increasingly used to inform decision making in clinical practice and public health [Buschman, BJ, *et al.* November 1998, Egger, M, *et al.* 1997]. Such reviews are, whenever possible, based on meta-analysis - defined as "a statistical analysis which combines or integrates the results of several independent clinical trials considered by the analyst to be combinable" [Yusuf, S, *et al.* 1985].

Although the underlying statistical methods are generally well developed [Whitehead, A 2002], the results and interpretation of such analyses remain difficult to understand for those who are less acquainted with statistics. The prevailing statistical approach to meta-analysis is to estimate the magnitude of combined results across studies (referred to as effect size estimate) with a confidence interval (CI) around it [Hedges, LV,

et al. 1992, Oakes, MW 1986]. This provides information not only on whether the null hypothesis (e.g. that the test treatment has no effect over the reference treatment) should be rejected at a given significance level or not, but also on whether the observed treatment effect is large enough to be considered of practical import. Between-study variation in effects can be treated as fixed or random [Hedges, LV, *et al.* 1985], depending on whether the model assumes that the population effect size is a single fixed value or a randomly distributed variable with its own mean and variance.

The test case: choosing antimalarial treatments

Malaria remains a substantial burden worldwide, placing half the global population at risk and killing a million children every year [WHO, 2010]. Artemisinin-containing combina-tion therapies (ACTs) are the World Health Organization (WHO) recommended first-line treatment of acute uncomplicated *Plasmodium falciparum* malaria [WHO, 2010], While this general recommendation is supported by systematic reviews of randomized clinical trials [Adjuik, 2004; Sinclair et al, 2009][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,59][25,37], summarising evidence in malaria treatment is challenging [Garner, 2009]. Treatment outcome may vary from place to place and over time with parasite susceptibility and host response; furthermore, decision makers in disease-endemic countries are faced with the problem of choosing the treatment that is best adapted to their situation and for this they need both relative and absolute outcomes. Currently systematic reviews provide results mainly in the form of forest plots of relative estimates.

Graphical presentation of results

Graphical displays are commonly used to present meta-analysis outcomes and heterogeneity [Bax, L, *et al.* 2009]. Results are typically displayed as forest plots which present summary statistics such as risk ratio (RR, also referred to as relative risk) or odds ratio (OR) with CIs for individual study comparisons and for aggregate meta-analysis [Yusuf, S, *et al.* 1985]. The pooled RR or OR is calculated as a weighted average of the treatment effects estimated in the individual studies using a fix or a random effects model - to account for heterogeneity between countries, duration and years of studies for instance [Smeeth, L, *et al.* 1999, The Cochrane Collaboration 2005]. In a typical forest plot each study is represented by a block (the point estimate of treatment effect) whose size indicates the weight assigned to that study in the meta-analysis. The horizontal line is the CI which indicates the range of treatment effects compatible with the study result and indicates whether the treatment effect is statistically significant.

Such display places emphasis upon differences in effects between comparators but absolute treatment effects are not obvious - the same OR or RR can be obtained by comparing interventions that can be either effective or ineffective, thus of use or no use in practice. So, in order to derive information on the practical applicability of the results one needs to undertake to extract absolute efficacy rates and calculate CIs.

Clearly, there is a need for ways of describing the results of meta-analyses which are more informative to decision-makers, and some authors have developed more user-friendly statistical graphics.

In the L'Abbé scatter plots [L'Abbe, KA, *et al.* 1987], the proportions of cured patients with the experimental intervention are plotted (Y axis) against the cure rate for the comparator drugs (X axis). Thus, each point on the graph

represents one trial. Differences in favour of either intervention appear as a point laying on either side of the line of equality which divides diagonally the graph area into an upper and a lower triangle. A point on the line of equality means the efficacies are the same. L'Abbé plots allow visualizing the absolute and relative efficacies of treatment regimens; the distribution of results also indicates the degree of agreement or disagreement (heterogeneity) between trials. Moore *et al.* [Moore, RA, *et al.* 1998] use the true underlying control event rate (CER) and experimental event rate (EER) obtained by simulations instead of the raw efficacies; others weigh the points of the l'Abbé plot with the sample size ("bubble diagrams"). However, despite being easy to prepare (they can be derived graphically in Excel® and similar non-commercial softwares, and are also provided for in statistical packages like Stata®) and to understand by non-specialists [Ferrer, RL 1998], L'Abbé plots are not particularly popular in meta-analysis papers. Reasons are that they do not quantify (i) the difference in estimates between the test and the control treatment and (ii) heterogeneity.

Heterogeneity

Heterogeneity is almost inevitable when pooling retrospectively data from different trials and is a general concern in meta-analysis especially those dealing with aggregate data. Heterogeneity occurs when the variability in effect size estimates exceeds the variability expected from sampling error - i.e. there are real differences between studies [Sutton, AJ, *et al.* 2008]. In the particular case of malaria treatment studies, heterogeneity is common given that parasite susceptibility may vary with space and time. Heterogeneity can be visually identified on forest and L'Abbé plots [L'Abbe, KA, *et al.* 1987], but assessing its the clinical and statistical significance is more difficult. The Cochran Q test [Higgins, JP, *et al.* 2003] is often used but has low power, and it is generally recommended to further explore heterogeneity using additional methods. The I^2 is derived from the Q as [(Q-df)/Q]*100 and has the advantage of creating categories (0-40% = may not be important; 30-60% = may represent moderate heterogeneity; 50-90% substantial; 75-100% considerable) but it should be noted that categories do overlap [Higgins, J, *et al.* 2005].

Purpose of the paper

This paper concerns alternative ways of expressing meta-analysis results so that they can easily be understood while capturing the necessary information for decision-making. Specifically, we wanted to (i) generate graphical displays of comparative trials combining both the absolute (e.g. treatment success or failure rates [FRs]) and the relative size of the effect; and (ii) investigate ways of expressing heterogeneity. In either case, there was also a desire that the analyses be easy to generate (needing simple and possibly non-proprietary software and relatively modest statistical knowledge) and to interpret.

METHODS

Malaria was used as case-study. Malaria treatment guidelines are issued by the World Health Organization (WHO) based on evidence generated whenever possible through systematic reviews and meta-analysis {WHO, 2010} and the Cochrane Collaboration has produced various systematic reviews of antimalarial trials [Bukirwa and Orton, 2005; Eisenhut and Omari, 2009; Hwang *et al.*, 2006 ; McIntosh and Jones, 2005; Nakato *et al.*, 2007; Obonyo *et al.*, 2007; Omari *et al.*, 2005; Omari *et al.*, 2006; Osei-Akoto *et al.*, 2005; Praygod *et al.*, 2008; Sinclair *et al.*, 2009; ter Kuile *et al.*, 2007]. A specific WHO recommendation is that antimalarials should have a minimum efficacy of 90% (i.e. a FR of no more than 10%) after discounting re-infections. Decision makers in the concerned countries should refer to this cut-off to decide whether they can use a given regimen for treating malaria locally [Olumese, 2006].

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Database

We used the data collected through a systematic review of clinical trials of the treatment of uncomplicated falciparum malaria with the antimalarial combination artesunate plus amodiaquine (AS-AQ), which identified 35 comparative studies enrolling 6,551 patients on AS-AQ and 10,101 on comparator drugs (unpublished). The objective of the meta-analysis was to evaluate the efficacy of AS-AQ versus other treatments by using the FRs as the study endpoint. For ease we use here day 28 PCR-corrected efficacy results -i.e. the FRs after genotyping recurrent isolates (those emerging after initial clearance) to identify re-infections from recrudescences (true failures) - as currently recommended by the WHO [WHO, 2010].

In addition, 6 different scenarios were simulated for 20 hypothetical studies in terms of a test and reference drug FRs and risk differences (RDs):

1. The test drug is effective enough (FR <10%) and both the test and reference drugs are stable across all studies
2. The test drug is effective enough (FR <10%) and stable across all studies; the FR in the reference drug increases over time - the RD decreases.
3. The FR in the test drug increases, whereas the FR in the reference drug remains stable over time - the RD also increases.
4. The FRs in both the test and reference drug increase - the RD tends to increase
5. The FR in the reference drug increases, whereas the FR in the test drug remains stable - the RD decreases.
6. The FR in the test drug increases, whereas the FR in the reference drug remains stable - the RD also increases.

Statistical methods

For each and every individual study the FR and the number of patients treated for each treatment arm were used. The standard errors (SE) of each FR was calculated as $SE_{FR} = \frac{\sqrt{FR \times (1 - FR)}}{\sqrt{n}}$

A weight was derived from the standard error as $w_{FR} = \frac{1}{SE_{FR}}$.

A pooled, meta-analytic, estimate of the FR (PFR) for each treatment could then be calculated as $PFR = \frac{\sum_{i=1}^{k} (w_{FRi} \times FR_i)}{\sum_{i=1}^{k} w_{FRi}}$. An artificial FR = 0.01 was used to allow for inclusion in the calculations of studies with FR = 0 (100% efficacy, no failures).

To evaluate the RD between ASAQ and each comparator, the standard error was first calculated by using the standard error of the FR of ASAQ and each comparator: $SE_{RD} = \sqrt{SE^2_{FRasaq} + SE^2_{FRcomp}}$ and the associated weight $w_{RD} = \frac{1}{SE_{RD}}$.

Then the RD for each comparison of ASAQ and one comparator was $RD = FR_{ASAQ} - FR_{COMP}$ and the pooled meta-analytic RD: $PRD = \frac{\sum_{i=1}^{k} (w_{RDi} \times RD_i)}{\sum_{i=1}^{k} w_{RDi}}$

The RD is also referred to by some as the absolute risk reduction to avoid confusion with the relative risk reduction which is obtained from 1 – the relative risk (RR).

Graphic Display

FR (y axis) with 95% CIs for each study are plotted against the RD for each comparative study. An RD = 0 means no difference between the test and the control treatment. In this presentation, the size of the point estimate for each study is proportional to the sample size to reflect statistical precision of the estimate.

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The graph defines four quadrants (see **Figure 1**). The two below a predetermined threshold (here 10% failure, meaning an efficacy rate of 90% for PCR-adjusted Day 28 outcome) identify the studies where the test regimen is of interest. Results to the left of the line of no-difference (RD < 0, bottom left quadrant) indicate that the test drug did better than the comparator and vice-versa (e.g. an RD of - 0.10 means the test drug is 10% better than the comparator in a particular study.)

Here, we analysed 33 studies with day 28 PCR-corrected outcomes enrolling 6,299 ASAQ patients and 9,600 patients on comparator regimens (details in **Table 1.**) For ease, the individual study comparisons are presented in three graphs (one each for single-agent, non ACT and ACT comparators, Figure 1b-d). The aggregated estimates of failures rates and RD for each comparator were plotted on a separate graph (**Figure 2**).

A colour code was assigned to the four quadrants and the number of sites falling in each categories presented graphically on a map (size of the symbol proportional to the size of the trial) (**Figure 1**).

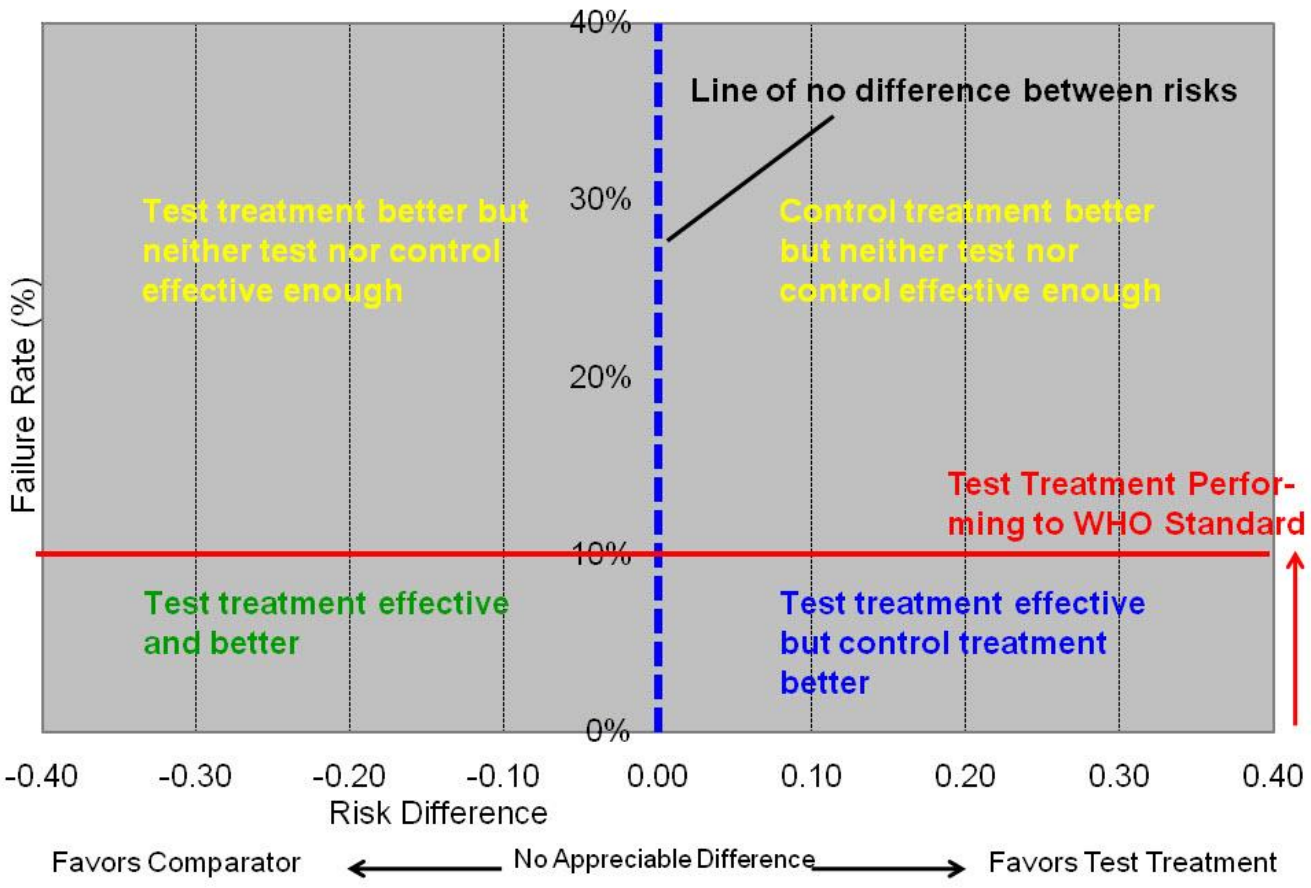

Figure 1. How to interpret the plot, a graphical display of FR against RD - the horizontal line = 10% FR (90% efficacy); the dotted vertical line = no difference (RD = 0). The horizontal line can be moved to reflect the desired failure/efficacy rate for each disease or condition.

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Heterogeneity

Heterogeneity was explored by using two alternative approaches:

(i) The traditional Higgins & Thompson's I^2; [Higgins, JP, *et al.* 2002] which was calculated for each pooled estimate of FRs and RD.

The Cochran Q can be estimated for the FRs by $Q_{FR} = \sum_{i=1}^{k} w_{FRi} \times (FR_i - PFR)^2$.

The Cochran Q can also be estimated for the RDs by $Q_{RD} = \sum_{i=1}^{k} w_{RDi} \times (RD_i - PRD)^2$

The I^2 is then evaluated from each Cochran Q as $I^2 = \frac{(Q - df)}{Q}$

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Table 1. Summary Day 28 PCR-adjusted outcomes and pooled estimates by comparator drug
Note that, since some studies had >2 arms, the number of AS-AQ patients should not be added as they may be counted twice.

	Product	Studies (n)	AS-AQ			Comparator			RD	95%CI	
			Events (n)	Patients (n)	FR	Events (n)	Patients(n)	FR			
Single-agent	Chloroquine	5	26	529	4,9%	210	382	55,0%	-0,48	-0,65	-0,31
	AQ	11	106	1367	7,8%	283	1296	21,8%	-0,13	-0,20	-0,05
	Sulfadoxine/pyrimethamine	7	37	639	5,8%	93	675	13,8%	-0,02	-0,05	0,01
	AS	4	2	193	1,0%	21	156	13,5%	-0,13	-0,49	0,23
	All	27	121	1663	7,3%	607	2509	24,2%	-0,13	-0,20	-0,06
Non-artemisinin combination	Chloroquine+ sulfadoxine/pyrimethamine	5	59	650	9,1%	260	535	48,6%	-0,5	-0,68	-0,32
	AQ + sulfadoxine/pyrimethamine	8	111	1375	8,1%	247	1424	17,3%	-0,1	-0,16	-0,04
	All	13	115	1506	7,6%	507	1959	25,9%	-0,17	-0,27	-0,07
Artemisinin-containing combination	AS + sulfadoxine/pyrimethamine	14	42	912	4,6%	92	1010	9,1%	-0,03	-0,07	0,00
	AS+mefloquine	1	0	151	0,0%	1	143	0,7%	-0,01	-0,02	0,01
	Artemether/lumefantrine 4 days	1	0	151	0,0%	5	117	4,3%	-0,01	-0,07	0,05
	Artemether+lumefantrine 6 days	14	74	1521	4,9%	45	1467	3,1%	0,01	0,00	0,01
	Diydroartemisinin/piperaquine	2	25	295	8,5%	14	317	4,4%	0,06	-0,04	0,15
	All	32	138	2659	5,2%	157	3054	5,1%	-0,02	-0,03	-0,01
All Treatments		72	289	4897	5,9%	1271	7522	16,9%	-0,07	-0,10	-0,04

(ii) The surface area defined by the individual FR and RD of each and every comparison for each comparator drug. This area can be defined as the area of an irregular polygon [Beyer, WH 1967]. The (signed) area of a planar non self-intersecting polygon with vertices (x1 + y1),…,(xn + yn) is $A = \left(\begin{vmatrix} x_1 & x_2 \\ y_1 & y_2 \end{vmatrix} + \begin{vmatrix} x_2 & x_3 \\ y_2 & y_3 \end{vmatrix} + \ldots \begin{vmatrix} x_n & x_1 \\ y_n & 1 \end{vmatrix} \right)$ which can be written as $A = \frac{1}{2} \sum_{i=1}^{n-1} \left(x_i y_{i+1} - x_{i+1} y_1 \right)$. The area of a convex polygon is defined to be positive if the points are arranged in a counter-clockwise order [Weisstein, EW 2009]. The formula was adapted to the coordinates defined by the FRs and the RD: $A = \frac{1}{2} \sum_{i=1}^{n-1} \left(RD_i FR_{i+1} - RD_{i+1} FR_1 \right)$.

Software

Data (for each individual study the FR and the number treated for each study arm) were keyed in an electronic spreadsheet, Microsoft Excel ®). Graphs were also generated using Excel®. We used Revman 5 [Higgins, J, *et al.* 2005] to calculate the RD and I^2.

RESULTS

The pooled estimates of the FRs of AS-AQ and the RDs of ASAQ against the comparators are presented in Table 1 and **Figure 3** by comparator drug and group of comparators (whether single-agent, non-artemisinin combinations or artemisinin-based combinations (ACTs)). Figure 1 explains how to interpret the plots. Figure 2 displays the results in terms of ASAQ absolute FRs and absolute difference between treatment arms by comparator type (Figure 2 a1, b1, c1), as well as heterogeneity (Figure 2 a2, b2, c2). Precision (1 – variance) is displayed by the size of the bubble for each individual study in Figure 2 a1, b1, c1.

For individual studies, the failures rates of ASAQ were generally below the 10% limit of efficacy recommended by the WHO, and in the bottom-left quadrant (negative RD), i.e. favouring ASAQ over monotherapies, and non-ACT combinations, except for some of the studies vs. another ACT (here AS-AQ failed also in >10% of cases in four studies). The pooled estimates favoured ASAQ over monotherapies and non-ACT combinations; the RD favoured slightly ASAQ over AS+SP, AS+MQ and AM+LF4 doses, and slightly AM+LF6 doses and DH+PQ over ASAQ (see Figure 3, Table 1).

Higgins & Thompson's I^2 were calculated for each comparator type for both the absolute FR of AS-AQ and the RD (**Table 2**) and plotted (**Figure 4**) along with those for the virtual studies. This scatter diagram has the I^2 for pooled estimate of the absolute FR plotted against that of the RD; the plot area defines four quadrants with studies with low heterogeneity lying in the bottom-left quadrant (I^2 <50% on both parameters). The studies from the AS-AQ database showed high heterogeneity for both FRs and RD, that was caused mainly by analysing individual studies by study site within each study [Olliaro, P, *et al.* 2009]; this results in the points in this graph being all in the top right-hand quadrant. The database of virtual studies included in conditions with variable heterogeneity, hence scattered across all quadrants (Figure 4).

The surface area defined by the points of each individual comparison was higher in the single agent comparisons, especially for SP, as well as for the non-artemisinin combination AQ+SP; the ACT comparators had a lower surface area than the two other comparator types (Table 2 and Figure 2).

The virtual study database is provided as supplementary information for the purpose of a sensitivity analysis. The FRs of AS-AQ and the RDs of AS-AQ against the comparators were calculated for the different scenarios referred to in the Methods section. The pooled estimates were then evaluated and plots drawn for each scenario (Figure 3). FRs were limited to 20% to confine RDs. The calculated areas were lower

than those for the real studies. Overall area estimations were low when the I^2 were low for the FRs and the RD and vice-versa.

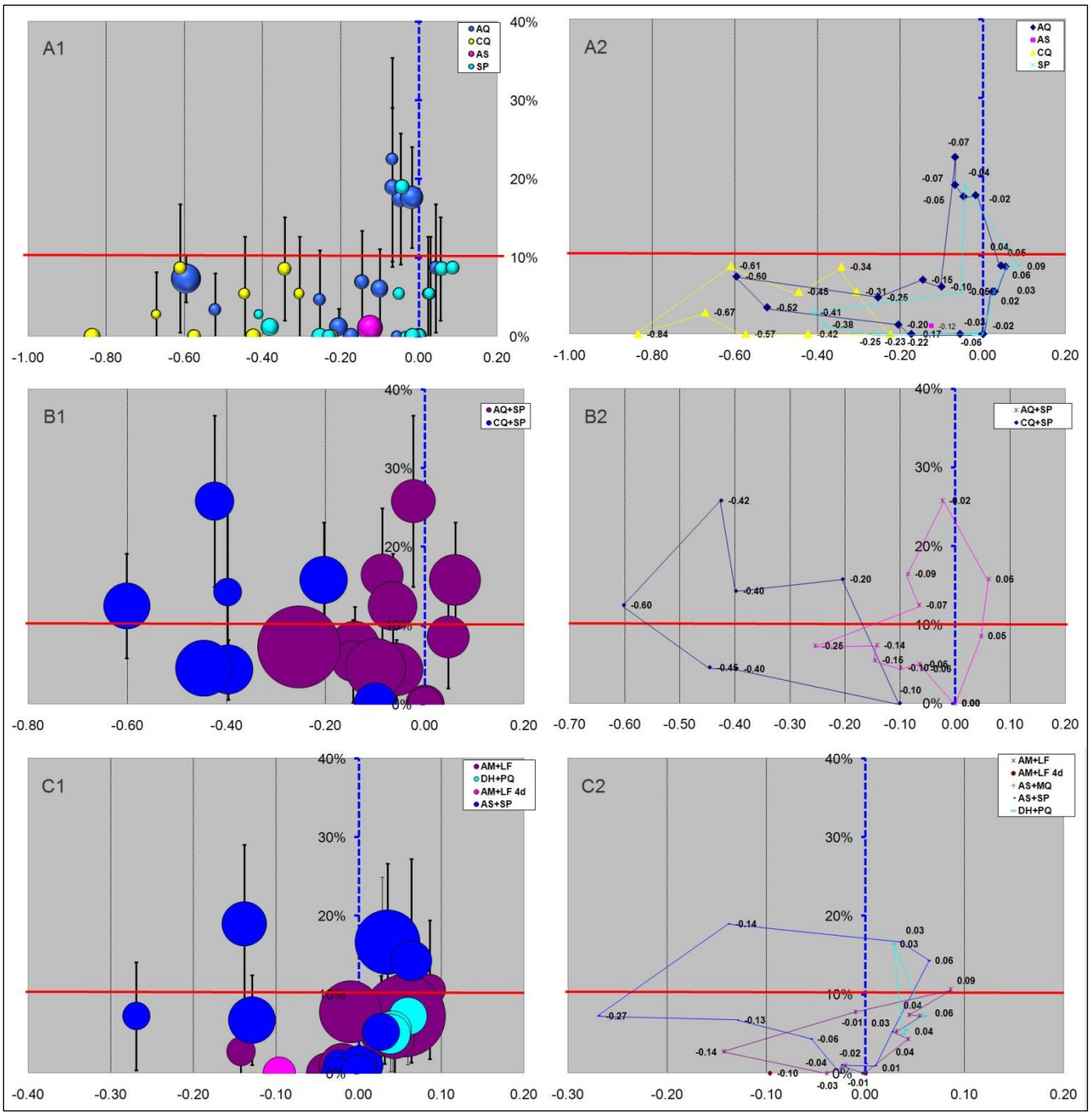

Figure 2. Day 28 PCR-adjusted FRs against RD ratios.

a = monotherapies, b = non-artemisinin combinations, c = ACTs. a1, b1, c1 = FRs with 95% CIs for AS-AQ. Note: Bubble size proportional to the sample size of the study. a2, b2, c2 = surface area of the spread of the plot by comparator drug - area defined by the coordinates of each point of a given comparator. Note: Diamonds: AQ=red, SP=pink, non-ACT. Triangles: AQ+SP=orange, CQ+SP=yellow). Squares=ACTs: AS+SP=dark green, AM+LF=dark blue, DH+PQ=purple.

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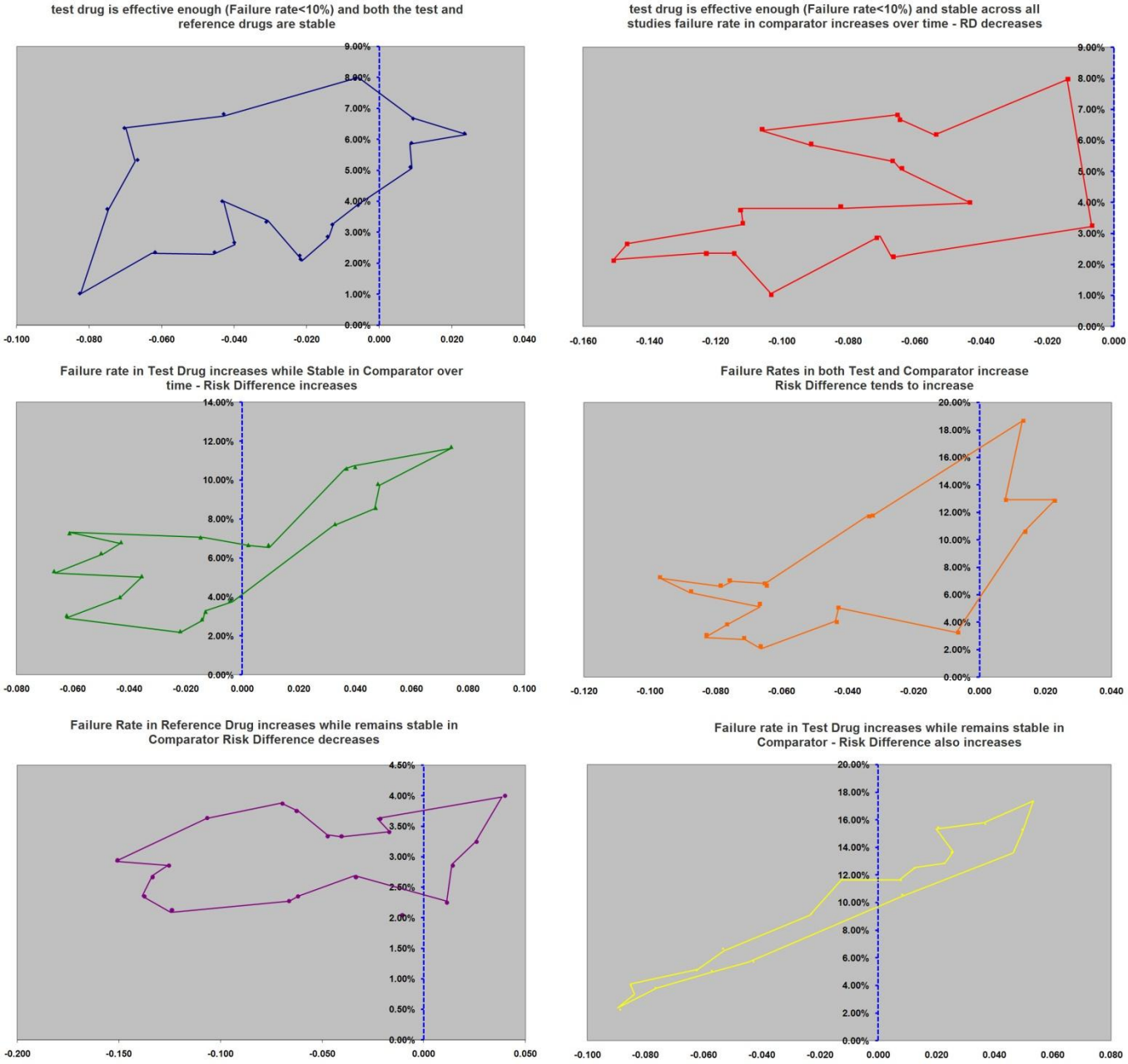

Figure 3. Virtual studies: surface area of the spread of the OVplot

DISCUSSION

Combining different types of presentations offers advantages in summarising clinical trial results de-rived from meta-analysis, and can help provide the information needed to support policy decision. This information can be presented in both graphical and numerical forms and have incremental levels of complexity [Bax, L, *et al.* 2009].

We propose here an alternative format for comparative trials combining absolute treatment effects and RD relative to the comparator treatment.

These analyses use simple, undemanding software and require a level of computer literacy which is within the reach of the unskilled user.

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Table 2. Heterogeneity - I^2 and surface area

		I^2		
		FRs	**RD**	**Area**
Comparator treatment in clinical trial dataset				
Single-agent	Chloroquine	88%	91%	14,33
	AQ	84%	96%	15,21
	Sulfadoxine/pyrimethamine	85%	91%	43,87
	AS	NA	NA	NA
Non-artemisinin combination	Chloroquine+ sulfadoxine/pyrimethamine	91%	91%	21,81
	AQ + sulfadoxine/pyrimethamine	90%	90%	11,65
Artemisinin-containing combination	AS + sulfadoxine/pyrimethamine	77%	78%	7,97
	AS + mefloquine	0%	0%	0,00
	Artemether/lumefantrine 4 days	0%	69%	0,00
	Artemether+lumefantrine 6 days	90%	77%	13,01
	Diydroartemisinin/piperaquine	48%	0%	0,53
Virtual studies	1 Test effective; both Test & Control stable	19%	10%	1,19
	2 Test effective & stable; Comparator failures increase	19%	35%	1,48
	3 Test failures increase; Comparator stable; RD increases	39%	38%	1,29
	4 Both Test & Comparator failures increase	64%	5%	2,76
	5 Test failures increase; Comparator stable; RD decreases	0%	80%	0,84
	6 Test failures increase; Comparator stable; RD increases	73%	41%	0,96

Although we used for convenience Microsoft Excel®, other non-proprietary products are available for free downloading (e.g., Gnumeric available at http://projects.gnome.org/gnumeric/ or open Office calc available at http://www.openoffice.org/). Revman is a free-download software of the Cochrane collaboration (http://www.cc-ims.net/RevMan) and can also be used for more refined analyses including options for fixed or random effect models, choices between various methods of deriving ORs and RRs, as well as for summary aggregate statistics. Calculations of exact binomial CIs were not added to the method because they are not straightforward to obtain in Microsoft Excel®. However, an add-in (confint.xla), available on http://statpages.org/confint.html, includes the calculation of binomial CIs.
The plot could include CIs for the RD for completeness; here, we decided not to display it, as it would make the graph too cumbersome.

Common effect measures for trials with dichotomous outcomes are risk ratio (RR), odds ratio (OR) and absolute RD. The RD [The Cochrane Collaboration 2005] is the difference between the proportions of individuals with an outcome (here treatment failure) in the two groups. It can be calculated for any trial, is straightforward to interpret and is considered by some to provide more complete information than relative measures [Sackett, DL, *et al.* 1997]. However, when using the RD one should be cognizant of its limitations: (i) its clinical importance may

depend on the underlying risk of events (i.e. the same RD may be proportionally larger or smaller depending on how frequent the event is); (ii) relative effect measures, in general, are considered more consistent than absolute measures [Deeks, JJ 2002, Engels, EA, *et al.* 2000]; (iii) because it measures differences between cure rates, similar values are obtained for widely different FRs (e.g. 20 vs 10% and 50 vs 60%). This limitation is remedied by having it plotted against the absolute value (the FR of the test treatment).

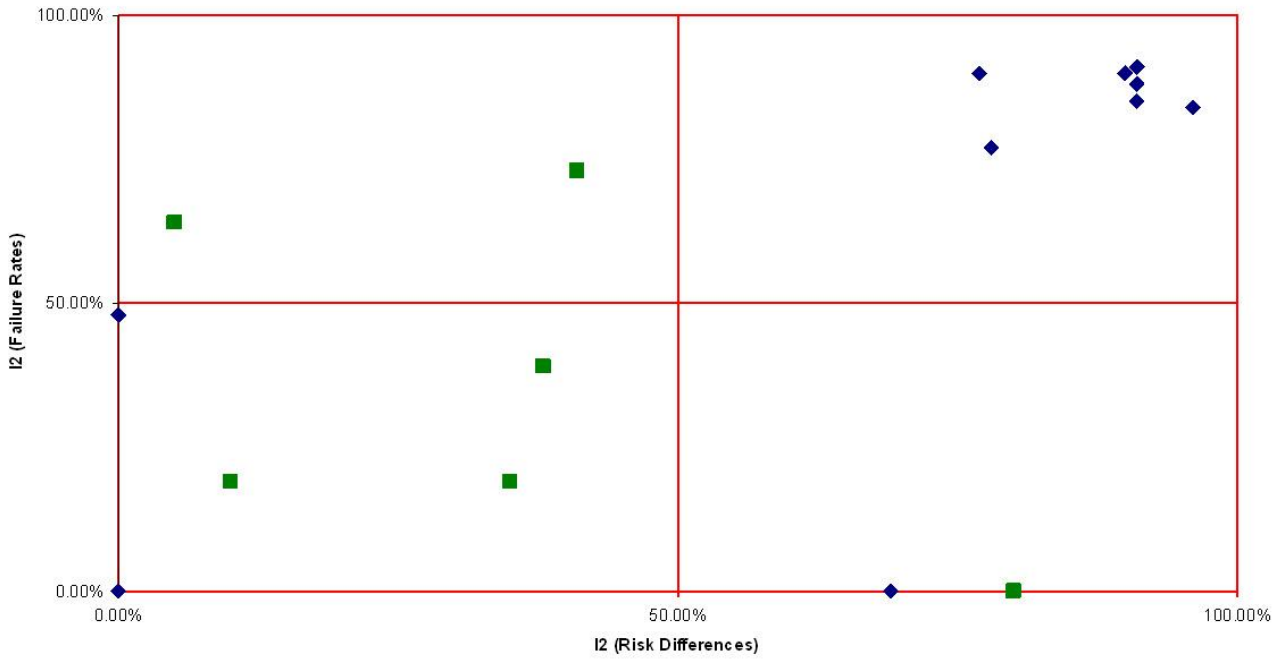

Figure 4. I^2 of RDs versus I^2 of FRs for studies from the ASAQ meta-analysis database (◊) and the virtual studies (□)

Presenting data as both absolute effect and RD of the outcome of interest has practical advantages: (i) both are straightforward and intuitive; (ii) the absolute FR gives an indication of the value of an intervention (in a particular setting or on aggregate) and the confidence around it (hence indirectly also on variability and the sample size); (iii) it allows identifying interventions which meet criteria for a predetermined threshold e.g. a 10% PCR-corrected FR on Day 28, the latest WHO recommendation of minimum efficacy for an antimalarial treatment {WHO, 2006 #21} and (iv) it allows quantifying the added benefit vs. the comparator treatment. If a drug is effective and better than the others, it will show in the bottom left part of the graph - to the left of the no-difference (the 0) line and below the line of the pre-determined required level of failure (here 10%). If using success rates, the inverse applies: the results of interest are those appearing in the top right part of the graph - to the right of the no-difference (the 0) line and above the line of the pre-determined required level of efficacy, 90% in this case). The sample size of the study features as the size of the bubble, and gives an idea of precision (1 - variance). Quantifying the difference in terms of RD on the same graph allows also to weigh additional elements involved in the decision. For instance, if the level of efficacy of the test treatment meets requirements (e.g. it fails in <10% of cases), a marginal benefit

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attained with the comparator treatment (i.e. a positive RD in this case) may not be enough to justify a change in policy. Alternatively, even in case of a sizable advantage (high negative value of the RD), neither the test nor the control intervention are worthwhile if the efficacy is below the desired threshold (i.e. if both fail in >10% of cases). Similarly, issues like price and safety can be included to add another dimension to the decision making process.

This graphical display allows also visualizing and quantifying heterogeneity as the area of the polygon defined by a circuit where the corners are the individual study results. Here, heterogeneity combines both the absolute efficacy of the test drug and the difference with respect to its comparator. Hence, it is difficult to relate it to the Higgins & Thompson's I^2 of each individual measure. While our polygonal area is intuitively proportional to the two-dimension heterogeneity, it has not been tested on a sufficiently large and representative database to gather information on possible breakpoints that would define the size of heterogeneity (as is the case for the I^2). Another potential limitation of this approach is that the polygon was drawn by visual inspection in such a way to obtain the largest surface area (i.e. worse case scenario for heterogeneity; a brief tutorial is provided in the supplementary file). Indeed several polygons could be drawn from a set of points. However the demonstration that a unique estimate of heterogeneity could be carried out was not the primary objective of this work. We are evaluating different approaches which would allow a unique estimate of heterogeneity reproducibly. While perfectible, the polygon areas are estimates of heterogeneity complementing the individual I^2 of FRs and RDs with the combined values for both.

Malaria treatment is a difficult case with regards to heterogeneity. Treatment response is highly dependent on characteristics which may vary with time and geographical location. Typically parasite sensitivity to a given treatment changes under drug pressure, so that efficacy is eroded (FRs in-crease) over time, but this process may evolve differently in different places due to multiple concurring factors. In the case of closely related drugs, resistance acquired to one drug may extend to the next one (cross-resistance). Treatment response depends also upon patient immunity, which develops with age and repeat encounters between the host's immune system and the parasite, and is thus conditioned by the level (intensity) of transmission. This dependency is reflected in marked differences in treatment responses that may be found between children and adults in areas of intense transmission, while they are equivalent in areas of low transmission and in travelers. Like parasite sensitivity, also exposure to infection can change over time; for instance, declining transmission intensity (e.g. as a result of control measures) means immunity against malaria will be acquired later in life, if ever.

Together, these issues make it problematic to generate general recommendations on malaria treatment based on meta-analyses [Garner, P, *et al.* 2009]. The ideal drug is highly efficacious in all ar-eas and age groups (i.e. all parasites are susceptible and cleared with no need for immunity). However, there may be substantial heterogeneity in the relative effects (OR, RR or RD) if different comparator drugs have variable efficacies. By exploring also the variability of the absolute effect of our drug we will acquire an important element in explaining heterogeneity - and the relevance thereof. In the case of our ideal drug recently introduced, FRs will homogenously be low. If we now followed the drug over time through the combined measures of both absolute and relative effects and their respective heterogeneities, it will be possible to monitor if the situation remains stable or changes with time and have elements to interpret these findings.

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We are living through an exciting time with new malaria drugs becoming available; while this holds promises of improved malaria control and hopes of elimination, it poses also significant challenges, including the choice of antimalarial treatments that can be recommended generally and are adapted to local conditions. In order to generate evidence-based recommendations, the WHO commissioned a Cochrane meta-analysis of current ACTs [Sinclair, D, *et al.,* 2009] which included the visual display presented here {Sinclair, 2010 #109}.

Disclaimer

The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the WHO or CRP-Santé.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio2010.08.004.

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Efficacy and safety of artesunate plus amodiaquine in routine use for the treatment of uncomplicated malaria in Casamance, southern Senegal

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Abstract

Background. There are no data on the long term use of an artemisinin combination treatment in moderate or high transmission areas of Africa.

Methods and Findings. Artesunate plus amodiaquine (AS-AQ) was used to treat slide-proven *Plasmodium falciparum*-infected patients of all ages in the Oussouye district, Casamance, Senegal, over a period of six years (2000 to 2005). Efficacy, by Kaplan Meier survival analysis (n=966), and safety (adverse event rates, n=752) were determined over 28 days. A weight-based dosing regimen was used for the loose tablets during 2000-2003 (n=731) and a commercially available co-blister was used during 2004-2005 (n=235).

Annual crude (non PCR corrected) cure rates remained stable over the study period [range 88.5-96.7%; overall 94.6 (95% CI 92.9-95.9)]. Nine co-blister treated patients (0.9%) withdrew because of drug-related adverse events; seven had gastrointestinal complaints of whom two were hospitalized for vomiting. By Day 28, the mean total bilirubin (n=72), AST (n=94) and ALT (n=95) values decreased. Three patients had Day 28 AST/ALT values > 40 < 200 IU/L. Changes in white cell counts were unremarkable (n=87).

Conclusion. AS-AQ in combination was highly efficacious and well-tolerated in this area and justifies the decision to use it as first line treatment. Long-term monitoring of safety and efficacy should continue.

BACKGROUND

Artemisinin-containing combination therapies (ACTs) are now being deployed in some 42 malaria endemic countries and a further 26 have agreed to adopt ACTs following the World Health Organization (WHO) recommendation that ACTs should be the first line drugs for treating uncomplicated falciparum malaria (data provided by WHO/GMP, February 2007).

Artesunate plus amodiaquine (AS-AQ) is one of the currently available ACTs and is in use in Indonesia and 18 African countries (Burundi, Cameroon, Congo, Côte d'Ivoire, Democratic Republic of Congo, Equatorial Guinea, Gabon, Ghana, Guinea, Liberia, Madagascar, Malawi, Mauritania, Senegal, Sao Tome & Principe, Sierra Leone, Sudan (South), Zanzibar).

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Because the WHO policy change is recent, there is, to date, little experience with the systematic use of these drugs in malaria endemic countries. The most reliable data regarding systematic use comes from the low transmission areas of the Thai Burmese border where artesunate plus mefloquine has been in continuous use since the early 1990s, well before the WHO recommendation. This combination has consistently produced high cure rates, achieved a reduction in the transmission of *Plasmodium falciparum* while the trend of increasing *in vitro* mefloquine resistance has been reversed [1, 2]. Similar results have been reported with the deployment of artemether/lumefantrine (Coartem®) in another low-transmission area on the South Africa-Mozambique border, where the malaria burden has fallen and where there has been a reduction in morbidity and mortality [3].

By contrast, there are no reported data on the long term use of ACTs from areas of higher malaria transmission. Having this information is important because areas of moderate and high transmission account for most of the global malaria burden and experience from areas of low transmission may not necessarily be applied to higher transmission settings [4]. In addition, the useful therapeutic life spans of the ACTs may vary with transmission intensity and with such factors like cost, compliance and treatment seeking behaviour.

This paper reports on the safety and efficacy of AS-AQ in the chloroquine-resistant, Oussouye district of southern Casamance, Senegal, during 2000-2005 for treating patients with parasitologically confirmed falciparum malaria.

METHODS

Study site characteristics

This study was conducted at the outpatient clinics of four dispensaries (Mlomp, Oussouye, Kabrousse and Djembereng) all situated in the District of Oussouye, southern Casamance. The total population of the district is circa 70,000, mostly farmers. Malaria transmission is perennial and mesoendemic with an increase in cases during the rainy season (July to December). The entomological inoculation rate is 25 infected bites per person-year [5].

In this area, the rate of chloroquine-resistant strains has remained stable at around 66% between 1997 and 2004 [6]. Despite this high rate, trials over the past decade have shown that AQ alone or combined with AS are efficacious [7-9].

Study methodology

This was a non-comparative assessment of the efficacy and safety of AS plus AQ conducted over 28 days. Potentially eligible patients of all ages who attended the clinic with either a history of fever or a confirmed fever (measured axillary temperature ≥ 37.5°C) and a positive Giemsa-stained thick film for *P. falciparum* were briefed about the study and those who gave their written informed consent were registered and their houses were mapped. Entry criteria included: weight >5kg; male and non-pregnant or breast-feeding female; living in the study area (for ease of follow-up); having given informed consent to participate; fever or history of fever; falciparum parasitaemia 1,000-200,000 parasites/μL; no antimalarial drug intake in the previous week; able to take oral drugs; no signs/symptoms of severe malaria; no major intercurrent illness or history of cardiac, hepatic or renal disorder; no known allergy to study drugs. The study was approved by the Senegalese National Ethical Committee.

All eligible patients were treated with AS + AQ. Initially (2000-2001), the use of AS-AQ was restricted to the rainy season, and later

extended (2002-2005) to all year round. During 2000-2003, loose tablets were used in combination and patients were dosed by body weight. Subsequently a blister pack containing both drugs became available during 2004-2005 and patients were dosed by age (except 30 subjects in 2004 who were dosed by weight). The drugs used were:

- loose combination: Arsumax® tablets (Sanofi-Aventis) containing 50 mg of AS and Camoquin® tablets (Parke-Davis) containing 200 mg of AQ base tablets. The doses used were 4 mg/kg/day (AS) and 10 mg/kg/day (AQ) both for three days.

- the blister pack: Arsumax® tablets (Sanofi-Aventis) containing 50 mg of AS and AQ tablets (Sanofi-Aventis) containing 153 mg of AQ base. There were three dosing blisters and both drugs were administered following the manufacturer's instructions: (i) children below one year of age = ½ tablet of each drug; (ii) children between one and six years of age = one tablet of each drug; (iii) children above six and below 14 years of age = two tablets of each drug, and (iv) above 14 years of age = four tablets of each drug.

All treatments were administered under supervision in the clinic, except in 2005 when only the first dose was supervised and patients were instructed to continue their treatment at home. Patients were seen on Days 0-3 inclusive during 2000-04 (Days 0, 2 and 3 in 2005), and then on Days 7, 14, 21, 28 or in between, as needed. Non attendees for scheduled clinic visits were actively sought by community health workers. Giemsa-stained thick films were read by trained microscopists and confirmed by one of the investigators (PB).

Study end points

The end point for efficacy was the Day 28 crude cure rate calculated using Kaplan-Meier survival analysis [10] on the Intent-to-Treat (ITT) dataset (all patients who entered the study) for the whole period under study and by year; the log rank test was used to test for significance between years. Success was defined as parasite clearance that was sustained through Day 28. Failure to clear parasites and recurrent parasitaemia were considered as failures. Genotyping parasites was not done to distinguish between recrudescences and reinfections. All study withdrawals due to adverse events, irrespective of their relationship to study drug, were considered as failures. All patients lost to follow up were censored on the date they were last seen. Parasitological failures were rescued with quinine.

Safety was assessed by: (i) recording treatment emergent sign/symptom (TESS, i.e. events which were not present pre-treatment or worsened with treatment) and (ii) measuring liver (alanine, ALT and aspartic, AST transaminases and bilirubin), renal functions (creatinine) (KONELAB 60I analyser, Konelab, Finland) and haematology (haematocrit, WBC total counts), aiming for about 30% of the patients enrolled to have at least one baseline and one post-treatment sample. The common toxicity criteria for adverse events (CTCAE Version 3.0 08/09/2006) were used to evaluate and grade the severity of clinical events and laboratory measurements. Shift tables were done to show changes in severity of CTC grades between Day 0 and Day 28.

Data analyses

Data were recorded in a case record form comprising demography, parasite counts, signs and symptoms, laboratory data and adverse events at each scheduled visit. They were double keyed in Excel® using an end-

user formatted sheet with online edit-checks.

Descriptive statistics are presented as counts, percentages, means and standard deviations, as appropriate. One-way ANOVA was used to assess the comparability of the patients' baseline characteristics between years and sites. Continuous data were assessed for normality using the Kolmogorov-Smirnov test; if significant, data were log-transformed and analysed using the 't' test, if normally distributed. Otherwise the Mann-Witney U test was used for paired comparisons. Homogeneity of variance was assessed with the Bartlett test. The Welch adjusted ANOVA was carried out if variances were unequal. Between groups comparisons of continuous data were further investigated with the Tuckey-Kramer post hoc test using means (normally distributed data) or ranks (skewed data). Dichotomous variables were analysed using chi-squared or Fisher's exact tests (Freeman-Halton if more than two categories) test if the expected counts were lower than five in any cell.

Kaplan-Meier survival analysis was performed to evaluate cure rates: (i) for all the years combined, (ii) between years, and (iii) between the loose and the co-blistered products [10]. The log rank test was used to test for homogeneity between survival curves of each year and the loose and the co-blistered products. A Cox proportional hazard model of the probability of failure was done to test for the contribution of the year of treatment and products. A descending stepwise manual modelling strategy based on the likelihood ratio test between subsequent models was carried out, beginning with a saturated model containing all factors.

A p value of <0.05 was considered statistically significant. All tests were two-tailed. Statistical analyses were conducted with the statistical package SAS version 9.3.1 (SAS Institute, Cary, NC, USA)

Because the dosing of AS and AQ changed during the study, the doses of AS and AQ taken by patients with the loose and the blister combinations are presented in relation to two dosing schedules: (i) the recommended weight based dosing of 4 mg/kg/d x 3d for AS and 10 mg/kg/d x 3d for AQ base, and (ii) the new age based dosing regimen with newly defined therapeutic windows of 2-10 and 7.5-15 mg/kg/d, respectively [11].

RESULTS

Baseline characteristics

During 2000-2005, 966 patients were enrolled, of whom 723 in Mlomp (75%), 110 in Diembereng, 27 in Kabrousse and 106 in Oussouye. The loose combination was given to 731 patients during 2000-03 and the co-blistered product to 235 patients during 2004-05. The dose was calculated on body weight for 761 patients (731 treated with the loose and 30 with the co-blistered products) and on age for 205 (all co-blister). Treatment was given supervised to 810 patients and unsupervised to 156 (co-blistered product in 2005). **Table 1** shows the patients' baseline characteristics. Overall, for all the years combined, there were 30% more male than female patients, the mean age, weight and temperature values were 13.8 years, 33.3 kg and 38.4 °C. The geometric mean baseline parasitaemia was 31,850/μL. The mean (±SD) daily doses of AQ and AS for all the study years were 359 (±179) mg and 131 (±66) mg, respectively.

There were significant statistical differences for certain baseline parameters between most of the years. In particular, there was a difference in (i) body weight between 2000-

2004, 2001-2003, 2001-2004, (ii) AQ dose between 2000-2004, 2000-2005, 2001-2003, 2001-2004, 2001-2005, 2002-2004, 2002-2005, and (iii) AS dose between 2001-2004 and 2004-2005.

The age structure of the population treated is presented in Figure 1. Overall, 16% of patients were < 5 years of age, 30% were 6-10 years of age, 27% were 11-15 years of age, 11% were 16-20, 8% were 21-30 and 7% ≥ 30 years of age. Using these categories there was no difference between years and sites ($p > 0.05$). Just over half, 57% (n=556), of the patients were aged between 6-15 years; 46% (n=449) were under 11 years of age.

Table 1. Patient's baseline characteristics overall and by year of enrolment

	year	2000	2001	2002	2003	2004	2005	2000-05
Enrolled	N	214	302	117	98	79	156	966
Sex ratio	F/M	0.6	0.92	0.75	0.69	1.03	0.7	0.77
Age (years)	mean	12.8	12.9	14	16.8	15.7	14	13.8
	std	9.5	10.5	11.6	14.8	9.8	11.9	11.2
Body weight (kg)	mean	31.9	31	33.3	38	38.9	33.8	33.3
	std	15.8	16.3	18	20.4	16.6	17.8	17.3
Parasites/µL	geomean	32866	48098	24775	24394	39679	17408	31850
	std	3.7	3.6	4.8	4.7	3.4	4.7	4.2
Body temp. (^{o}C)	mean	38.1	38.7	38.6	38.6	37.8	38.3	38.4
	std	1.1	0.6	0.9	1	1.2	1.2	1
AQ dose (mg/d)	mean	333	320	351	414	437	399	359
	std	156	152	178	242	170	189	179
AS dose (mg/d)	mean	128	124	134	140	155	130	131
	std	64	65	71	73	59	62	66

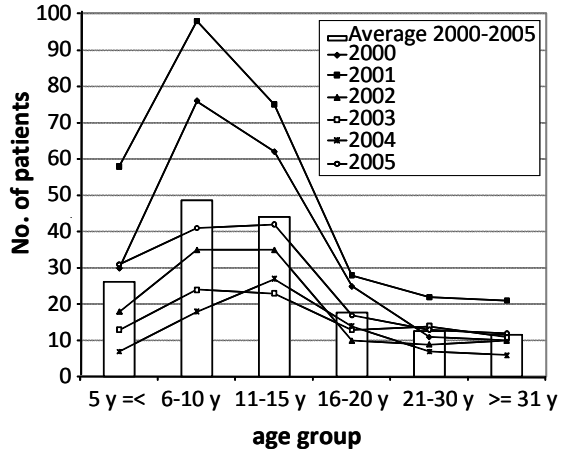

Figure 1. Age structure of the malaria cases treated by year of enrolment and mean over 2000-05

The doses of AS and AQ taken by patients compared with the weight- and age-based dosing regimens are shown in **Figure 2**. With both products used, doses were well within the newly defined, therapeutic windows for both drugs. For AS, doses with the loose product and the co-blistered product were similar and very close to the target dose of 4 mg/day; the co-blister mean doses were slightly lower with wider 95% CIs. For AQ, doses were higher with both products than the target dose of 10 mg/day with a tighter 95% CIs for the loose combination except for 11-15 years old.

Efficacy evaluation

The Kaplan-Meier estimates of the crude cure efficacy rate was 94.6 % (95% CI 93.0; 95.9) for all years combined. By individual years cure rates were 96.7% [93.2; 98.4] in 2000, 94.0% [90.6; 96.2] in 2001, 95.7% [90.0; 98.2] in 2002, 94.9% [88.2; 97.8] in

2003, 88.5% [79.0; 93.8] in 2004 and 95.9% [91.1; 98.1] in 2005. There were no differences (p=0.12) in cure rates between years by the log rank test for homogeneity over time (**Figure 3**).

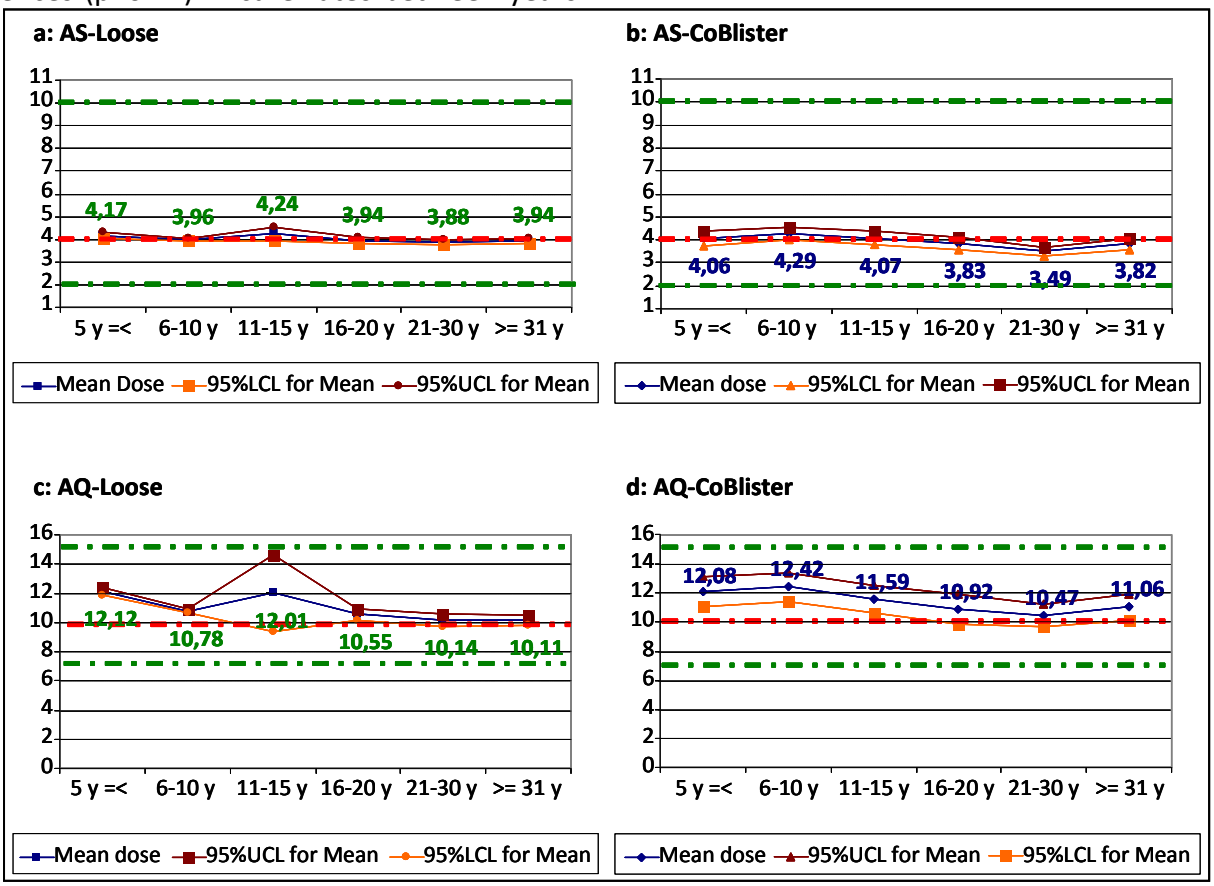

Figure 2. Mean (95% CI) doses of AS and AQ taken by patients treated with the weight based loose and aged based co-blistered drug regimens as a function of age
a=AS-Loose, b=AS-Blister, c=AQ-Loose, d=AQ-Blister

All patients cleared their parasites by Day 3 (no early treatment failure, ETF); 36 patients returned with parasites during follow-up (late treatment failures, LTF) and nine were withdrawn due to an adverse event (considered as failures in our analysis - see below); 32 were lost to follow-up (censored) (**Table 2**). All treatment failures were re-treated successfully with injectable quinine. All 36 LTFs occurred in patients under 16 years of age (20 in the age range 6-10). Efficacy was 95% in 0-10 years and 97% in 11 and above (log rank test, p=0.03). In 2005 the losses to follow up amounted to 15%, while they were ≤3% in the other years.

The Kaplan-Meier estimates of the crude cure efficacy rates were similar (p=0.14) between the loose (n=731) and the co-blistered (n=235) products: 95.1% (93.3;96.5) vs. 93.1% (88.8;95.8), respectively. This held true whether dosing was weight (n=761) or age (n=205) based: 94.8% (92.9;96.2) vs. 94.2% (89.7;96.7), respectively (p=0.51) and whether treatment was supervised (n=810) or unsupervised (n=156): 94.5% (92.7;95.9) vs. 95.9% (91.1;98.1), respectively (p=0.75).

Year of study and product used were non significant contributors to failure in a Cox proportional hazard model. Using a saturated model containing year, product, site,

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age, sex, weight, dose AS and dose AQ, the contribution to hazard of failure was border-line for year (p=0.06) but significant for the total daily dose of AS (p=0.004) for a hazard of failure of 0.993 [0.988; 0.998]. Only year 2004 was statistically different from the reference year 2000 (p=0.003): the hazard of failure was four times greater in 2004 than in 2000 (hazard ratio=4.496 95% CI = [1.661; 12.168]).

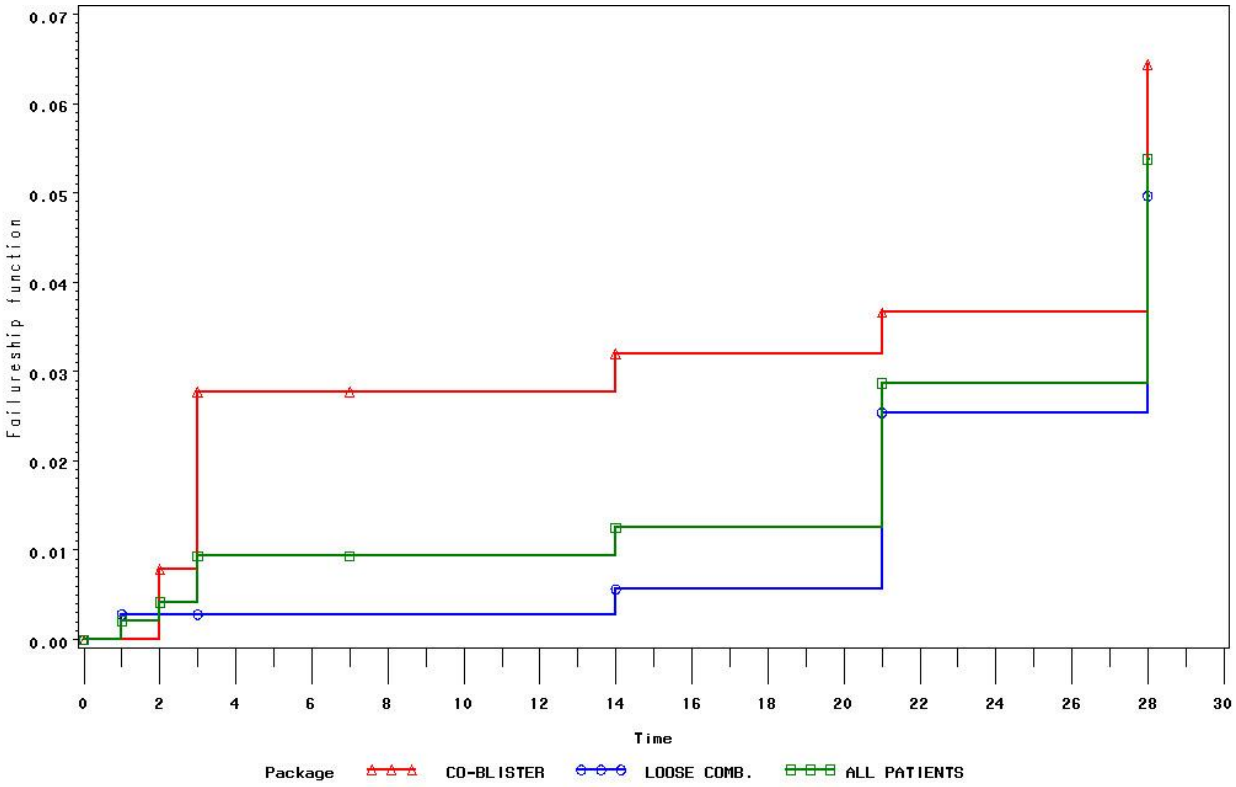

Figure 3. Kaplan-Meier of one minus survival curves to show cumulative parasitological failure rates overall (2000-05) and by year of treatment (all ages combined)

Table 2. Distribution of treatment failures and losses to follow-up by year of study

(a) by year	2000	2001	2002	2003	2004	2005	2000-05
KM estimate of success (95%CI)	**96.7 (93.2-98.4)**	**94 (90.6-96.2)**	**95.7 (90.0-98.2)**	**94.9 (88.2-97.8)**	**88.5 (79.0-93.8)**	**95.9 (91.1-98.1)**	**94.6 (93.0-95.9)**
LTF	7	17	2	4	5	1	36
withdrawn due to AE	0	0	0	0	4	5	9
lost to follow up	2	1	3	2	1	23	32

Treatment failures were late parasitological failures [LTF] and withdrawals in the Kaplan-Meier analysis. Losses to follow-up were censored in the KM analysis. See KM analysis in figure 3.

Safety evaluation

Complete safety records are available for 752 patients enrolled during 2001-2005. At presentation (Day 0), all patients reported fever or had a measured fever in the clinic. Other malaria associated symptoms/signs

on presentation were weakness [n=296 (29%)], headache [n=291 (29%)], vomiting [n=133 (13%)] and nausea [n=113 (11%)]. There were no significant differences in the frequency of symptoms/signs between the different age groups or sexes.

After treatment, 69 patients (7.1%) experienced at least one treatment emergent sign/symptom (TESS) which was either not present pre-treatment or worsened post-treatment: 54 patients suffered one TESS, 14 had two and 1 had three for a total of 85 TESSs: 36 were vomiting, 19 vertigo, 11 asthenia, 8 pruritus without a rash, 5 abdominal pain, 3 diarrhoea, 2 headaches and 1 nausea (**Table 3**). TESSs were more likely to be reported by the co-blister recipients: 54 (5.6%) vs. 15 (1.5%) (p<0.0001) and were independent of age. For the co-blister 39 patients suffered one TESS during the study, 14 suffered 2 TESSs and one suffered 3 TESSs; of patients treated with the loose combination, 15 suffered one TESSs (p<0.0001).

Table 3. Type, frequency and severity of Treatment Emergent Signs and Symptoms (TESS) and number of patients experiencing at least one episode

Symptom	Intensity				Total n(%) of TESS
	Mild	Moderate	Severe	Verysevere	
Abdominal pain		5 (10.2%)			5 (5.9%)
Asthenia		2 (4.1%)	9 (53%)		11(13%)
Diarrhoea		3 (6.1%)			3 (3.5%)
Headache		1 (2%)	1 (5.9%)		2 (2.4%)
Nausea				1 (33.3%)	1 (1.2%)
Pruritus		4 (8.2%)	3 (17.6 %)	1 (33.3%)	8 (9.4%)
Vertigo	4 (25.%)	13 (26.5%)	2 (11.8%)		19 (22.3%)
Vomiting	12 (75%)	21 (42.9%)	2 (11.8%)	1 (33.3%)	36 (42.3%)
Total	16 (18.9%)	49 (57.6%)	17 (20%)	3 (3.5%)	85 (100%)

Nine patients, all treated with the co-blistered product (two weight-based and seven age-based), were withdrawn from the study because of a TESS for an overall, crude withdrawal rate of 0.9%: 3.8% [(co-blister) vs. 0% (loose), p<0.0001 (**Table 4**)]. All events were considered probably related to AS-AQ except one case of vomiting (possibly related). In five such cases, the daily dose of AQ exceeded the target dose by 20% or more. Two patients with Grade 2 vomiting were admitted to hospital for intravenous quinine and symptomatic treatment; they recovered well. These hospitalizations define these AEs as serious adverse events (SAEs).

For the laboratory investigations, pretreatment results were available in 33%, 22% and 17% of patients for haematocrit (Hct), total white blood cells (WBC) and biochemistry, respectively (**Table 5**). No CTC grade 4 values were present at baseline and at Day 28. One patient had a grade 4 creatinine value on Day 7 [16.5 > 6 x ULN, ULN=1,20 mg/dL] and returned to grade 0 at Day 28.

There were no shifts between Day 0 and Day 28 from grades 0 – 2 to grades 3 or 4 (**Table 6**). Out of 12 changes in the total

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WBCs, three patients changed from grade 0 to grade 1 and one patient from grade 0 to grade 2, while the remaining eight patients changed to lower grades. There were 28 changes in CTC grade for AST; four had increased grades, from grade 0 to 1 (n=3) and from grade 0 to grade 2 (n=1). Out of 29, only two increased shifts were observed with ALT; one from grade 0 to grade 1 and one from grade 1 to grade 2. One shift from grade 0 to grade 1 was observed for the serum creatinine. For total bilirubin there were one change in CTC grades from 0 to 1 and two from 0 to 2. There were also nine decreases from grade 1 to grade 0 and four decreases from grade 2 to 0.

Table 4. TESS requiring withdrawal from the study. All patients received the co-blistered product.

									Dose (mg/d)				
									AS		AQ		
pt#	dosed by	super vised	day withdrawn	reason	AE (Day= grade)	imputability	measure taken	Age (Years)	Weight (Kg)	Actual	Target	Actual	Target	Δ%
108	age	yes	3	vomiting	D0=2; D1=2; D2=1	possible	metopimazine i.m.	19	49	200	196	612	490	20%
167	age	yes	2	vomiting	D1=2	probable	metopimazine i.m. + quinine i.m.	18	47	200	188	612	470	23%
186	weight	yes	2	abdominal pain	D1=2	probable	phloroglucinol p.o+quinine i.m.	19	77	200	308	612	770	-26%
212	weight	yes	2	abdominal pain + weakness	D1=2	probable	phloroglucinol p.o+quinine i.m.	14	49	200	196	612	490	20%
266	age	no	3	vomiting	D1=2	probable	hospitalized: meto-pimazine i.v. + quinine i.v.	11	36	100	144	306	360	-18%
464	age	no	3	vomiting	D1=2	probable	hospitalized: meto-pimazine i.v. + quinine i.v.	1 1/2	8.8	50	35.2	153	88	42%
116	age	no	2	vomiting	D1=2	probable	metopimazine i.m. + quinine i.m.	18	47	200	188	612	470	23%
8	age	no	3	vertigo	D1=2, D2=2	probable	none	16	53	200	212	612	530	13%
87	age	no	3	pruritus	D1=2, D2=2, D3=2	probable	dexchlorphe- nira-mine p.o.	16	59	200	236	612	590	4%

D0 = pre-treatment

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There were no significant changes in mean values between Day 0 and Day 28 for the haematocrit (mean diff. 0.31 ± 6.10 %, p= 0.51) and total WBC (mean diff. -395 ± 2940, p= 0.21) but there were significant decrease from Day 0 to Day 7 (2.52 ± 5.56 %, p<0.0001) and increase from Day 7 to Day 28 (-1.91 ± 4.70 %, p<0.0001) for haematocrit. The mean AST, ALT and bilirubin decreased significantly while there was a small (0.16 mg/dL) but statistically significant increase in the mean creatinine values between Day 0 and Day 28 (**Figure 4**).

Table 5. Clinical laboratory values on D0 (pre-treatment), Day 7 and Day 28 (mean, standard deviation) and mean (95CI) changes between Day 0 and Day 28

					Difference vs. D0				
		N	mean	std dev	N	Mean	95% CI		Paired t-test
Haematocrit	D 0	323	39	6					
	D 7	233	36.3	5.6	228	-2.5	-3.2	-1.8	<.0001
	D 28	171	38.8	5	168	-0.3	-1.2	0.6	0.51
WBCs	D 0	212	6421	2929					
	D 7	120	6746	2509	120	-78.9	-602.6	444.8	0.76
	D 28	88	6987	2598	86	395.5	-234.8	1026	0.22
ASAT	D 0	167	40.2	38.8					
	D 7	134	23	41	123	-7.4	-14.4	-0.3	0.04
	D 28	106	21	22	101	-15.2	-22.1	-8.3	<.0001
ALAT	D 0	169	21.4	16.3					
	D 7	133	15	29.9	126	-4	-9.2	1.1	0.12
	D 28	105	11.7	11.7	103	-9.4	-12.7	-6.2	<.0001
Creatinine	D 0	166	0.6	0.3					
	D 7	132	0.8	1.4	118	0.2	-0.1	0.4	0.23
	D 28	106	0.7	0.3	100	0.1	0	0.2	0.02
Bilirubin	D 0	156	7	6.2					
	D 7	123	3.3	2.2	104	-3.9	-5	-2.8	<.0001
	D 28	75	4.2	4.3	71	-3.4	-5.1	-1.6	0.0004

D0 = pre-treatment
std dev = Standard Deviation
95% CI = Confidence Interval at 95% level for estimated mean change

DISCUSSION

The favourable results of this study support the policy decision of using AS-AQ in Senegal. It is premature to speculate how long this treatment will last but after six years of use, AS-AQ is still highly effective in the district of Oussouye where the rate of *in vitro* chloroquine resistance is > 60% [12]. The patients from this study represent approximately one third of the more than 3000 patients who have already received AS-AQ as part of its deployment as first-line treatment at the district level. Reassuringly, the *in vitro* susceptibility of *P. falciparum* parasites to desethyl-AQ and artemisinin has not changed under drug pressure during this initial phase of deployment [12].

The efficacy of AS-AQ was well above the 90% threshold recommended by the WHO despite the conservative approach used in analysing the data: ITT dataset, adverse events considered as treatment failures and no adjustment for PCR proven new infections. As such, this study reflects more the effectiveness of AS-AQ in the field, while probably underestimating the real efficacy

of this treatment. Interestingly, failure rates even in children under 10 years of age were considerably lower than those detected in 1998 in the same age group [7]. It is difficult to explain these differences; in both cases, 28 day crude, non PCR-adjusted were measured, while patients were recruited during the rainy season in 1998 and all year round in 2000-05. Reinfections would be less frequent outside the rainy seasons; furthermore, the number of malaria cases has been decreasing after 2000.

Table 6. Shift tables of CTC grades from Day 0 to Day 28 by CTC grading

	WBCDay 28			ASATDay 28			ALATDay 28			Creatinine Day 28		Total Bilirubin Day 28		
Day 0	**G.0**	**G. 1**	**G.2**	**G.0**	**G.1**	**G.2**	**G.0**	**G.1**	**G.2**	**G.0**	**G.1**	**G.0**	**G.1**	**G.2**
Grade 0	61	3	1	63	3	1	84	1	0	23	1	53	1	2
Grade 1	5	2	0	20	5	0	17	0	1	0	0	9	0	0
Grade 2	1	0	1	5	4	0	0	0	0	0	0	4	0	1
Grade 3	1	0	1	0	0	0								

CTC grading: G0 = none; G1 = mild; G2 = moderate; G3 = severe; G4 = very severe

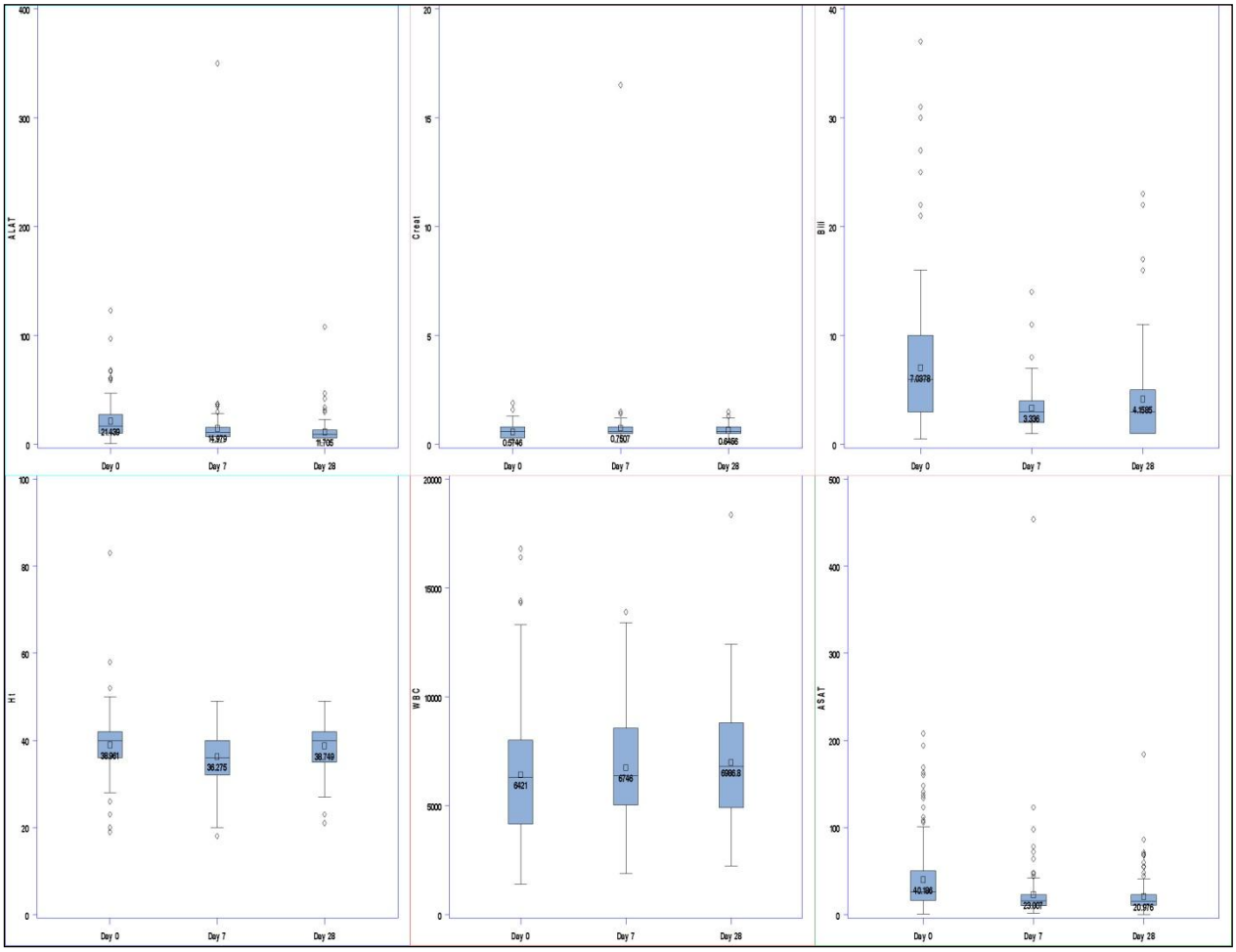

Figure 4. Boxplots of laboratory parameters over time (ALT = aspartate transaminase; ALT = alanine transaminase)

Despite the good parasitological efficacy, treatment did not produce haematological recovery, a softer marker of efficacy. This may have been due to a combination of a relatively high mean, pretreatment haematocrit, the negative effect of AS on reticulocytes, and the short follow up period. Haematological recovery requires more than four weeks in some malaria settings [13]. Treatment was well-tolerated as testified by the very low withdrawal rate (<1%), including two hospital admissions, because of drug induced toxicity. No significant clinical laboratory toxicities were detected.

AS-AQ was equally effective whether given based on body weight as loose combination or by age as co-blistered products (as per the manufacturer's instructions); however, the latter induced more TESS and all the treatment withdrawals due to intolerance. The use of the co-blister did result in patients receiving doses of both drugs, in mg/kg, that were close to the recommended, weight based, mg/kg dosing regimen, though the co-blistered AQ mg/kg dose looked to be a little higher than the loose AQ and had a broader spread of the 95% CIs. Gastrointestinal complaints accounted for seven of the nine withdrawals and were probably AQ rather than AS related, given that AS has excellent tolerability. Five patients received AQ doses ≥ 20% than the weight based dose of 10 mg/kg and four were within the 15 mg/kg upper limit of the newly defined therapeutic window. Never the less, the AQ dose may have contributed to their gastro-intestinal complaints.

The results obtained when patients are dosed more loosely by age are important because more practical regimens may expose patients to drug doses that are outside of the recommended, strictly defined, weight based doses. A more refined practical dosing has been developed for a new, aged dosed, fixed dose combination of AS-AQ [14]. It is generally accepted that making treatments easier to understand and use by patients or e.g. their parents results in better compliance and that using fixed dose combinations enhances this [15, 16]. The AS-AQ fixed-dose combination will become available later in 2007 and plans are afoot to test it in Casamance. Its tolerability will be evaluated.

No cases of hepatitis or severe leukopenia (as a surrogate marker of neutropaenia) were detected, the two amodiaquine-associated toxicities that have caused fatalities in the past, when used as prophylaxis in travelers [16]. However, the number of closely monitored patients was too low to detect rare toxicities, and differential WBC counts could not be done. Intensified monitoring of possible AS-AQ related AEs needs to be conducted in parallel with its widespread deployment, a practice which should be adopted systematically with all other ACTs.

This study confirms that, in this area of moderate/intense transmission, the risk of clinical malaria is present throughout life and that children between 6-15 years of age are most affected. This is important as clinical studies in these areas normally enroll patients up to 10 years old, and thus would miss an important segment of the patient population. In this study, although treatment was statistically less efficacious in children under 11 years of age than in older patients, efficacy rates were still high in both, 95% and 97%, respectively.

To conclude, this field study has shown a high and stable cure rate for AS-AQ even when dosed by age. Tolerability was good but continued intensive safety monitoring is still required in large numbers of patients for this ACT. Further research is planned to continue on the AS/AQ fixed dose combination.

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Competing interest

The author(s) declare that they have no competing interests.

Authors' contributions:

All authors read and approved the final manuscript.

- P Brasseur was the Principal Investigator of the study. He contributed to the concept, protocol, analysis and reporting of the study, and contributed to the preparation of the manuscript. He personally contributed to the treatment, follow-up of patients and quality control of the study.
- P Agnamey contributed personally to the treatment and follow-up of patients.
- O Gaye participated in designing the concept and protocol of the study and supervised study conduct.
- M Vaillant designed and conducted the analyses, contributed to the preparation of the manuscript.

WRJ Taylor and P Olliaro contributed to the concept of the project; design of the protocol and analyses, reporting of the study, and to the preparation of the manuscript.

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Dosing accuracy of artesunate and amodiaquine as treatment for falciparum malaria in Casamance, Senegal.

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Summary

Objectives. Several products of artesunate plus amodiaquine (AS-AQ) are being deployed in malaria-endemic countries for treating uncomplicated *falciparum* malaria but dosing accuracy and consequential effects on efficacy and tolerability have not been examined.

Methods. Patients with parasitologically confirmed, uncomplicated *falciparum* malaria were treated/followed by research teams or local health centre staff in Casamance, Senegal. AS-AQ was given as: (i) loose combination (AS 50 mg, AQ 200 mg), dosed on body weight, or (ii) co-blistered product (AS 50 mg, AQ 153 mg) dosed by weight or age. Target doses were: (i) AS 4 (2-10) mg/kg/d and (ii) AQ 10 (7.5-15) mg/kg/d. Patients receiving therapeutic doses defined dosing accuracy. Treatment-emergent signs and symptoms (TESS) were recorded.

Results. 3,277 patients were treated with loose (n=1,972, weight-dosed) or co-blistered (n=1,305, 962 age-dosed, 343 weight-dosed) AS-AQ by the research team (n=966) or clinic staff (n=2,311). AS was dosed correctly in >99% with all regimens. Loose AQ by weight was 98% correct. The co-blister AQ overdosed 18% of patients when dosed by age and underdosed 13% by weight. Lower weight was an independent risk factor for overdosing. The co-blister had significantly more TESS than the loose product [117/1305 (9%) vs. 41/1972 (2%), relative risk = 4.3 (95% CI 3.0-6.1, p<0.0001)]. TESS occurred mostly within one day (72%) and were mild or moderate (75%).

Conclusion. AS is easier to dose than AQ. Currently available age-dosed, co-blistered AS-AQ tends to overdose AQ, is less well tolerated than loose tablets and may not be the optimal presentation of AS-AQ.

Keywords falciparum malaria, artesunate, amodiaquine, dosing presentation, Senegal

INTRODUCTION

Artemisinin-containing combinations (ACTs) are being actively implemented in 42 countries for the treatment of acute, uncomplicated *Plasmodium (P.) falciparum* malaria based on 28-day, ACT efficacy trials in Africa (Adjuik *et al.* 2004).

Experience with large scale, long term deployments of ACTs is limited to the low transmission areas of the on the Thailand-Myanmar border (Brockman *et al.* 2000; Nosten *et al.* 2000) and the Kwazulu-Natal border, where artesunate-mefloquine and artemether-lumefantrine, re-

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spectively (Barnes *et al.* 2005), have resulted in large reductions of the malaria burden (Price *et al.* 1996). By contrast, no such data exist from areas of moderate or high transmission.

Artesunate-amodiaquine (AS-AQ) is one ACT that is being used in several African countries and is national policy in Senegal since 2006. Although its efficacy varies across regions, in chloroquine-resistant Casamance, southern Senegal, both AQ alone and AS-AQ have consistently resulted in high cure rates (>90%) in recent years (Adjuik *et al.* 2002; Agnamey *et al.* 2005; Agnamey *et al.* 2006; Brasseur *et al.* 1999; Sokhna *et al.* 2001). The combination is available as either loose tablets dosed by weight, necessitating the use of multiple tablet fractions, or the same drugs distributed in a blister pack and dosed by age, with tablet fractions only used for children <1year.

Little attention has been paid to the dose of drugs actually taken by patients and how this might affect tolerability, efficacy, and parasite sensitivity. These are important questions because ACTs are being used on a wide scale. Using a limited number of age-based dosing categories is easier than weight-based dosing but may result in the systematic dosing errors because some patients will receive doses below or above the recommended therapeutic dose ranges. As part of the development of a new fixed-dose combination (FDC) of AS-AQ of different tablet strengths, new therapeutic ranges for AS (2-10 mg/kg/d) and AQ (7.5-15 mg/kg/d) were defined based on published and unpublished safety and efficacy data. Computer modelling of a large African anthropometric database predicted that 99.9% and 83.4% of patients would receive correct doses of AS and AQ, respectively (Taylor *et al.* 2006). There are no data examining the dosing accuracies of loose AS and AQ using the conventional doses (4 and 10 mg/kg/d, respectively) or the newly defined recommended doses.

Herein, we report the accuracy of the age and weight-based dosing regimens of the currently available loose and blister-packed AS-AQ in malaria patients and the ways this affected efficacy and tolerability.

METHODS

Study methodology

From 2000 to 2006 in Oussouye District, Casamance (southern Senegal), patients seen at health posts with parasitologically confirmed (Giemsa-stained thick blood smear) *P. falciparum* malaria were enrolled into one of two studies:

(i) Dispensary study. Dispensary staff, supervised by district medical officers MC and MB, used a simplified 3-day protocol. Supervised treatment was given on Days 0, 1, and 2 and patients reviewed on Day 3 (thick blood smear). Efficacy was defined as parasite clearance on Day 3. Age, sex, weight, doses given, Days 0 and 3 parasitaemia, clinical status, and adverse events were recorded on a case record form.

(ii) Efficacy and safety 28-day study conducted by PB and PA. Study methodology is detailed elsewhere (Brasseur *et al.* 2007). Patients were seen on Days 0-3, 7, 14, 21 and 28 for clinical and parasitological (thick blood smear) assessments. Efficacy was defined as sustained parasite clearance to Day 28.

In both studies, patients were treated with either the loose or blister-packed AS-AQ once daily for 3 days (dosed 4 mg/kg/d for AS and 10 mg/kg/d for AQ) with the following drugs:

- Loose weight-based regimen: Arsumax® 50 mg artesunate tablets (sanofi-aventis, France) 4 mg/kg/d, and Camoquin® 200 mg AQ base tablets (Parke-Davis, France), 10 mg/kg. Tablet fractions were used as appropriate.
- Age- and weight-based blister regimen Arsucam®: Arsumax®(as above) and Flavoquine® 153 mg amodiaquine base tablets (sanofi-aventis).

Treatment was given according to the manufacturer's instructions: (i) for children <1 year (weight <10 kg) = ½ tablet of each drug; (ii) 1 to <6 years (10-20 kg) = one tablet of each drug; and (iii) ≥6 to <13 years (21-40 kg) = two tablets of each drug, and (iii) ≥13 years (>40 kg) = four tablets of each drug.

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Ethical Approval

The study was approved by the Senegalese National Ethical Committee.

Statistical Methods

Data analyses

Data were double-entered into Microsoft Excel, checked and analysed with SAS version 9.3.1 (SAS Institute, Cary, NC, USA). All tests were two-tailed. A p value of <0.05 was considered significant. Non normally distributed data, assessed by the Kolmogorov-Smirnov test, were analysed by the Mann-Witney U test or the geometric least square mean ratio (parasite counts). Dichotomous variables were analysed using the chi-squared test.

Dosing assessment

Dosing accuracy was assessed by determining the proportions of patients who received AS and AQ doses within the , therapeutic dosing ranges (Taylor *et al.* 2006) of 2-10 mg/kg/d (AS) and 7.5-15 mg/kg/d (AQ) supplemented by box plots [median, mean, interquartile ranges (IQ), fence (within 1.5 x IQ), outliers (outside 1.5 x IQ)] of the actual dose in mg/day versus target dose range for each patient by age. The magnitude of under and overdosing for all patients combined was determined by calculating the difference between the mean doses received (mg/d) outside the therapeutic range and the mean doses of the therapeutic range. A logistic model explored possible risk factors for dosing below and above the therapeutic ranges. A saturated model was adjusted by age and weight categories, type of study, product and year of study and method of dosing (by age or weight). A descending stepwise modelling based on the likelihood ratio test between subsequent models was carried out.

Tolerability

Treatment-emergent signs and symptoms (TESS) were defined as events which were not present pre-treatment or worsened with treatment. Information from the patient was solicited from a pre-defined symptoms list and any other symptoms reported. Symptom intensities were graded as 0-4 (none, mild, moderate, severe, very severe). The incidence of the different symptoms pre-treatment (Day 0) was tabulated by year. TESS were analysed by age category, intensity, type of study and day of occurrence. The TESS frequency and the number of patients with $\geq$1 TESS were tabulated.

Drug doses received and outcomes

The relationships between an under and overdose were explored with: (i) the occurrence of TESS between the dispensary and research team studies, (ii) the proportions of aparasitaemic patients on Day 3, and (iii) the Day 28 cure rate.

RESULTS

Study overview

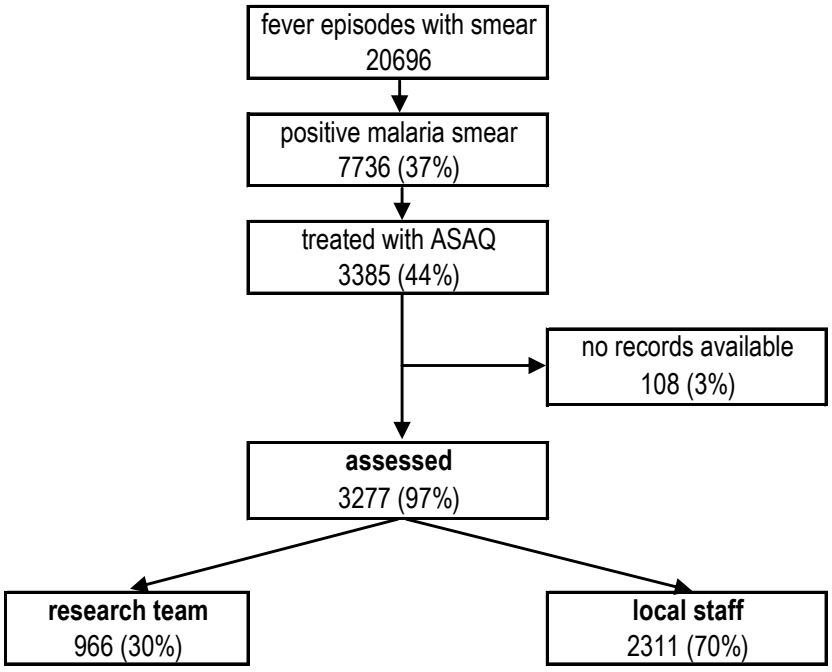
From 2000 to 2006, 20,696 blood smears were done; 7,736 (37%) patients were positive for *P. falciparum* and 3,385 were treated with AS-AQ. Other patients were treated mostly with quinine or chloroquine (Agnamey *et al.* 2005). Records were unavailable for 108 (3%), leaving 3,277 patients who could be assessed: 966 (30%) and 2,311 (70%) were treated and followed up by the research and local teams, respectively (Figure 1). All 966 patients contributed to the Day 28 efficacy analysis but safety was assessed in 752 (78%) patients (Brasseur *et al.* 2007). Safety data are available for the 2,311 recruited patients and 2,245 (97%) had a smear on Day 3.

Treatments administered

Of the 3,277 patients assessed, 1,972 (60%) received loose AS and AQ and were dosed by weight and 1,305 (40%) were treated with the co-blistered product, 962 dosed by age and 343 by weight (Figure 2).

Baseline characteristics

Nearly half (49%) of patients were aged 6-15 years; patients <11 years constituted 42% of the total. The age structure of the population was consistent throughout the period of the study. Few patients (4%) weighed ≤10 kg; 45% weighed 11-30 kg. When comparing the baseline characteristics of patients treated with



	Product		TOTAL
731 (76%)	Loose	1241 (54%)	1972 (60%)
235 (24%)	Co-Blister	1070 (46%)	1305 (40%)

	Regimen		
779 (81%)	weight-based	1536 (66%)	2315 (71%)
187 (19%)	age-based	775 (34%)	962 (29%)

Figure 16. Trial structure

the loose or the co-blistered product, or enrolled by the research or local team (Table 1), statistically significant but clinically irrelevant differences were seen for age, weight, sex and pre-treatment parasitaemia. The mean doses administered by research and local teams were very similar for AQ (10.7 +/- 1.8 vs. 10.4 +/- 1.5 mg/kg/d) and AS (4.0 +/- 0.5 vs. 4.0 +/- 0.6 mg/kg/d).

Dosing accuracy

Body weight was available for 1,962 (99%), 337 (98%) and 359 (37%) of the patients treated with the loose, co-blister weight-based and co-blister age-based, respectively. Based on the newly defined therapeutic dose range, the weight-based loose drugs were the most accurate for AQ resulting in 98% of patients dosed correctly; other proportions were 85.2% (blister-packed by weight), 79.9% (blister-packed by age), and 87.6% if the new FDC had been used (Table 2). All regimens dosed AS correctly between 99.2% and 100%. The co-blister pack under-dosed AQ in 12.8% of patients when given by weight and overdosed 17.8% when dosed by age.

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The overall mean AQ under-doses were 67.9 ± 44.3 mg/day (n=8), 33.5 ± 32.7 mg/day (n=43), and 55.5 ± 38.9 mg/day (n=18) for co-blister age-based, co-blister weight-based, and loose combinations, respectively. The corresponding mean figures for overdosing were 54.7 ± 63.8 mg/day (n=64), 33.1 ± 58.8 mg/day (n=7) and 46.7 ± 59.7 mg/day (n=22).

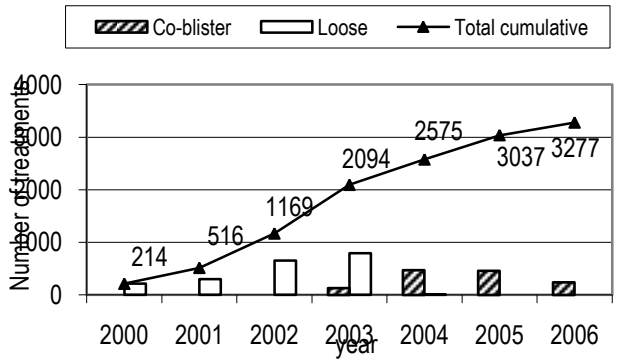

Figure 17. Yearly and cumulative number of treatments with the loose and co-blistered AS-AQ combination, 2000-06

The age-dosed, co-blistered AQ under-dosed 7 patients, 4 in age group ≥7-13 years, and 3 in age group ≥14 years. Of the 64 overdosed, 13 patients (3.6%) were overdosed by a mean 43 mg (1-6 years group), 30 (8.4%) by 40 mg (≥7-13 years) and 21 (5.8%) by 83 mg (≥14 years group). When dosed by weight, the AQ co-blister underdosed 7 patients (2.1%) by a mean 25 mg (1-6 years), 11 (3.3%) by 25 mg (≥7-13 years), and 25 (7.4%) by 40 mg (≥14 years) (Figure 3).

The logistic model identified weight, type and year of study as risk factors for incorrect dosing. Weighing <9 kg carried a higher risk of receiving an inadequate dose relative to patients weighing 18-35.9 kg (OR 6.7 [95% CI=1.6; 27.5]) and 9-18 kg (1.6 [1.0; 2.5]) but not compared to patients with weights >36 kg. Patients enrolled in the dispensary study had a lower risk of being inaccurately dosed (0.6 [0.4; 0.9]). From 2002 onwards (year of the introduction of the co-blister), each year except 2003 (borderline) carried a supplementary risk with respect to 2000.

Table 1. Baseline characteristics

	N (%total)	All patients	Loose combination	Co-blister	*p-value*	Recruited by research team	Recruited by local staff	*p-value*
Age (years)*	3215 (98%)	15.1 +/- 12.1	15.0 +/- 12.3	15.2 +/- 11.9	*<0.001 §*	13.8 +/- 11.2	15.6 +/- 12.5	*<0.001 §*
Sex F:M	3276 (100%)	45.5% : 54.5 %	46.7% : 53.3%	43.7% : 56.3%	*0.09 #*	43.4% : 56.6%	46.4% : 54.6%	*0.11 #*
Weight (kg)*	2659 (81%)	35.7 +/- 18.7	35.8 +/- 19.1	35.6 +/- 17.5	*0.83 §*	33.3 +/- 17.2	37.1 +/- 19.3	*<0.001 §*
**parasitaemia Day 0 **	3219 (98%)	23763 [11346-60281]	24538 [11601-66421]	22586 [10910-52414]	*0.12 £*	31854 [12001-93801]	20965 [10801-54841]	*<0.001£*
temperature Day 0*	3223 (93%)	38.5 +/- 6	38.4 +/- 1.1	38.5 +/- 9.4	*<0.001 §*	38.4 +/- 1.0	38.5 +/- 7.1	*0.62 §*
Signs and Symptoms Day 0 Patients with at least 1	3277	1619	1060 (65.5%)	559 (34.5%)	0.0005 #	1003 (61.9%)	606 (38.1%)	*<0.001 #*
Signs and Symptoms Day 0	3277	609 (18.6%)	368 (18.7%)	241 (18.5)	0.89 #	311 (32.2%)	298 (12.9%)	*<0.001 #*

LEGEND: * mean +/- std; ** geomean [Q25-Q75]; § Mann-Withney; # Chi-square; £ Geometric least square mean ratio

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Table 2 Frequency of patients wo received dose within, under and over the therapeutic ranges for amodiaquine and artesunate for the loose and co-blistered drugs

	Dose mg/kg/d						
	Amodiaquine >=7.5 to =<15			Artesunate >=2 to <=10			
	under	adequate	over	under	adequate	over	total
loose weight-based	18	1922	22	1	1961	0	1962
%	0.9%	98.0%	1.1%	0.1%	99.9%	0.0%	
co-blister weight-based	43	287	7	0	337	0	337
%	12.8%	85.2%	2.1%	0.0%	100.0%	0.0%	
co-blister age-based*	8	287	64	2	356	1	359
%	2.2%	79.9%	17.8%	0.6%	99.2%	0.3%	
FDC age-based**	137	2170	169	4	2472	0	2476
%	5.5%	87.6%	6.8%	0.2%	99.8%	0.0%	

A simulation for the new FDC combination when dosed by age was also done

* age groups by manufacturer: 1-6; >6-13; >13 yr

** simulation with ASAQ FDC and age groups as per Taylor et al (2006): 0-<1; 1-6; >6-13; >13

AQ, amodiaquine; AS, artesunate; FDC, fixed dose combination

Treatments administered by the research team resulted in a dose outside the therapeutic range for AQ in 62/941 (6.6%) patients as compared to 77/1,536 (5.0%, p=0.098) for the dispensary staff (NB: the actual dose could not be calculated for 603/962 (63%) of patients given the co-blister by age because weights were not recorded).

Tolerability

One hundred and fifty eight (4.8%) of the 3277 patients experienced 207 TESS: 165 (blister) vs. 42 (loose, p=0.09). 117/1305 (9%) of the blister-treated patients had $\geq$1 TESS compared to 41/1972 (2%) in the loose group (p<0.0001) for a relative risk of 4.3 [95% CI 3.0-6.1)]. Overall, 2/158 (1.3%) of the patients with $\geq$ 1 TESS had AQ under-dosed, 48/158 (30.4%) had AQ over-dosed, and 108/158 (68.3%) had a therapeutic dose. TESS frequencies and gradings are detailed in ; most (75%) were mild or moderate in severity and 149/207 (72%) occurred on the first day of treatment; 53 events were recorded on Day 2, 3 events on Day 3, and 2 events on Day 4. In particular, of the 64 cases of vomiting, 52 (81.25%) occurred on Day 1 and 11 on Day 2. Nine blister-treated patients discontinued treatment owing to an adverse event; all were dosed by age and 7 had AQ within the therapeutic range (**Error! Reference source not found.**).

Efficacy

The crude, PCR-unadjusted efficacy rates are 94.6% [95% CI 92.9-95.9] and remained stable over the period under study, ranging from 88.5% to 96.7% (Brasseur *et al.* 2007). Cure rates were independent of dose received (Table 4). There were no failures in AQ-overdosed patients but 7 (10.6%) were lost to follow up. Of the 16 AQ under-dosed patients, one failed and none were lost to follow-up. Of the 3,191 patients who had a slide taken on Day 3, 10 (0.3%) were positive for *P. falciparum*: 0.62% (blister pack dose by weight), 0.44% (blister pack dose by age), and 0.2% (loose dose by weight, p=0.18).

DISCUSSION

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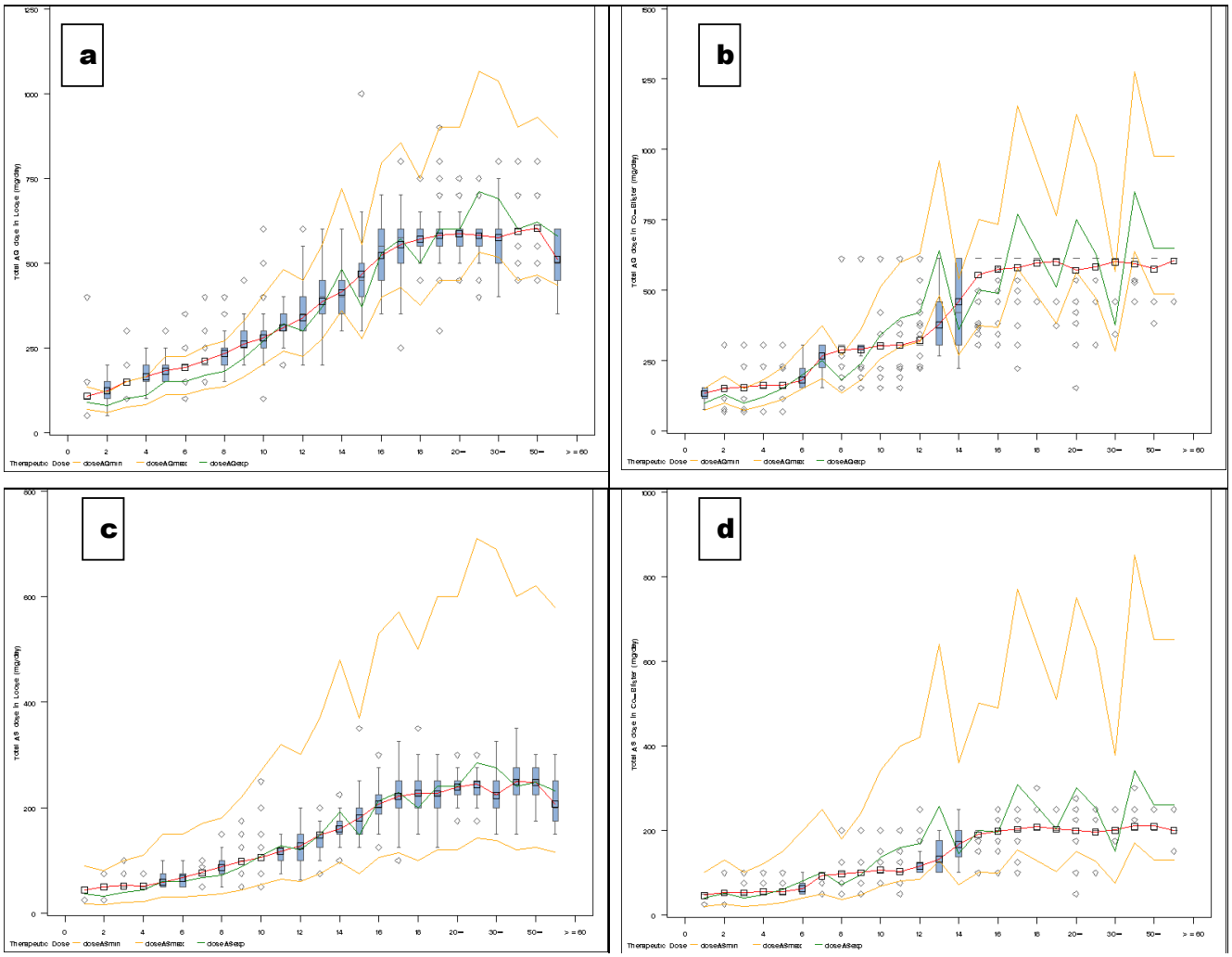
We have shown that dosing accuracy varies markedly with different AS-AQ presentations when using a newly defined and expanded therapeutic dosing range for each drug. Co-blistered AS-AQ dosed less accurately and was associated with more side effects; however, this did not lead to more drug withdrawals. This is, to our knowledge, the largest dataset prospectively documenting the use of different dosing programs and presentations of the AS-AQ combination, their dosing accuracies, and consequential effects on efficacy and tolerability from an area of moderate malaria transmission. The patient and malariometric

Figure 3 Boxplots of dose (mg/d) by age of AQ administered as (a) loose and (b) co-blistered product and AS as (c) loose and (d) co-blistered product. The orange lines represent the dosing limits using the new therapeutic dosing ranges for each drug (2-10 mg/kg/d AS. 7.5 to 15 mg/kg/d AQ). The green line represents the drug dose that would be given if using strictly the target doses for each drug (4 mg/kg/d AS, 10 mg/kg/d AQ). The blue line is the average actual dose



characteristics during this study were similar to those before the introduction of AS-AQ (Agnamey *et al.* 2005). *P. falciparum* was detected in 37% of the patients with suspected malaria and the age distribution of patients has not changed; about half of the malaria patients were 6-15 years old, consistent with moderate malaria transmission and an entomological inoculation rate of 25 bites per person-year (Sokhna, *et al.* 2001).

Dosing accuracy

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When deploying a new drug, combination, or dosing method of established drugs, it is important to document dosing accuracy and to determine if patients are systematically under-dosed (potentially resulting in treatment failure and the setting of conditions for the selection of drug-resistant parasites), or overdosed (potentially producing drug toxicity.) This is not unusual for antimalarials since they are not normally developed on pharmacokinetic/dynamic evidence; 76% of Kenyan children <5 years were found to be systematically under-dosed when sulfadoxine/pyrimethamine was given following the internationally recommended age-based dosing schedule (Terlouw *et al.* 2001) and this may have contributed to the emergence of parasite resistance to the drug.

Table 3 Treatment Emergent Signs and Symptoms (TESS) by intensity (206/207 TESS with intensity recorded)

	intensity					
	Mild	Moderate	Severe	Very severe		
Symptoms	Grade 1	Grade 2	Grade 3	Grade 4	Total	%
Abdominal pain	5	5	0	0	10	4.9%
Anorexia	2	2	9	0	13	6.3%
Diarrhoea	5	7	2	1	15	7.3%
Headache	18	2	5	1	26	12.6%
Nausea	1	1	3	1	6	2.9%
Pruritus	7	9	10	1	27	13.1%
Rash	0	0	1	1	2	1.0%
Vertigo	15	23	4	1	43	20.9%
Vomiting	17	37	8	2	64	31.1%
Total	70	86	42	8	206	
%	34.0%	41.7%	20.4%	3.9%		

Table 4 Frequencies of patients who received adequate or inadequate doses based on the new therapeutic dose ranges of 7.5-15 mg/kg/d of amodiaquine (AQ) and 2-10 mg/kg/d artesunate (AS) daily and their outcomes during 28 days of follow up

	AQ dose				AS dose			
	under	adequate	over	Total	under	adequate	over	Total
LTF	1	35	0	36	0	36	0	36
	6.25	3.96	0		0	3.81	0	
Success	14	817	58	889	1	870	18	889
	87.5	92.42	87.88		100	92.06	90	
lost to follow up	0	25	7	32	0	30	2	32
	0	2.83	10.61		0	3.17	10	
withdrawn due to AE	1	7	1	9	0	9	0	9
	6.25	0.79	1.52		0	0.95	0	
Total	16	884	66	966	1	945	20	966

The recommended target doses for AS and AQ are 4 and 10 mg/kg/d, respectively. Their respective therapeutic windows have been extended to 2-10 mg/kg/d and 7.5-15 mg/kg/d based on a review of available clinical data and computer modelling of an anthropometric database numbering ~88,000 individuals (Taylor *et al.* 2006).

During the course of the staggered deployment of AS-AQ in Casamance, patients received first a loose combination of individually packaged AS (50 mg tablets) and AQ (200 mg base) dosed by weight, using tablet fractions if necessary, fol-

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lowed by co-blistered AS (50 mg) and AQ (153 mg base) dosed either by age or weight, according to the manufacturer's instructions. These drug doses and dosing instructions differ from those of the newly developed FDC (Taylor *et al.* 2006). The therapeutic index is wide for AS, so dosing accuracy was high, but narrower for AQ and dosing accuracy varied markedly between ~80% for the age-dosed blister and 98% for the weight-dosed, loose AQ. The former overdosed ~18% of patients by a mean of 55 mg/d. The FDC would be dosed accurately in 87.6% of these patients, performing slightly better than the 83.4% predicted by the database used by Taylor *et al.* (2006). The AQ tablet strength of 153 mg in the co-blister was clearly less flexible than the 200 mg loose tablets and the 67.5/135 mg tablet strengths of the FDC.

Based on these results, particular care should be taken when treating patients with a low body weight (weighing <9 kg was a risk factor for overdosing) because these presentations are not adapted to small children.

The health posts in Oussouye have weighing scales and health workers dosed AS-AQ remarkably well, but dosing by age is more expedient. Offering age- and weight-based dosing would seem to be a reasonable strategy in Senegal and similar settings, but in many tropical areas dosing by age is the only option. With the current co-blister this carries the highest risk of inaccurate dosing because it uses approximated age/weight correlations, whereas the FDC has specifically designed age- and weight-based regimens; the latter ensures that patients receive AQ within the 7.5 to 15 mg/kg/day therapeutic window, a distinct advantage.

Monitoring treatment effects

AS-AQ was generally well tolerated: <5% patients complained of side effects, which were generally of mild or moderate intensity. The co-blister pack was less well tolerated than the loose combination and was about 4 times more likely to cause toxicity. Over and under-dosing were not significantly associated with either drug toxicity, failure, or parasite positivity on Day 3 but the sample sizes were small and these events were rare. A small number of patients had unremarkable haematology and biochemistry results (Brasseur *et al.* 2007) insufficient to detect possible neutropaenia or drug-induced hepatitis; both tend to be asymptomatic. Future studies must monitor the total and differential white cell counts and liver enzymes.

Implications for research and practice

There are limitations to these analyses. Patients were not randomized to either study, one of which had more intensive monitoring compared to the more pragmatic, dispensary study. The blister pack currently distributed in Senegal by the National Malaria Control Programme is not the same brand of the one used in this study but has the same presentation, tablet strength and dosing instructions. Loose products should not be available, as advocated by the WHO (Olumese 2006).

It will be important to document clinically manifested toxicity, but also to monitor neutropaenia and hepatitis, and explore the effects of repeated treatment over time. Senegal is fortunate to have a good health infrastructure and is able to supervise ACT treatment. However, dosing accuracy, drug effectiveness, and patient adherence should also be assessed with unsupervised AS-AQ dosed by age, as this would be common practice. Essential information (e.g. age, weight, dose, and treatment effects) should be systematically collected to enable data pooling and analysis (Price *et al* 2007; Sibley *et al.* 2007). Drug levels should also be measured in order to define the relationships between dose and treatment outcomes.

To conclude, dosing the currently available co-blister by age was the least accurate, exceeding the upper dose limit in ~18% of patients and causing more side effects. If the new, age-based AS-AQ FDC were deployed in the same population, AQ would be correctly dosed in ~88% with under and overdosing in equal proportions (~6 and ~7%, respectively). The long-term, population-wide implications of the under- and over-

dosing of antimalarials demonstrate the need for more research.

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Plasmodium falciparum In vitro Susceptibility to Antimalarial Drugs in Casamance (South-western Senegal) during the First 5 Years of Routine Use of Artesunate-Amodiaquine.

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We have monitored the *in vitro* sensitivities of *Plasmodium falciparum* isolates pre- and during the deployment of artesunate plus amodiaquine treatment in Mlomp, Casamance (south-western Senegal) during 2000-2004. Parasites remain susceptible to both drugs. Chloroquine resistance levels are high but stable. Quinine continues to be effective.

Parasite resistance to chloroquine (CQ) is widespread in Sub-Saharan Africa. Artemisinin-based combinations (ACTs) are currently recommended instead for the treatment of uncomplicated falciparum malaria [13]. In southern Senegal *in vitro* and *vivo* CQ resistance is established [9, 11] causing excess burden [12]. We compared in controlled trials CQ and amodiaquine (AQ) [4], and artesunate-amodiaquine (AS-AQ) and AQ [1] in children in Mlomp (Casamance, south-western Senegal). Subsequently, we deployed AS-AQ in 2000 for all age-groups, first during the rainy seasons and then year-round [2]. AS-AQ has been consistently efficacious and well-tolerated. With several countries scaling-up the use of ACTs, it is important to know whether parasite sensitivity is affected by the widespread use of these drugs. Therefore we monitored the *in vitro* susceptibility of local isolates to CQ, quinine (QN), artemisinin (ART) and the AQ metabolite monodesethylamodiaquine (MdAQ) using the DELI test [7] pre-ACT (1997) and during deployment (2000-2004).

Malaria is mesoendemic in Mlomp (25 infective bites/person-year). Malaria transmission occurs year-round with a peak during the rainy season (July-December.)

Isolates were collected a regular intervals pre-treatment from consecutive subjects with a *Plasmodium falciparum* mono-infection and parasitaemia $\geq$0.2% [6] and used for the *in vitro* assay within 4 hours. Methodology was as in Brasseur *et al.* [3], except for 0.5 % Albumax supplementation (Gibco BRL Grand Island, NY) instead of human serum. Drug stock solutions were prepared in RPMI and serially diluted two-fold to obtain final concentrations ranging 14.6-3750nM (CQ), 14.4-3692.3nM (QN), 5.9-1518.9nM (MdAQ) and 1-532nM (ART). Parasite growth in control and treated wells was measured as optical density (OD) reflecting pLDH level [7]. Drug activity was expressed as the concentration of drug resulting in a 50% inhibition of parasite growth (IC_{50}), as calculated from maximal OD values from test wells compared to control wells. The cut-off values of IC_{50} for resistance were: 100 nM (CQ), 500 nM (QN), 60 nM (MdAQ) and 15 nM (ART) [10].

IC_{50s} were log-transformed; ANOVA was used to generate Least Squares Means (LSM); Geometric LSM (GLSM) were obtained by anti-logarithm transformation of LSMs. The ratio of GLSM (with 95%CIs) were estimated between years for each treatment (no difference was concluded if the 95%CI of the comparison included 1.0.) For each treatment a Generalized Linear Model (GLM) was estimated with year as the categorical factor using 1997 as reference. Changes in the proportion of resistant isolates over time were explored by the Cochran-Armitage test for trends. Statistical significance was at $p < 0.05$ (two-tailed.).

IC_{50s} were obtained for 242 subjects (CQ), 236 (QN), 250 (mdAQ), and 183 (ART.) Different analyses concur to show that CQ resistance is established but steady; IC_{50s} are higher than in 1997 for QN but otherwise stable during 2000-04, decreased for MdAQ and are consistent for ART.

Fig 1 (a-d) shows log-transformed IC_{50s}. CQ values were stable (1a), QN increased from 1997 to 2000, then remained stable during 2000-04 (1b), MdAQ decreased during 1997-2004 (1c), and ART showed discreet fluctuations over time (1d).

GLSM and pair-wise GLSM ratios are in table 1. CQ values were constant, except between 2000/1997 and 2002/2001. For QN differences were statistically significant between 1997 and all years 2000-2004 (95%CIs of the ratios >1, thus IC_{50s} increased *versus* 1997), with occasional differences between years. For MdAQ differences were significant between 1997 and

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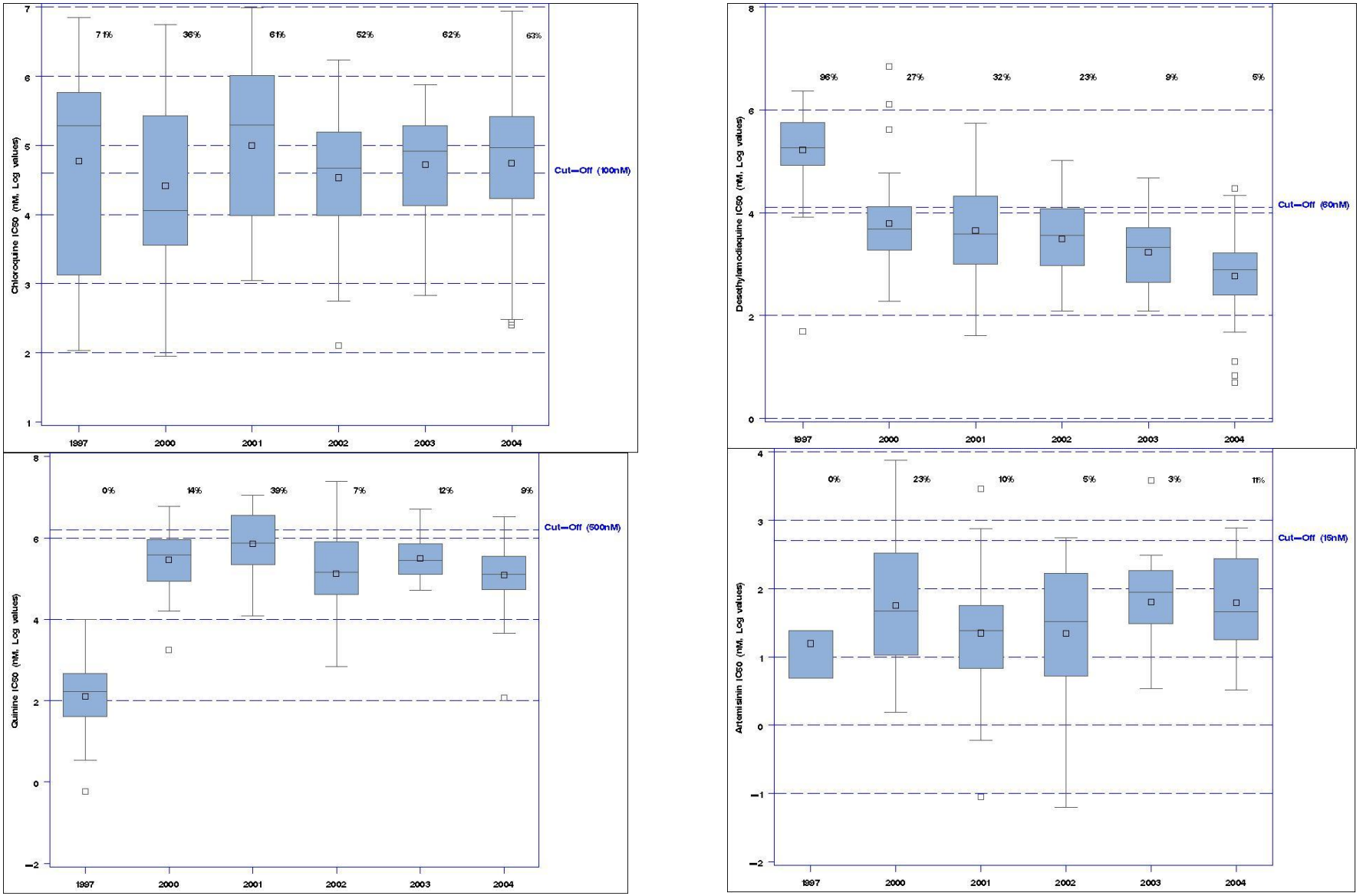

Figure 1 : Box Plots of Chloroquine (a), mdAmodiaquine (b), Quinine (c) and Artemisinine (d) IC50 (nM) log-transformed values versus years (1997, 2000-2004). *The top (or the base) of the whisker correspond to the largest (or the lowest) observed value within the upper fence (1.5 x Interquartile Range). Observations outside the fences are identified with a special symbol (described in Chapter 2 of Tukey, 1977).*

TABLE 1. GLSM Results

	GLS Mean (95%CI)			
	Chloroquine	Quinine	Desethylamodiaquine	Artemisinin
1997	119.0 (86.7 ;163.4)	8.2 (6.8 ;10.1)	185.7 (151.1 ;228.2)	3.3 (2.2 ;5.1)
2000	82.6 (60.8 ;112.4)	238.2 (184.8 ;306.9)	44.5 (34.9 ;56.6)	5.8 (4.5 ;7.5)
2001	148.4 (108 ;203.8)	350.1 (275.1 ;445.5)	38.5 (30.0 ;49.4)	3.9 (3.0 ;5.0)
2002	93.3 (71.9 ;121.0)	168.4 (136.2 ;208.2)	33.1 (26.8 ;40.8)	3.9 (3.1 ;4.8)
2003	112.7 (83.3 ;152.5)	245.0 (194.6 ;308.3)	25.3 (20.0 ;32.1)	6.1 (4.8 ;7.8)
2004	115.0 (92.7 ;142.7)	162.2 (136.0 ;193.5)	15.9 (13.3 ;18.9)	6.0 (4.7 ;7.6)

TABLE 2. GLSM Ratios

		GLS Mean ratio (95%CI)			
		Chloroquine	Quinine	Desethylamodiaquine	Artemisinin
Vs. 1997	2000	0.7* (0.4 ;0.9)	28.9* (19.0 ;38.7)	0.2* (0.2 ;0.3)	1.8 (0.8 ;2.7)
	2001	1.2 (0.8 ;1.7)	42.4* (27.5 ;57.4)	0.2* (0.1 ;0.3)	1.2 (0.5 ;1.8)
	2002	0.8 (0.5 ;1.1)	20.4* (14.3 ;26.5)	0.2* (0.1 ;0.2)	1.2 (0.5 ;1.8)
	2003	0.9 (0.6 ;1.3)	29.7* (19.7 ;39.7)	0.1* (0.1 ;0.2)	1.8 (0.8 ;2.9)
	2004	1 (0.6 ;1.3)	19.7* (13.9 ;25.4)	0.1* (0.1 ;0.1)	1.8 (0.8 ;2.8)
Vs. 2000	2001	1.8 (1.2 ;2.4)	1.5 (1.0 ;2.0)	0.9 (0.5 ;1.2)	0.7* (0.4 ;0.9)
	2002	1.1 (0.7 ;1.5)	0.7* (0.5 ;0.9)	0.7 (0.5 ;1.0)	0.7* (0.4 ;0.9)
	2003	1.4 (0.9 ;1.8)	1 (0.7 ;1.4)	0.6* (0.4 ;0.8)	1.1 (0.7 ;1.4)
	2004	1.4 (0.9 ;1.9)	0.7* (0.4 ;0.9)	0.4* (0.2 ;0.5)	1 (0.7 ;1.4)
Vs. 2001	2002	0.6* (0.4 ;0.8)	0.5* (0.3 ;0.7)	0.9 (0.6 ;1.2)	1 (0.6 ;1.3)
	2003	0.8 (0.5 ;1.0)	0.7* (0.5 ;0.9)	0.7* (0.4 ;0.9)	1.6* (1.0 ;2.1)
	2004	0.8 (0.5 ;1.0)	0.5* (0.3 ;0.6)	0.4* (0.3 ;0.6)	1.6* (1.0 ;2.1)
Vs. 2002	2003	1.2 (0.8 ;1.6)	1.5 (1.0 ;1.9)	0.8 (0.5 ;1.0)	1.6* (1.1 ;2.1)
	2004	1.2 (0.9 ;1.6)	1 (0.7 ;1.3)	0.5* (0.3 ;0.6)	1.6 (1.0 ;2.1)
Vs. 2003	2004	1 (0.7 ;1.4)	0.7* (0.4 ;0.9)	0.6* (0.4 ;0.8)	1 (0.7 ;1.3)

[a] Comparison year GLSM/reference year GLSM. *, statistically significant (P<0.05) increase (GLSM ratio > 1 or decrease (GLSM ratio < 1) from reference year to comparison year

2000-2004 (95%CIs <1, IC_{50s} decreased *versus* 1997), and sporadically between years (always decreasing with time). For ART, erratic differences were seen in either directions.

The GLM confirmed the above results: Spearman correlation coefficients showed no relationship for CQ, significant relationship for QN (positive estimates: p<0.0001, r=0.34) and AQ (negative estimates: p<0.0001, r=-0.63), limited fluctuation for ART (p=0.03, r=0.16).

The proportions of resistant isolates (IC_{50} > cut-off) are in Fig 1(a-d). The Cochran-Armitage trend test was non-significant for all drugs except MdAQ (significant decrease of resistant isolates over time, p<0.0001).

Some methodological issues of this study deserve further comments.

We used artemisinin (from which all artemisinin derivatives are synthesized), and monodesethylamodiaquine (AQ main metabolite); both are intrinsically less bioactive than artesunate [8] and AQ [5], respectively.

There are no well-established methodologies to analyse *in vitro* sensitivity data. To confidently classify a strain as resistant/sensitive requires validated thresholds; while adopting pre-defined cut-off values (>2 standard deviations above the mean) [10], their clinical significance is doubtful, especially for ART and somewhat MdAQ. In addition, comparing IC_{50s}, particularly trends over time, poses problems. We explored several approaches (including Welch-adjusted ANOVA or non-parametric Kruskall-Wallis sign rank test as appropriate) and opted for presenting the GLSM ratios complemented with GLM. We are confident in the results presented as all test converge to pointing to the same conclusions.

In the past decade QN (i.m. twice a day for 3 days) has been preferred over failing CQ in this area [2]. Attendance registries in Mlomp report a total of 6642 first-line

treatments of fevers with antimalarials during 1996-2000, 96% of which were consistently QN and 4% CQ. In 2000, we started using AS-AQ for parasitologically-confirmed falciparum malaria. During the first 5 years of deployment, close to 2800 treatments have been delivered in Mlomp. In 2004 nearly all treatments of confirmed cases were AS-AQ, but QN use continues particularly when a parasitological diagnosis cannot be made. AS-AQ has consistently been >90% parasitologically and clinically effective [2].

To date, the level of CQ *in vitro* resistance is stable (50-60%) and there is little resistance *in vitro* to QN, AQ and ART. IC_{50s} are decreasing for MdAQ and fluctuate for ART; it is too early to determine whether these changes have any clinical significance. These *in vitro* data are consistent with *in vivo* responses. Our data allow us to conclude that parasite sensitivities have not been adversely altered by this initial phase of deployment of the AS-AQ. QN should still be used for severe malaria cases. The combination of geometric least square mean ratios (with 95%CIs) and generalized linear model is useful for the analysis of *in vitro* sensitivity data.

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Geometric least squares means ratios for the analysis of *Plasmodium falciparum in vitro* susceptibility to antimalarial drugs

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Abstract

Background. The susceptibility of microbes such as *Plasmodium falciparum* to drugs is measured *in vitro* as the concentration of the drug achieving 50% of maximum effect (IC_{50}); values from a population are summarized as geometric means. For antimalarial drugs, as well as for antibiotics, assessing changes in microbe susceptibility over time under drug pressure would help in-form treatment policy decisions, but no standard statistical method exists as yet.

Methods. A mixed model was generated on log_e-transformed IC_{50} values and calculated geometric least squares means (GLSM) with 90% confidence intervals (CIs). In order to compare IC_{50}s between years, GLSM ratios (GLSMR) with 90%CIs were calculated and, when both limits of the 90%CIs were below or above 100%, the difference was considered statistically significant. Results were compared to those obtained from ANOVA and a generalized linear model (GLM).

Results. GLSMRs were more conservative than ANOVA and resulted in lower levels of statistical significance. The GLSMRs approach allowed for random effect and adjustment for multiple comparisons. GLM was limited in the number of year-to-year comparisons by the need for a single reference year. The three analyses yielded generally consistent results.

Conclusion. A robust analytical method can palliate inherent limitations of *in vitro* sensitivity testing. The random effects GLSMRs with adjustment for multiple comparisons and 90%CIs require only assumptions on the mixed model to be applied. Results are easy to display graphically and to interpret. The GLMSRs should be considered as an option for monitoring changes in drug susceptibility of *P. falciparum* malaria and other microbes.

BACKGROUND

Plasmodium falciparum, the species causing most of the malaria burden in the world, can be grown in culture, which makes it possible to measure parasite drug susceptibility *in vitro* [1]. Results are generally expressed as IC_{50} (the concentration achieving 50% of maximum effect). Turnidge *et al.* [2] presented a new method to evaluate cut-off values to define antibacterial resistance. However, while methodologies are well established for antimicrobials [3], it is not possible, for the majority of drugs, to confidently classify a strain as resistant or sensitive be-cause validated thresholds are not available, unlike the situation with antibiotics [4] .

Of particular interest in *P. falciparum* drug susceptibility is not the sensitivity profile of an isolate from a given patient, but rather the monitoring of the sensitivity patterns to drugs when submitted to drug pressure in a

given area [5]. This information may contribute to inform decision as to choice of drugs for the treatment of malaria. However, evaluating trends over time poses methodological problems, mainly because (i) the high variability of results makes conventional statistical methods, such as the analysis of variance (ANOVA), inadequate; (ii) the statistical unit is not the individual patient isolate but the composite population of parasites within an individual. Due to this distribution variability, these data are usually presented as geometric mean. A key issue in the analysis of these data is detecting trends over time. However, there is still no standard statistical approach for this [6], without loss of information.

Methods used in pharmacokinetic studies deal with similar problems in dealing with datasets with respect to drug disposition in an individual or a population of individuals [7], [8], [9]. Bioequivalence between drug formulations is typically assessed by using the analysis of variance of $\log_e$-transformed data from a cross-over design with the null hypothesis expressed by: H_0: $\mu_T = \mu_R$ where μ_T and μ_R represent the log-transformed expected bioavailability parameters of the test and reference formulations respectively [10] [11]. A 90% confidence interval (CI) for the ratio test/reference for bioavailability parameters is constructed by using the equation: $\mu_T - \mu_R \pm S\sqrt{\frac{2}{nt_{0.05(1),\upsilon}}}$ where S is the square root of the mean square error from the analysis of variance, n is the number of subjects per period, $t_{0.05(1),\upsilon}$ is the critical value of t at α=0.05 with υ the degrees of freedom.

In the case of susceptibility testing conducted over time, when subjects are grouped by the year of sampling, the variance in the ANOVA of parasite susceptibility (expressed as IC_{50}) can be separated out into the contributions of the parasite, the subject and the time of measurement. The ANOVA will allow for the year of measurement and test whether the variability between years occurs at random or not. The ratio of the mean sum of squares of the parameter (e.g. IC_{50}) to the error mean sum of squares in the ANOVA will give an F-statistic to test the null hypothesis: H_0: $\mu_{year} = \mu_{year0}$. This will provide a test of whether the arithmetic mean of IC_{50}s measured from a given year is identical to the mean of IC_{50}s obtained in the reference year.

An underlying assumption in order to use the ANOVA is the normality of the residuals (the difference between an individual value and the mean of the sample it belongs to, $x_i - \bar{x}$). However, verifying the null hypothesis of identity between means may not be possible with distributions of residuals that are generally neither normal nor log-normal. Furthermore, the sample size is most often too small with respect to the variance for the comparison of means. The high variability of data leads to large error variability in terms of error sum of squares. Thus, the detection of a difference will be difficult to interpret since it will be a function of the variability of data and sample size for each year. Mixed models can be used when there are different levels of clustering in the observations. One can assume that there is a grouping per year and a random part of measurements within years due to the subject and the parasites strains the subject is infected with. It allows the user to analyse samples (here: years) with un-equal sample sizes and to relax the assumption of independently and identically distributed residuals while accounting for the data structure in a more flexible way [12].

For malaria, with the introduction of new treatment regimens such as the Artemisinin-containing Combination Therapies (ACTs) [13], it is important to evaluate whether, with parasites being exposed to

drug pressure, the amounts of drug needed to inhibit parasite growth departs from that of a reference year, prior to and during deployment to monitor the evolution of drug susceptibility. In addition, appropriate statistical methods are needed to account for the variability in the determination of IC_{50}s in order to properly inform treatment policy decisions.

Several approaches were explored to describe the trends over time of parasite *in vitro* drug susceptibility of the parasite using a dataset collected in Casamance (south-western Senegal) during 2000-2004 slightly adapted for the purpose from Agnamey *et al.* [14].

METHODS

Mixed model

A fixed effect model can be expressed as $y_{ij} = \mu + t_j + e_{ij}$ where j is the year of measurement, y_{ij} the observation on year j for patient I, μ the overall mean (also referred to as intercept in statistical software), t_j the relative effect of year j, $\mu + t_j$ is the mean effect for the year j. and e_{ij} the residual variance for year j on the i^{th} patient [12]. The model allows for the patient effect, in which case the formula becomes $y_{ij} = \mu + p_i + t_j + e_{ij}$ where p_i represents the i^{th} patient effect. Instead of defining some effects as constants in the model, one could consider them as arising from independent samples with a normal distribution [12], i.e. as random effects. The model containing both fixed and random effects can then be referred to as a mixed model [12] [15]. The underlying assumptions to using mixed models are: normally distributed residuals, normally distributed random effects and residuals independent of the random effects.

GLSMRs calculations

A mixed linear model of log_e-transformed values was estimated whereby the year was considered as fixed and the intercept as a random effect. The isolates were from different subjects each year.

From the mixed model, t statistics of standardized pair wise differences were calculated as $\bar{y}_i - \bar{y}_j \Big/ \hat{\sigma}_{ij}$ where *i* and *j* are the indices of two years, $\bar{y}_i$ and $\bar{y}_j$ are the least square means (LSM) for years *i* and *j* and σ_{ij} is the square-root of the estimated variance of $\bar{y}_i - \bar{y}_j$. In this model, LSMs are predicted population means from log_e-transformed values. Consequently, assuming that $Log_e \frac{y_i}{y_j} = Log_e y_i - Log_e y_j$ and that the geometric mean is the antilog of the mean of log_e-transformed values, the geometric least squares means ratio (GLSMR) can be calculated with the antilog of the $\bar{y}_i - \bar{y}_j$ (LSMs differences) extracted from the model (where y_i and y_j can be expressed as linear combination $l_i'b$ and $l_j'b$ of the parameter estimates). From these linear combinations the parameter estimates that define the LSMs, σ_{ij}, (i.e. the standard deviation of the LSMs difference), can be estimated by $\sigma_{ij}^2 = s^2 l_i'(X'X)^- l_j$. The confidence interval can be derived as $(\bar{y}_i - \bar{y}_j) \pm t_{\nu, \frac{\alpha}{2}} \sigma_{ij}$.

GLSM ratios (GLSMRs) were calculated for each between-year comparison. An adjustment for multiple comparisons was done in order to control for the overall type 1 error rate using the Tukey-Kramer method (chosen because it allows for unequal sample size between years). GLSMRs were considered statistically different if both bounds of the CIs fell on either side of the value of 1 (or 100% in percentage values). Previously [14], we had used GLSMRs calculated without this adjustment and evaluated the 95%CIs.

Standard statistical methods

Standard methods such as the one-way ANOVA were also used with the year as fixed factor to analyse the variations of IC_{50}s over time. For non-normally distributed data (significant Kolmogorov-Smirnov test), a $\log_e$-transformation was applied. The Levene test for homogeneity of the variance was used and, in case of non-homogeneity, a Welch adjustment for the ANOVA. A non-parametric Kruskall-Wallis sign rank test was used when parametric analyses were not suitable. Pair-wise mean comparisons between years for each treatment were carried out following ANOVA with a Tukey adjustment. Normality of residuals was checked with a non significant Shapiro-Wilk test and normal probability plots. Concurrently a generalized linear model (GLM) was also estimated with the year as fixed factor using a normal probability function and an identity link function parameterization[16].

A p-value of <0.05 was considered statistically significant. All tests were two-tailed. Statistical analyses were carried out with the statistical package SAS® System version 9.1.3 (SAS Institute, Cary, NC, USA).

Dataset

The dataset is an update of the one described in Agnamey *et al.* [14]. Briefly, *in vitro* susceptibility of local isolates to chloroquine (CQ), quinine (QN), artemisinin (ART) and the amodiaquine metabolite monodesethylamodiaquine (MdAQ) were monitored the using the DELI test [17] before (1997) and during the deployment (2000-2004) of artesunate+amodiaquine combination in Mlomp, a village in the district of Oussouye in Casamance, Southern Senegal, where malaria is mesoendemic (25 infective bites/person-year) and transmission occurs year-round with a peak during the rainy season (July-December). Samples for the *in vitro* assay were from consecutive patients recruited as part of an observational study [18] with a *P. falciparum* mono-infection and parasitaemia > 0.2% [19].

RESULTS

IC_{50}s from 242 subjects for CQ, 250 subjects for QN, 236 subjects for MdAQ and 183 subjects for ART were used in these statistical analyses [14]. The number of subjects with *in vitro* results was different among years and products tested (**Table 1**). Means with two standard deviations along with data distributions are plotted in **Figure 1**. Mean, Geometric means and GLSMs are presented together in Table 1. For all products except log-transformed ART, values were not normally distributed (Kolmogorov-Smirnov test $p<0.05$).

Results of the analyses using the different methods

The ANOVA pair wise means comparisons (**Table 2**) showed no significant differences for CQ IC_{50} values. For MdAQ, statistically significant increases were observed between 1997 and 2000-2004, while there were decreases in IC_{50}s between 2000-2004, 2001-2004 and 2002-2004. For QN, a statistically significant increase was observed between 1997 and 2001, and a decrease between 2001-2002 and 2001-2004 (Figure 1). For ART, no statistically significant differences were observed.

Table 1. Means of raw and $\log_e$-transformed IC_{50}s, geometric means and Geometric Least Squares Means

	1997			2000			2001			2002			2003			2004			Normality
Variable	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	**N**	**Mean**	**STD**	**p-value**
Means (raw values)																			
CQ	31	236	203.4	33	173.2	223	31	251.4	236.7	46	131.3	104	34	143.8	91.03	67	171.5	157.2	0.01
QN	46	234.6	150.2	28	305.8	210.4	31	444	286.2	40	252.6	273.6	34	280.8	162.8	58	207.5	149.8	0.01
MdAQ	45	12.8	12.4	33	85.1	167.8	31	57.7	63.2	43	42.9	32.3	34	31.1	21.3	64	21.7	17.6	0.01
ART	10	3.6	0.8	31	9.8	12.0	31	6.1	6.6	40	5.7	4.5	34	7.4	5.9	36	7.5	5.0	0.01
Means (natural log values)																			
CQ	31	4.9	1.4	33	4.4	1.2	31	5	1.2	46	4.5	0.9	34	4.7	0.8	67	4.7	1	0.01
QN	46	5.2	0.8	28	5.5	0.8	31	5.9	0.7	40	5.1	0.9	34	5.5	0.5	58	5.1	0.8	0.04
MdAQ	45	2.1	0.9	33	3.8	1.0	31	3.6	0.9	43	3.5	0.7	34	3.2	0.6	64	2.8	0.9	0.04
ART	10	1.2	0.3	31	1.8	1.0	31	1.3	1	40	1.3	1.0	34	1.8	0.6	36	1.8	0.7	0.15
Geometric means																			
CQ	31	130.3	3.9	33	82.3	3.5	31	148.4	3.2	46	93.7	2.5	34	112.2	2.2	67	115.6	2.7	-
QN	46	181.3	2.3	28	237.5	2.2	31	350.7	2.1	40	169.0	2.6	34	244.7	1.7	58	162.4	2.1	-
MdAQ	45	8.5	2.5	33	43.8	2.7	31	38.5	2.4	43	33.1	2.1	34	25.3	1.9	64	15.8	2.4	-
ART	10	3.5	1.3	31	5.8	2.7	31	3.9	2.7	40	3.9	2.8	34	6.1	1.9	36	6.0	2.0	-
Geometric Least Squares Means																			
CQ	31	130.8	1.2	33	82.6	1.2	31	148.4	1.2	46	93.3	1.2	34	112.7	1.2	67	115	1.1	-
QN	46	181.3	1.1	28	238.2	1.2	31	350.1	1.1	40	168.4	1.1	34	245	1.1	58	162.2	1.1	-
MdAQ	45	8.5	1.1	33	44	1.2	31	38.5	1.2	43	33.1	1.1	34	25.3	1.2	64	15.9	1.1	-
ART	10	3.5	1.3	31	5.8	1.2	31	3.9	1.2	40	3.9	1.1	34	6.1	1.2	36	6.0	1.1	-

P-values are from the Kolmogorov-Smirnov test for normality. CQ = chloroquine; QN = quinine; MdAQ = monodesethylamodiaquine; ART = artemisinin

Using the GLM with 1997 as the reference year (**Table 3**), no relationship was found for CQ and ART, a significant positive estimate for MdAQ (IC_{50}s decreased) and a significant positive estimate for QN in 2001, followed by a negative estimate (IC_{50}s increased) in 2002.

GLSMR (**Table 4**) of CQ IC_{50}s showed no significant differences, consistent with a stable response to CQ over the study period (**Figure 2**). For MdAQ, there were statistically significant increases between 1997 and 2000-2004. From 2000 to 2004, there was a decrease in IC_{50}s which was significant between 2000 and 2004, 2001 and 2004, as well as 2002 and 2004. QN showed an increase between 1997 and 2001 and 2001-2002, and a significant decrease between 2002-2004. For ART, no statistically significant changes were found (Figure 2).

Comparison of the results obtained with the different methods used

GLSMRs and ANOVA generated consistent results. With the GLM, results were similar to the two other tests for CQ and ART; for MdAQ, the GLM identified only a decrease between 1997 and 2001; for QN, it showed an increase between 1997 and 2001, while the ANOVA and GLSMRs revealed a decrease for 2001-2002 and 2001-2004 periods respectively.

For comparison, when this set of data is analysed with GLSMRs with no random intercept and 95%CIs calculated without adjustment for multiple comparisons, results are slightly different from the current GLSMRs. With the former, significant differences are found for 1997-2000 for CQ and ART, between 2001-2002 for CQ and 2002-2003 for ART. There are two significant differences with 2000 as reference year in comparison with 2002 and 2004, which are not found with the current GLSMRs. Similarly, 2001 is different from 2003 and 2003 from 2004. In the case of ART, the year 2003 is significantly different from the oth-

ers while it was not with in the current GLSMRs.

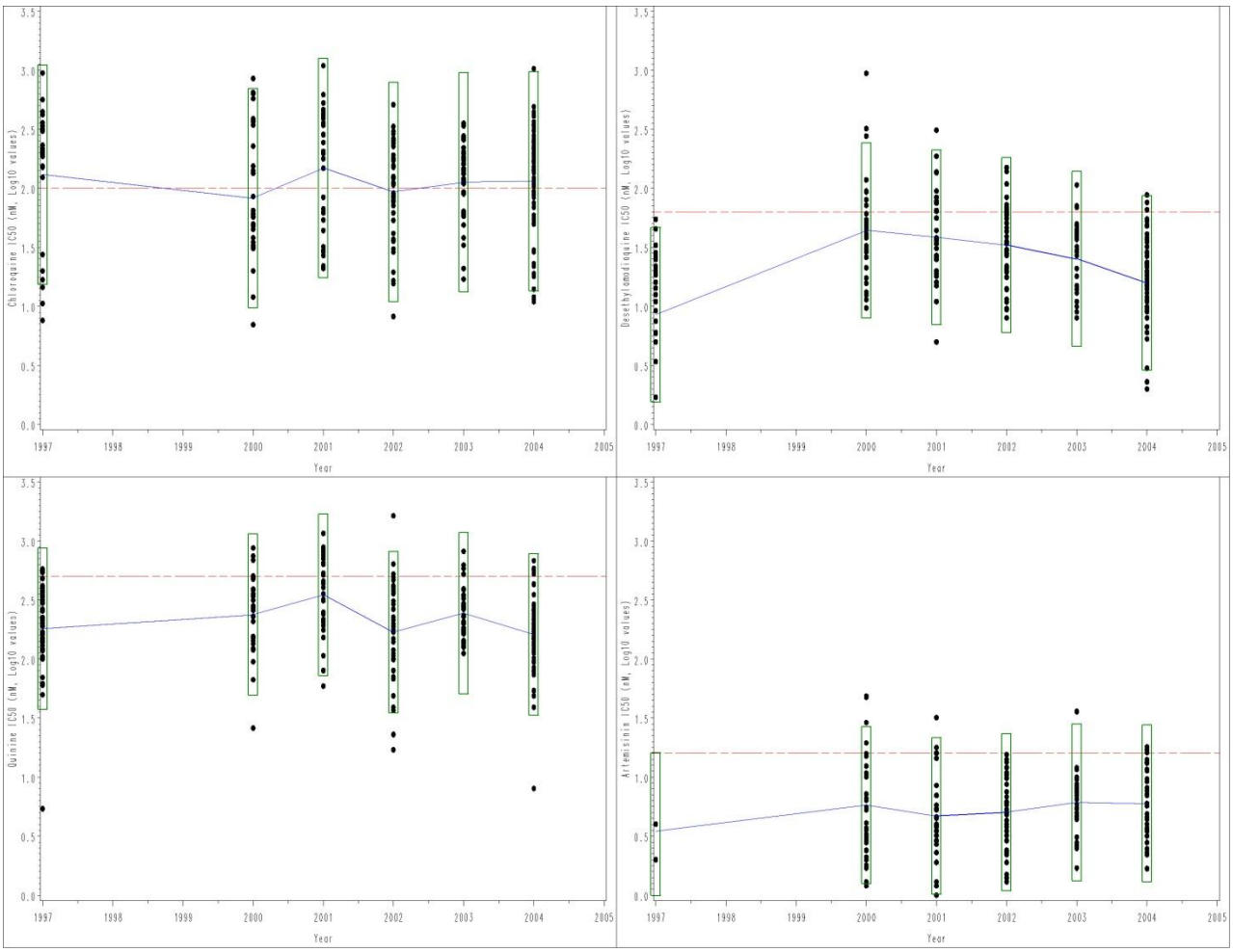

Figure 1. Scatterplots of the log_e-transformed values of the quinine (QN), chloroquine (CQ), monodesethylamodiaquine (MdAQ), and artemisinin (ART) IC_{50}s between years

The straight line is the cut-off value for susceptibility to the products (here: 100 nM for CQ, 60 nM for AQ, 500 nM for QN and 15 nM for ART [4]). The means of each year are connected with a trend line. The box around each year distribution of values represents two standard deviation of the mean.

DISCUSSION

In this study, three different statistical methods to assess changes of IC_{50} over time (ANOVA, GLM, GLSMRs) were compared. The use of data from a single site of moderate to high transmission (25 infecting bites per person-year), and with consistent treatment policies and practices, meant that all patients were expected to be infected with parasites having been under the same degree of drug pressure.

Normality assumption

Data were not normally distributed, even after log_e-transformation, for all drugs except log_e-transformed ART.

Table 2. Pairwise Least Squares Means comparisons following ANOVA of chloroquine (CQ), mono-desethylamodiaquine (MdAQ), Quinine (QN) and Artemisinin (ART) log_e-transformed IC_{50} (nM) between years

	MdAQ	CQ			QN			ART					
year Comparison	Difference Between Means	Simultaneous 95% Confidence Limits		Sig.	Difference Between Means	Simultaneous 95% Confidence Limits	Sig.	Difference Between Means	Simultaneous 95% Confidence Limits		Sig.	Difference Between Means	Simultaneous 95% Confidence Limits	Sig.
1997 - 2000	-1.6	-2.3	-1.0	***	0.5	-0.4	1.4	-0.3	-0.9	0.4		-0.5	-1.6	0.5
1997 - 2001	-1.5	-2.2	-0.8	***	-0.1	-1.0	0.8	-0.7	-1.3	0.0	***	-0.1	-1.2	0.9
1997 - 2002	-1.4	-2.0	-0.7	***	0.3	-0.5	1.2	0.1	-0.5	0.6		-0.1	-1.1	0.9
1997 - 2003	-1.1	-1.7	-0.4	***	0.1	-0.7	1.0	-0.3	-0.9	0.3		-0.6	-1.6	0.5
1997 - 2004	-0.6	-1.2	-0.1	***	0.1	-0.6	0.9	0.1	-0.4	0.6		-0.5	-1.6	0.5
2000 - 1997	1.6	1.0	2.3	***	-0.5	-1.4	0.4	0.3	-0.4	0.9		0.5	-0.5	1.6
2000 - 2001	0.1	-0.6	0.8		-0.6	-1.5	0.3	-0.4	-1.1	0.3		0.4	-0.3	1.1
2000 - 2002	0.3	-0.4	0.9		-0.1	-0.9	0.7	0.3	-0.3	1.0		0.4	-0.3	1.1
2000 - 2003	0.6	-0.1	1.2		-0.3	-1.2	0.6	0.0	-0.7	0.6		-0.1	-0.8	0.7
2000 - 2004	1.0	0.4	1.6	***	-0.3	-1.1	0.4	0.4	-0.2	1.0		0.0	-0.7	0.7
2001 - 1997	1.5	0.8	2.2	***	0.1	-0.8	1.0	0.7	0.0	1.3	***	0.1	-0.9	1.2
2001 - 2000	-0.1	-0.8	0.6		0.6	-0.3	1.5	0.4	-0.3	1.1		-0.4	-1.1	0.3
2001 - 2002	0.2	-0.5	0.8		0.5	-0.4	1.3	0.7	0.1	1.4	***	0.0	-0.7	0.7
2001 - 2003	0.4	-0.3	1.1		0.3	-0.6	1.2	0.4	-0.3	1.0		-0.5	-1.2	0.3
2001 - 2004	0.9	0.3	1.5	***	0.3	-0.5	1.0	0.8	0.2	1.4	***	-0.4	-1.2	0.3
2002 - 1997	1.4	0.7	2.0	***	-0.3	-1.2	0.5	-0.1	-0.6	0.5		0.1	-0.9	1.1
2002 - 2000	-0.3	-0.9	0.4		0.1	-0.7	0.9	-0.3	-1.0	0.3		-0.4	-1.1	0.3
2002 - 2001	-0.2	-0.8	0.5		-0.5	-1.3	0.4	-0.7	-1.4	-0.1	***	0.0	-0.7	0.7
2002 - 2003	0.3	-0.4	0.9		-0.2	-1.0	0.6	-0.4	-1.0	0.2		-0.5	-1.1	0.2
2002 - 2004	0.7	0.2	1.3	***	-0.2	-0.9	0.5	0.0	-0.5	0.6		-0.4	-1.1	0.2
2003 - 1997	1.1	0.4	1.7	***	-0.1	-1.0	0.7	0.3	-0.3	0.9		0.6	-0.5	1.6
2003 - 2000	-0.6	-1.2	0.1		0.3	-0.6	1.2	0.0	-0.6	0.7		0.1	-0.7	0.8
2003 - 2001	-0.4	-1.1	0.3		-0.3	-1.2	0.6	-0.4	-1.0	0.3		0.5	-0.3	1.2
2003 - 2002	-0.3	-0.9	0.4		0.2	-0.6	1.0	0.4	-0.2	1.0		0.5	-0.2	1.1
2003 - 2004	0.5	-0.1	1.1		0.0	-0.8	0.7	0.4	-0.2	1.0		0.0	-0.7	0.7
2004 - 1997	0.6	0.1	1.2	***	-0.1	-0.9	0.6	-0.1	-0.6	0.4		0.5	-0.5	1.6
2004 - 2000	-1.0	-1.6	-0.4	***	0.3	-0.4	1.1	-0.4	-1.0	0.2		0.0	-0.7	0.7
2004 - 2001	-0.9	-1.5	-0.3	***	-0.3	-1.0	0.5	-0.8	-1.4	-0.2	***	0.4	-0.3	1.2
2004 - 2002	-0.7	-1.3	-0.2	***	0.2	-0.5	0.9	0.0	-0.6	0.5		0.4	-0.2	1.1
2004 - 2003	-0.5	-1.1	0.1		0.0	-0.7	0.8	-0.4	-1.0	0.2		0.0	-0.7	0.7

Table 3. Generalized linear model (SAS System Proc Genmod) of chloroquine (CQ), monodesethy-lamodiaquine (MdAQ), quinine (QN) and artemisinin (ART) IC_{50} (nM) with the year as independent parameter (log_e-transformed values and 1997 as the reference value)

		CQ			MdAQ			QN			ART		
Parameter		**Estimate**	**Std Error**	**p**	**Estimate**	**Std Error**	**p**	**Estimate**	**Std Error**	**p**	**Estimate**	**Std Error**	**p**
Intercept		4.9	0.2	<.0001	2.1	0.1	<.0001	5.2	0.1	<.0001	1.2	0.3	<.0001
ear*	**2000**	-0.5	0.3	N.S.	1.6	0.2	<.0001	0.3	0.2	N.S.	0.5	0.3	N.S.
	2001	0.1	0.3	N.S.	1.5	0.2	<.0001	0.7	0.2	0.0003	0.1	0.3	N.S.
	2002	-0.3	0.2	N.S.	1.4	0.2	<.0001	-0.1	0.2	N.S.	0.1	0.3	N.S.
	2003	-0.1	0.3	N.S.	1.1	0.2	<.0001	0.3	0.2	N.S.	0.6	0.3	N.S.
	2004	-0.1	0.2	N.S.	0.6	0.2	0.0001	-0.1	0.2	N.S.	0.5	0.3	N.S.
Scale		1.1	0.0	-	0.8	0.0	-	0.8	0.0	-	0.8	0.0	-

The ANOVA can be used on non-normally distributed values (although in this case conclusions are less robust), and allows for multiple pair wise comparisons adjustment (but then residuals must be checked for independence and normality as well as homosedasticity, i.e the condition whereby variances are equal.) The GLM requires normally distributed values when using an identity link function (i.e. an assumption of normal distribution for the studied parameter) for comparison with a unique reference (baseline) value. For non-normally distributed data, a different model must be used for each different reference value of the independent factor. The GLSMRs approach can use non-normally distributed values and allows adjusted multiple comparisons between years.

GLSMR

The GLSMR had broader applicability than the other methods because, even if a mixed model is used to obtain the LSMs, it does not require either normally distributed IC_{50}s or variance homogeneity. However, one needs to verify the assumptions needed for a mixed model such as the normality of the residuals, the normality of the random effects, and the independence of the residuals and the random effects. As log_e-transformation serves the purpose of deriving GLSMs of the results, the linear mixed model allowed to relax the assumption of independence of the model residuals and to account for the inherent variability of the data structure in a more flexible way. The *in vitro* test reflects the susceptibility of the whole parasite population in one isolate, and thus cannot separate the effects of the various parasite clones in a given infected subject. Therefore one cannot parameterize the within-subject effect or within-parasite population effect in the linear mixed model. However, it can be argued that these effects are taken into account in the overall mean, which was defined as random in the model.

GLSMRs calculated at a 5% level without an adjustment for multiple comparisons are more likely to wrongly detect a significant difference because of the multiplicity of the statistical tests performed than the GLSMRs at a 1% level and with an adjustment for multiplicity.

Linear models

ANOVA and pair wise means comparisons between years were good indicators of a difference between years. Noteworthy, in bioequivalence studies GLSMR are generally computed using an ANOVA model, but effects specified as random in a linear model are treated as a fixed factor as they serve the sole purpose of producing the corresponding expected mean squares [20].

Table 4. IC $_{50}$s for chloroquine (CQ) quinine (QN), monodesethylamodiaquine (MdAQ) and artemisinin (ART) in nM. Geometric Least Squares Means Ratio between years

	MdAQ				CQ			QN			ART		
Variable	**2 one-sided 90% limits**	**GLS Mean Ratio (%)**	**90%CI**	**2sided t p-value**	**GLS Mean Ratio (%)**	**90%CI**	**2sided t p-value**	**GLS Mean Ratio (%)**	**90%CI**	**2sided t p-value**	**GLS Mean Ratio (%)**	**90%CI**	**2sided t p-value**
1997 vs. 2000	[80,125]	516.7	[266.4,1002.3]	<0.0001	63.2	[25.5,156.7]	N.S.	131.4	[69.3,249.2]	N.S.	166.8	[57.2,486.0]	N.S.
1997 vs. 2001	[80,125]	452.3	[230.3,888.2]	<0.0001	113.5	[45.1,285.4]	N.S.	193.1	[103.8,359.2]	0.005	110.9	[38.0,323.0]	N.S.
1997 vs. 2002	[80,125]	388.2	[209.6,719.2]	<0.0001	71.3	[30.7,165.8]	N.S.	92.9	[52.1,165.4]	N.S.	110.8	[39.2,313.4]	N.S.
1997 vs. 2003	[80,125]	297.1	[154.0,573.0]	<0.0001	86.2	[35.0,212.3]	N.S.	135.1	[73.9,247.2]	N.S.	175.6	[61.0,505.7]	N.S.
1997 vs. 2004	[80,125]	186.3	[106.1,326.9]	0.003	88.0	[40.0,193.5]	N.S.	89.5	[52.8,151.6]	N.S.	172.5	[60.3,493.5]	N.S.
2000 vs. 2001	[80,125]	87.5	[42.5,180.4]	N.S.	179.6	[72.4,445.3]	N.S.	147.0	[73.3,294.9]	N.S.	66.5	[31.5,140.3]	N.S.
2000 vs. 2002	[80,125]	75.1	[38.5,146.7]	N.S.	112.9	[49.3,258.3]	N.S.	70.7	[36.6,136.5]	N.S.	66.4	[32.9,134.2]	N.S.
2000 vs. 2003	[80,125]	57.5	[28.4,116.5]	N.S.	136.4	[56.1,331.1]	N.S.	102.9	[52.0,203.3]	N.S.	105.3	[50.7,218.5]	N.S.
2000 vs. 2004	[80,125]	36.1	[19.4,67.0]	<0.0001	139.2	[64.3,301.2]	N.S.	68.1	[36.8,125.9]	N.S.	103.4	[50.3,212.5]	N.S.
2001 vs. 2002	[80,125]	85.8	[43.4,169.6]	N.S.	62.8	[27.0,146.1]	N.S.	48.1	[25.4,91.1]	0.002	100.0	[49.4,202.0]	N.S.
2001 vs. 2003	[80,125]	65.7	[32.0,134.7]	N.S.	75.9	[30.8,187.0]	N.S.	70.0	[36.0,135.8]	N.S.	158.4	[76.3,328.7]	N.S.
2001 vs. 2004	[80,125]	41.2	[21.9,77.5]	0.000	77.5	[35.2,170.5]	N.S.	46.3	[25.6,83.9]	<0.0001	155.6	[75.7,319.8]	N.S.
2002 vs. 2003	[80,125]	76.5	[39.4,148.6]	N.S.	120.8	[53.2,274.6]	N.S.	145.5	[78.0,271.2]	N.S.	158.5	[79.8,314.7]	N.S.
2002 vs. 2004	[80,125]	48.0	[27.1,84.8]	<0.0001	123.3	[61.5,247.2]	N.S.	96.3	[55.7,166.8]	N.S.	155.7	[79.2,305.9]	N.S.
2003 vs. 2004	[80,125]	62.7	[33.9,115.8]	N.S.	102.1	[47.5,219.2]	N.S.	66.2	[37.2,117.9]	N.S.	98.2	[48.6,198.4]	N.S.

These expected mean squares lead to the traditional ANOVA components without accounting for the random effect in the variance.

In the GLSMRs calculations, the mixed linear model was computed using restricted maximum likelihood to evaluate variance parameters, which are in general preferred to ANOVA estimates [20]. Furthermore, mixed models are commonly used when there are different levels of clustering in the observations. The sole level of grouping was the year (treated as fixed in the model), so no other particular variable (or grouping level) was de-fined as random. The reasons for treating the time variable as discrete (i.e. by calendar year and not as a continuous variable) are: (i) there was a 3-year gap between 1997 (the baseline) and 2000 (the first of a series of five consecutive years.); (ii) only qualitative or discrete variables allowed for the model to simply extract estimates for the different categories of the year effect; (iii) the majority of malaria cases and treatments cluster between July-November during the wet season. Hence, subjects were grouped by year and it was assumed that there was a random part of measurements within years due to the contribution of the subject and the parasite strains subjects were infected with. Specifically, the number of isolates for each year was not the same and IC_{50}s varied considerably from year to year (Table 1).

The GLM appears not to be suitable to compare IC_{50}s because it treated values as if there were repeat measures from the same subjects, while isolates came from different individuals. In addition, IC_{50}s were non-normally distributed despite $\log_e$-transformation.

Estimates of the IC_{50}

It is clear that customary approaches are not satisfactory as the difficulty in the analyses is that the data are not time series or longitudinal data. They are also not normally distributed -a necessary condition to use parametric statistical tests, and a critical point of this work. In a recently published paper, Kaddouri *et al.* [21] developed a new inhibitory sigmoid Emax statistical model to estimate more precisely the IC_{50} of a given subject. However, it requires cut-off values for resistance of the studied parasites strains to a range of treatment, an element which is not easy to derive for antimalarial drugs. A Bayesian approach was also proposed recently to provide a correction of the estimate of the true IC_{50} [22]. This work was based on the assumption that resistance is systematically overestimated because: (i) the precision of the estimated IC_{50} value of the most resistant isolate will usually be the poorest of all the isolates assayed, (ii) sigmoid curve fitting or probit analysis of a unique isolate takes no account of other isolates in the series tested. This approach requires the distribution of measured IC_{50} to be established before using it to produce a large number of points and estimate the median as the true IC_{50}. In doing so, one is inevitably faced with the problem of normality and the choice to apply data transformations such as log or Box-Cox [23]. This work offers an alternative way to deal with data transformation and normality condition in the context of the evolution of microbial susceptibility to drugs.

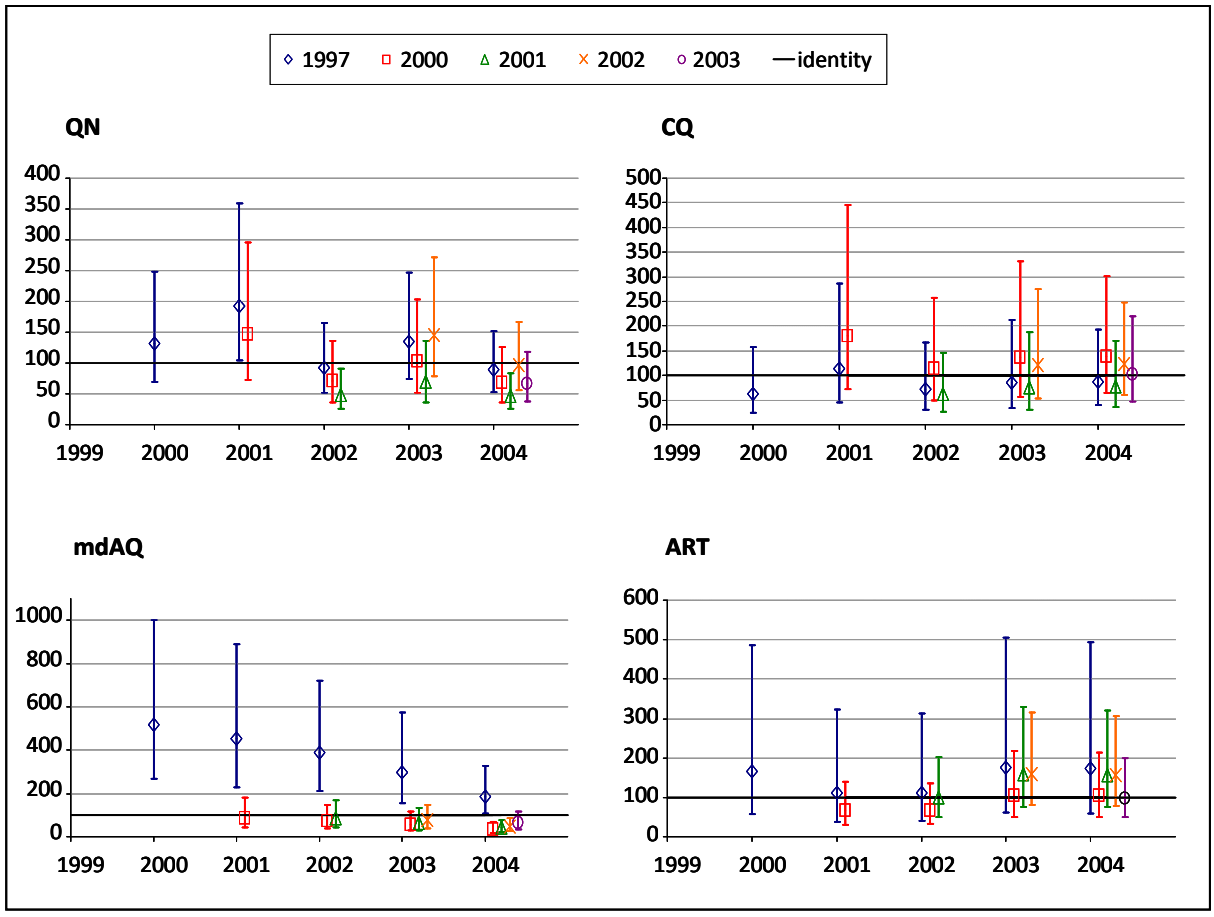

Figure 2. Geometric Least Squares Means Ratios of the IC_{50}s to quinine (QN), chloroquine (CQ), monodesethylamodiaquine (MdAQ), and artemisinin (ART)

The legend indicates the reference year for the ratio. The x-axis indicates the year tested in the ratio. A logarithmic scale was applied for QN

Expression of results

GLSMRs were found to be more intuitive, as results are expressed as percentage difference (increase or decrease) between two years, while with LSMs comparisons increases and decreases from the reference appear as inverted (they are marked with a negative and a positive sign, respectively). As geometric means are generally used to express IC_{50}s of a pool of isolates, GLSMR are naturally easier to understand and interpret than the other statistical methods tested here.

Two different plots were also produced in order to illustrate the difficulties in interpreting trends over time in drug susceptibility. Figure 1 is a traditional way of plotting the distribution of IC_{50}s with cut-off values above which a strain is resistant to a given drug. This makes the reader falsely interpret means against the cut-off, while there is a great variability in the data and no validated thresholds for the majority of antimalarial drugs. In addition, this display does not provide any indication of the significance (or lack thereof) of change between years. Figure 2 is based on the GLSMR results and provides a direct comparison of GLSM between years; it does not need the cut-off values; it depicts visually a statistical test as the confidence intervals of each GLSMR against the line of identity between sets of data (here: years).

Conclusion

Treatment policy decisions would benefit from reliable information on changes in susceptibility of parasite or bacterial isolates

to drugs over time. This entails an adequate statistical method, which can also account for the inherent variability of *in vitro* drug susceptibility tests (Figure 2). This is particularly important for antimalarial drugs and cases alike where validated thresholds for resistance are not available. The underlying linear mixed model of GLSMRs allowed accounting for this variability and for unequal number of isolates collected during field testing. Based on these data GLSMRs appear to be more accurate and to offer advantages over other tests for the "longitudinal" analysis of IC_{50}s. We used a simple statistical model which produces easily interpretable results and can be found in any statistical software. Finally, the utility of GLSMRs in monitoring drug susceptibility of not only malaria parasites but also other microbes should be further tested.

Competing interests

The author(s) declare that they have no competing interests.

Authors' contribution

All authors read and approved the final manuscript.

MV conceived the methodology and conducted the analyses; PO contributed to the concept; both contributed to the writing of the paper. PO is a staff member of the World Health Organization. The authors alone are responsible for the views expressed in this publication and they do not necessarily represent the decisions, policy or views of the World Health Organization.

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Management of fever in rural dispensaries in Southern Senegal following malaria treatment policy change

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ABSTRACT

Background. Current policy for treating uncomplicated falciparum malaria is artemisinin combination therapy (ACT) upon parasitological confirmation. Implementing such policy has proved challenging in several settings. In Senegal, artesunate plus amodiaquine is now the first-line treatment. Practices in two rural dispensaries in Southern Senegal were studied and compared over the period 2004-08. Mlomp started artesunate plus amodiaquine on parasitological diagnosis with operational research support since 2000; Elinkinde followed the national policies (amodiaquine-sulfadoxine/pyrimethamine in 2004-06; artesunate-amodiaquine from 2006, rapid diagnostic tests from 2007).

Methods. Data were retrieved from clinic registries and categorised in five case management classes. Analyses were done by semester (corresponding to low and high malaria transmission seasons). Logistic models were used to calculate Odd Ratios with 95% confidence intervals for changes of case management practices over time and for the comparison of the two dispensaries.

Results. On average community access to the facilities was similar (0.8 consultations per person/year; stable in Mlomp while it almost tripled in Elinkinde between 2004-08); 13,509 and 6923 consulted for fever in Mlomp and Elinkinde respectively. Mlomp dispensed 8221 antimalarial treatments (22% parasitologically confirmed), Elinkinde 4085 (2% confirmed). Artesunate plus amodiaquine accounted for 28% and quinine for 53% of treatments in Mlomp, compared to 36% and 46% in Elinkinde.

The prevalence rates of total consultations, consultations for fever, antimalarial treatments were significantly higher in Mlomp until 2006, similar or higher in Elinkinde until mid 2008. In Mlomp the odds of antimalarial treatment being given with no test done or despite a negative test did not decrease over the study period.

Conclusions. Malaria prevalence and treatments are decreasing in this rural district but deviations from the national guidelines are observed. Antimalarial treatments continue to be given without parasitological confirmation despite clear policies and training; use of non-artemisinin monotherapy, though decreasing, continues. In Mlomp, initial staggered, research-supported implementation facilitated adherence to policy but achievements are being eroded. Corrective actions are needed to reduce unnecessary treatments and their negative consequences (costs, potential toxicity, case mismanagement).

INTRODUCTION

Artemisinin-based combination treatment (ACT) is the WHO-recommended first-line treatment of uncomplicated falciparum malaria [World Health Organization 2006] and has become policy in malaria-endemic countries [World Health Organization 2008], but problems are being faced when implementing the policy locally.

The malaria treatment policy in Senegal changed in 2006 [Ministère de la Santé et de la Prévention Sociale du Sénégal 2007] from chloroquine or quinine given on clinical diagnosis to artesunate plus amodiaquine or artemether/lumefantrine upon parasitological confirmation for patients of all ages, but rapid diagnostic tests (RDTs) only became available in 2007. Prior to the full deployment of the new ACT policy, an interim measure consisting of amodiaquine plus sulfadoxine/pyrimethamine was used from 2004-05. Since 2006, only artesunate plus amodiaquine and quinine are made available to public sector facilities. Drugs and RDTs are procured through tenders by the country central pharmacy (Pharmacie Nationale d'Approvisionnement, PNA) and channelled through the regional and district pharmacies to be supplied to hospitals and peripheral health centres.

On occasion of the policy change, the national malaria control programme (Programme National de Lutte contre le Paludisme, PNLP) organized training courses for all categories of health providers (doctors, nurses, health workers). Regular refresher courses are also organized [Ministère de la Santé et de la Prévention Sociale du Sénégal. Programme National de Lutte Contre le Paludisme (PNLP) Juin 2007]. Compliance to policies is regularly verified by inspection of clinic registries by a district supervisor.

This papers reports fever management practices in two village health centres of southern Senegal during 2004-08. Here, malaria is holoendemic, with low transmission during the dry season (January-June) and higher intensity during the rainy season (July-December). The last entomological survey, conducted before the policy changes, showed 25 infectant bites person-year [Sokhna, CS 2000].

MATERIALS & METHODS

The district of Oussouye (Basse Casamance region, South-western Senegal) has four main peripheral dispensaries and one hospital for 44,600 inhabitants. The dispensaries of Mlomp and Elinkinde serve a population of ~6,000 and ~3,500 respectively. Each dispensary has one nurse, a technician and community health worker, supervised by the district medical doctor (M.Cisse until 2006,

M.Badiane thereafter, based in Oussouye). Neither village have private pharmacies or vendors. The dispensary in Mlomp (but not the one in Elinkinde) has a trained microscopist. The RDT used is Paracheck ® Pf rapid test (based on the detection of *Plasmodium falciparum* Histidine Rich Protein II (HRP II)). All treatments are dispensed and supervised by a nurse at the dispensary.

Data were extracted from clinical registries on site and aggregated on a monthly basis over the study period, specifically: number of consultations; reason for consulting (fever or other symptoms); diagnosis (malaria on clinical ground, parasitological confirmation, other diagnosis); and treatment administered. Five case management categories were identified: (1) patients with fever, a positive malaria test (microscopy or rapid enzymatic test) and an antimalarial treatment (FT+AT) (= antimalarial treatment for parasitologically-confirmed malaria), (2) patients with fever, a negative test and an antimalarial treatment (FT-AT) (= antimalarial treatment despite a negative test), (3) patients with fever, a negative test and a treatment other than antimalarial (FT-OT) (= other treatment after excluding malaria on a negative test), (4) patients with fever, no test done and an antimalarial treatment (FNTAT) (= antimalarial treatment on clinical ground), (5) patients with symptoms diagnosed as other than malaria and treated with drugs other than antimalarials (FOS) (= other treatment after excluding malaria on clinical ground).

Two approaches were used to study case management over time: (a) comparing all categories to category 5 (non-malaria fever and treatments FOS) as the reference group within each semester; (b) within-category comparison using 2004 as the reference. Calculations were made separately for the two dispensaries (for Elinkinde restricted to category 4 and 5 as parasitological diagnosis was introduced only in 2007).

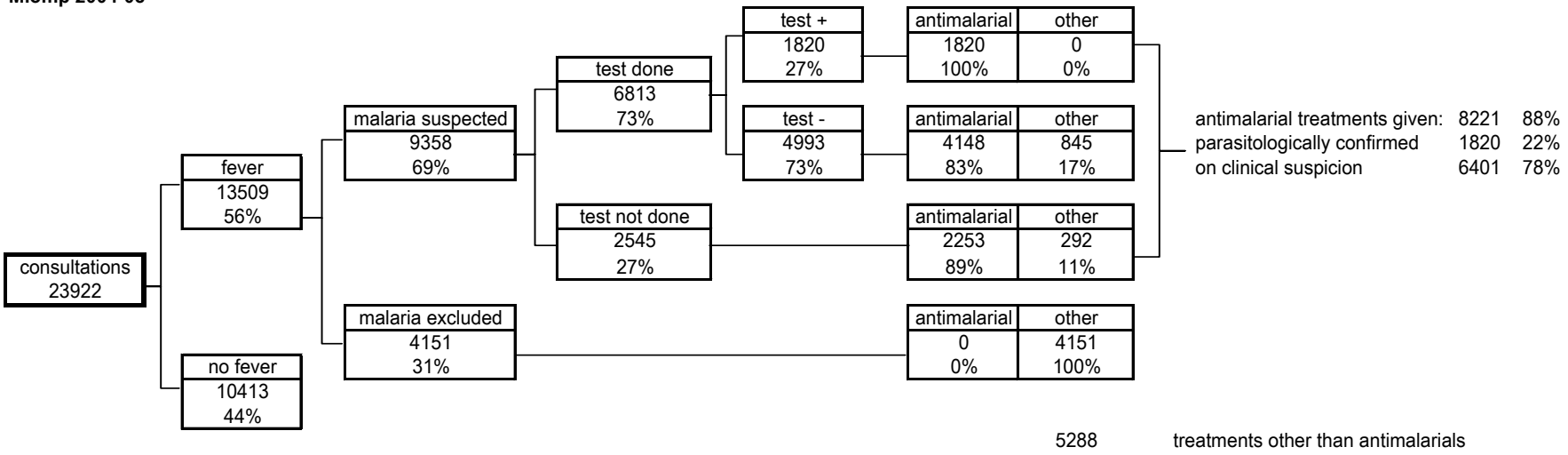
For both approaches, a logistic model was used with the semester as the time unit and with the response variable being the ratio between fevers and total consultations. The model integrates the interaction between time and case management strategies (1-5 above) and estimates the Odds Ratio with 95% confidence intervals (OR, 95%CI). The semester was selected after comparing different time units (month, trimester, semester, year) using the Akaike criteria for the best goodness of fit. The semesters also correspond to the transmission seasons (low and high). Sensitivity analyses were conducted in order to detect possible bias introduced by the transmission season on the relationship between fever counts and case management categories.

The two dispensaries were compared in terms of 6-month prevalence of consultations, fevers and malaria treatments (occurrence of the above corrected for the population in each village).

ORs of prevalences in Mlomp versus Elinkinde were evaluated with a logistic regression for each semester; the model included site, semester and interaction between site and semester.

Figure 1. Summary findings in the two dispensaries

Mlomp 2004-08



Elinkinde 2004-08

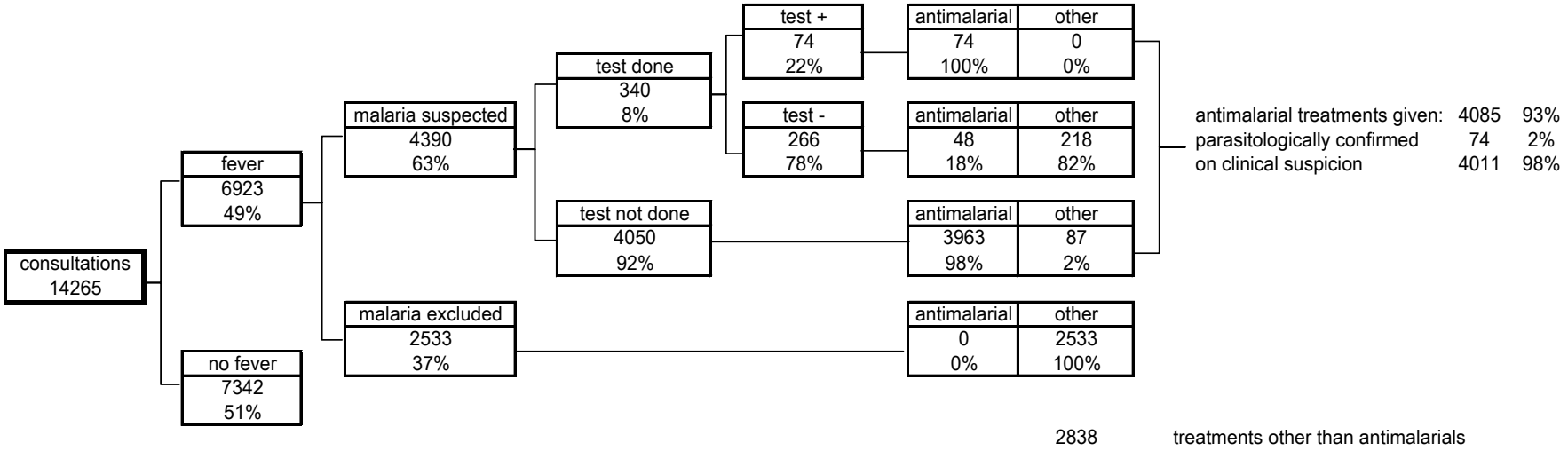


Table 1. Summary findings by year for the two dispensaries

	Mlomp						Elinkinde					
	2004	**2005**	**2006**	**2007**	**2008**	**TOT**	**2004**	**2005**	**2006**	**2007**	**2008**	**TOT**
TOTAL CONSULTATIONS	5566	5212	4131	4528	4485	23922	1454	1890	2876	4524	3521	14265
TOTAL FEVERS	3216	3307	2089	2705	2192	13509	924	1003	1515	2344	1137	6923
% fever/consultations	*58%*	*63%*	*51%*	*60%*	*49%*	*56%*	*64%*	*53%*	*53%*	*52%*	*32%*	*49%*
antimalarial treatment	2595	2672	1090	979	885	8221	689	772	1130	1305	189	4085
parasitologically positive	581	524	324	198	193	1820	0	0	0	17	57	74
parasitologically negative	1366	1539	488	545	210	4148	0	0	0	44	4	48
parasitological diagnosis not done	648	609	278	236	482	2253	689	772	1130	1244	128	3963
% fevers with parasitological diagnosis (pos or neg)	*75%*	*77%*	*74%*	*76%*	*46%*	*73%*	*0%*	*0%*	*0%*	*5%*	*32%*	*NA*
% of antimalarial treatments in test-negatives / tests done	*70%*	*75%*	*60%*	*73%*	*52%*	*70%*	*NA*	*NA*	*NA*	*72%*	*7%*	*39%*
% fevers treated with an antimalarial	*81%*	*81%*	*52%*	*36%*	*40%*	*61%*	*75%*	*77%*	*75%*	*56%*	*17%*	*59%*
other treatment, diagnosis unspecified	37	84	368	160	488	1137	0	0	0	0	305	305
diagnosis other than malaria	584	551	631	1566	819	4151	235	231	385	1039	643	2533
% infections other than malaria/total fevers	*18%*	*17%*	*30%*	*58%*	*37%*	*31%*	*25%*	*23%*	*25%*	*44%*	*57%*	*37%*
% fevers with a parasitological test done	*62%*	*65%*	*56%*	*33%*	*30%*	*49%*	*0%*	*0%*	*0%*	*3%*	*25%*	*5%*
% test positive / done	*29%*	*24%*	*28%*	*22%*	*30%*	*27%*	*NA*	*NA*	*NA*	*28%*	*20%*	*22%*

RESULTS

During 2004-2008 a total of 23,922 patients attended the Mlomp dispensary, 13,509 (56%) with fever or history of fever, compared to 14,265 and 6923 (48%) in Elinkinde (Table 1 shows the yearly counts and proportions of the main parameters collected). Consultations averaged 0.8 per person-year in both places over the study period. However, in Mlomp they decreased slightly from 0.92 to 0.74, while in Elinkinde they increased dramatically from 0.41 in 2004 to 1.28 in 2007 and then 0.99 in 2008. The 6-month counts of total consultations, all fevers, total antimalarial treatments (whether on clinical or parasitological grounds) and low respiratory tract infections are presented in Figure 2a for Mlomp and Figure 2b for Elinkinde.

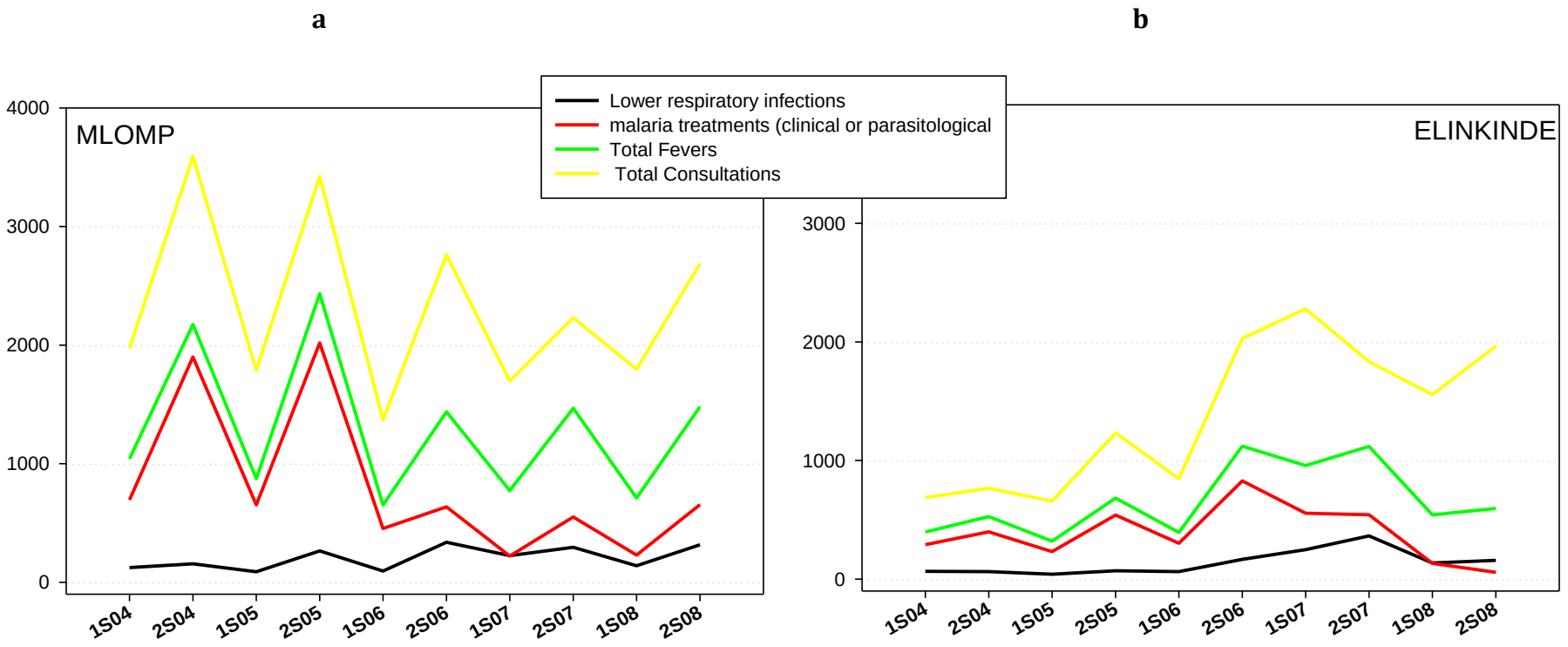
Case management practices

Mlomp. At the time of this study, the ACT policy was expected to be fully implemented in Mlomp. Treatment with artesunate plus amodiaquine on parasitological diagnosis (microscopy) had been introduced in 2000 on a pilot scale and then at full scale from 2001[Brasseur, P, *et al.* 2007] under an operational research programme; since 2007 routine diagnosis of malaria is now done by RDT.

During 2004-08 consultations numbered 23,922 of which 13,509 (56%) were for fever; malaria was suspected in 9358 of these (69% of fevers) and a parasitological diagnosis was done in 6813 (73% of suspected malaria cases) which tested positive for *Plasmodium falciparum* in 27% (n=1820), all treated with an antimalarial (5280 smears, 27.2% positive and 1132 RDTs, 26.1% positive). An additional 6401 antimalarial treatments were given to patients who tested negative (4148/4993, 83%) or had no test done (2253/2545, 89%) to give a total of 8221 antimalarial treatments (61% of the 13,509 cases of fever or 88% of the 9358 suspected malaria cases). Thus 22% of all antimalarial treatments were given on the basis of parasitological confirmation. When malaria had been excluded a-priori based on clinical presentation, no antimalarial was prescribed. An additional 5288 non-malaria treatments (39% of fevers) were dispensed (Figure 1.)

The annual breakdown is reported in Table 1. The annual number of consultations decreased by ~20% from 5566 to 4485 and the number of patients presenting with fever by ~32% from 3216 to 2192 between 2004-2008. The contribution of fevers to general consultations also dropped from 58% in 2004 to 49% in 2008.

Figure 2. Counts of total consultations, all fevers, total malaria treatments (whether on clinical or parasitological grounds) and lower respiratory infections by semester in Mlomp(a) and Elinkinde (b).



The number of antimalarial treatments dispensed decreased by 66% from 2595 in 2004 to 885 treatment courses in 2008, and the proportion of fevers treated with an antimalarial decreased from 81% to 40%. Concomitantly, while the proportion of fevers parasitologically tested dropped by half from 62 to 30%, the proportion of positive tests remained stable at an average 27%.

The most common antimalarial treatment dispensed in Mlomp was quinine (4516/8221, 55%), followed by artesunate plus amodiaquine (2303 treatments, 28%). Overall, 1663 of the 1820 treatments for parasitologically confirmed malaria (91%) were with either Quinine (855, 47%) or artesunate plus amodiaquine (808, 44%). Quinine accounted for 57% and 58% of the cases in which parasitology was negative or not done, respectively, compared to 22% and 26% for artesunate plus amodiaquine. In addition, 256 patients received a treatment of quinine followed by artesunate plus amodiaquine; chloroquine continued to be used sporadically (n=446), while the use of amodiaquine+sulfadoxine/pyrimethamine was limited (n=388) (Table 2.) The drop in antimalarial treatments observed over time (see above) was largely accounted for by a decrease in the use of quinine (from 1442 to 476). The use of artesunate plus amodiaquine fluctuated over time, but the general trend was towards a relative increase (from 21% to 46% of treatments); the number of treatments with artesunate plus amodiaquine is now exceeding the number of confirmed malaria cases since 2005 (in 2008 there were more than twice as many treatments as confirmed cases).

Elinkinde. In Elinkinde the interim treatment policy (amodiaquine plus sulfadoxine/pyrimethamine) was introduced in 2004 and the ACT policy (artesunate plus amodiaquine) in 2006, complemented by an RDT since 2007.

The total number of consultations in Elinkinde was 14,265 of which 6923 for fever (49%); malaria was suspected in 4390 of these (63% of fevers) and an RDT was done in 340 (8% of suspected malaria cases) which tested positive for *Plasmodium falciparum* in 22% (n=74). This corresponds to 2% of the total of 4085 antimalarial treatments dispensed (4011 on clinical suspicion). An additional 2838 non-malaria treatments (41% of fevers) were dispensed (Figure 1.)

The number of consultations increased from 1454 in 2004 to 3521 in 2008 with a peak at 4524 in 2007, which was paralleled by fevers (924 to 1137, with a peak of 2344 in 2007), while the contribution of fevers to consultations decreased markedly over time from 64% to 32%.

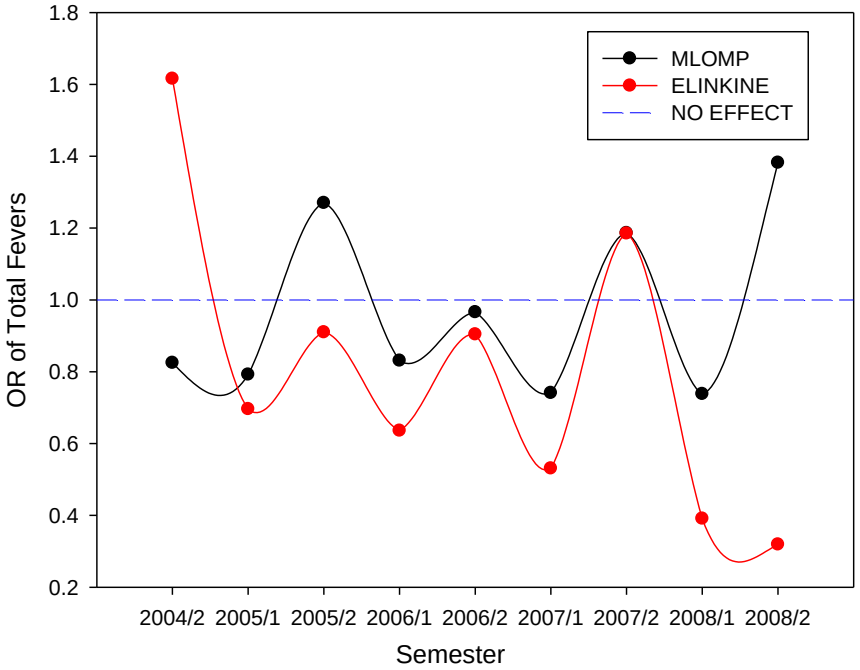
The number of antimalarial treatments initially rose from 689 to 1305 and then dropped to 189 in 2008 (Table 1.) The proportion of fevers treated with an antimalarial was stable at ~75% during 2004-06, and then dropped to 56% in 2007 and 17% in 2008.

Table 2. Antimalarial treatments dispensed during 2004-08 according to the type of parasitological diagnosis

	Mlomp								Elinkinde								ALL	
	smear +		smear -		smear not done		total		smear +		smear -		smear not done		total			
QN	855	(47.0%)	2355	(56.8%)	1306	(58.0%)	4516	(54.9%)	38	(51.4%)	10	(20.8%)	1145	(28.9%)	1193	(29.2%)	5709	(46.4%)
CQ	13	(0.7%)	292	(7.0%)	141	(6.3%)	446	(5.4%)	0	(0.0%)	0	(0.0%)	7	(0.2%)	7	(0.2%)	453	(3.7%)
ASAQ	808	(44.4%)	901	(21.7%)	594	(26.4%)	2303	(28.0%)	32	(43.2%)	38	(79.2%)	2062	(52.0%)	2132	(52.2%)	4435	(36.0%)
QN+ASAQ	102	(5.6%)	113	(2.7%)	41	(1.8%)	256	(3.1%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	0	(0.0%)	256	(2.1%)
AS/SP	7	(0.4%)	280	(6.8%)	101	(4.5%)	388	(4.7%)	0	(0.0%)	0	(0.0%)	725	(18.3%)	725	(17.7%)	1113	(9.0%)
Others	35	(1.9%)	207	(5.0%)	70	(3.1%)	312	(3.8%)	4	(5.4%)	0	(0.0%)	24	(0.6%)	28	(0.7%)	340	(2.8%)
Total	**1820**		**4148**		**2253**		**8221**		**74**		**48**		**3963**		**4085**		**12306**	

Legend: QN = quinine; CQ = chloroquine; AS = artesunate; AQ = amodiaquine; SP = sulfadoxine/pyrimethamine

Figure 3. Comparison of the 6-month prevalence rates of consultations for fever weighted by the total number of consultations in Mlomp and Elinkinde. Odd Ratios between the first semester of 2004 (reference) and all subsequent semesters through 2008. Horizontal dotted line at 1 = no difference



Over the entire study period, the most common treatment was artesunate plus amodiaquine (2132/4085 52%), essentially without parasitological diagnosis (n=2062). Quinine was the second most used drug (1193, 29%), followed by amodiaquine plus sulfadoxine/pyrimethamine (725, 18%) (Table 2). During 2004-08 the use of quinine decreased consistently (from 380 to 49) while amodiaquine plus sulfadoxine/pyrimethamine ceased to be used in May 2006 when artesunate plus amodiaquine became available.

Risk Of Fever As Reason For Consulting. Figure 3 shows the variations in the consultations for fever (weighted for total consultations) for each semester referred to the first semester of 2004, both in Mlomp and in Elinkinde. In Mlomp, the ORs fluctuated overtime and seasonally, with the second semester of 2005, 2007 and 2008 having a higher risk and all the others a lower risk of fever than in the first semester of 2004. In Elinkinde, there was a general decline in the ORs of fevers through the period of study with the exception of the second semester of 2004 and 2007.

Febrile Illnesses Other Than Malaria. 4151 and 2533 cases were diagnosed clinically as non malaria fevers in Mlomp and Elinkinde, respectively, corresponding to 31% and 37% of fevers. In both places, lower respiratory infections were predominant (2199 (53%) and 1417 (56%), respectively).

Case management over time

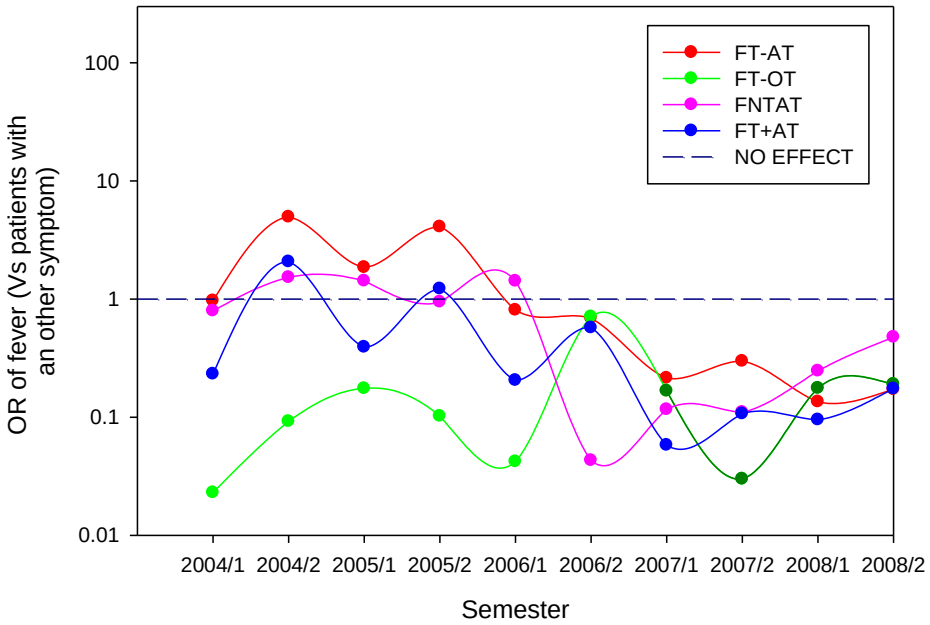
Mlomp. Overall, considering the entire period under observation (2004-08), all fever management types (1-4) were significantly different from fever other than malaria (category 5, FOS). The OR was <1 for fevers with a positive malaria test treated with an antimalarial (FT+AT, OR=0.99) and fevers with a negative test with another treatment (FT-OT, OR=0.05) and was >1 for fevers treated with an antimalarial whether the test had not been done (FNTAT, OR=1.11) or negative (FT-AT OR=2.62).

The ORs of the interactions between time and fever management category are plotted for each semester in Figure 4**Error! Reference source not found.**a and 4b. All comparisons were significant.

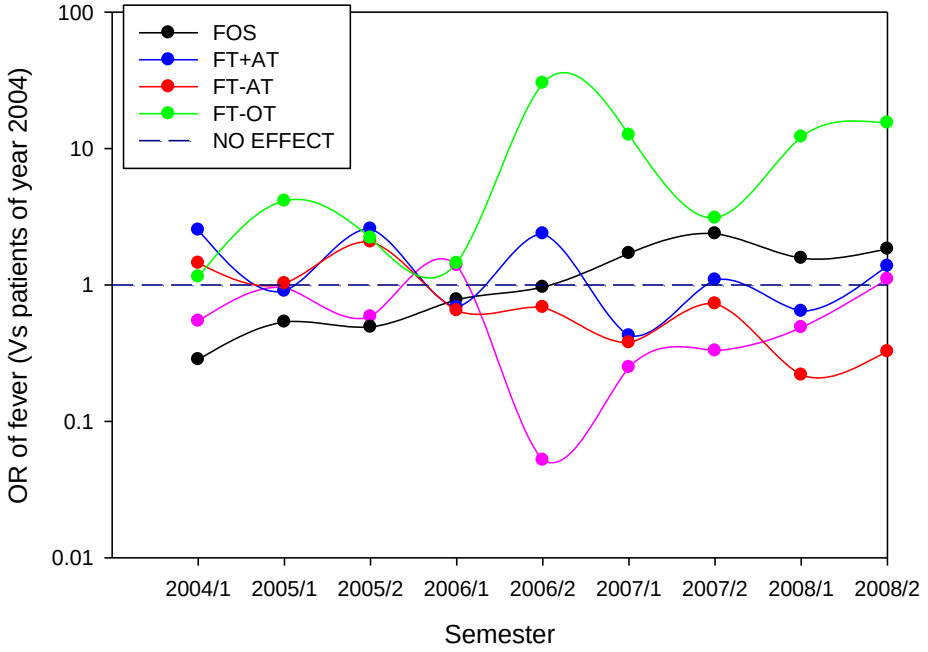
Error! Reference source not found.a shows the odds of belonging to categories 1-4 over the entire study period with respect to category 5 (FOS, non-malaria fever and treatment). For treatment with non antimalarial drugs of suspected malaria with a negative malaria test (category 3), the odds were consistently lower throughout the period under observation.

Figure 4. Management of fever over time in Mlomp. Odds of occurrence of each fever management category (a) referred to fever other than malaria (FOS) within each semester; (b) with reference to the same category in 2004. Odd ratios are on a logarithmic scale. Horizontal dotted line at 1 = no difference

a



b



From 2006, there was a steady decrease in the odds of receiving an antimalarial treatment despite a negative test (category 2). The same happened to category 4 (receiving an antimalarial without a test done) and 1 (antimalarial treatment on a positive test); for the latter two, however, there was a tendency for a relative increase from 2007.

Figure 4b presents the interaction between the fever management category and time when comparing each category to itself in 2004. The odds for an antimalarial treatment on a negative test (category 2) decreased consistently over time, whereas the odds for an antimalarial treatment on a positive test (category 1) or no test (category 4) were essentially the same as in 2004 in the second semester of 2008 after fluctuating in between. In contrast, the odds of being diagnosed with a non-malaria fever (category 5) and receiving a non-malaria treatment on a negative test (category 3) increased steadily over time (though with wider fluctuations for the latter.)

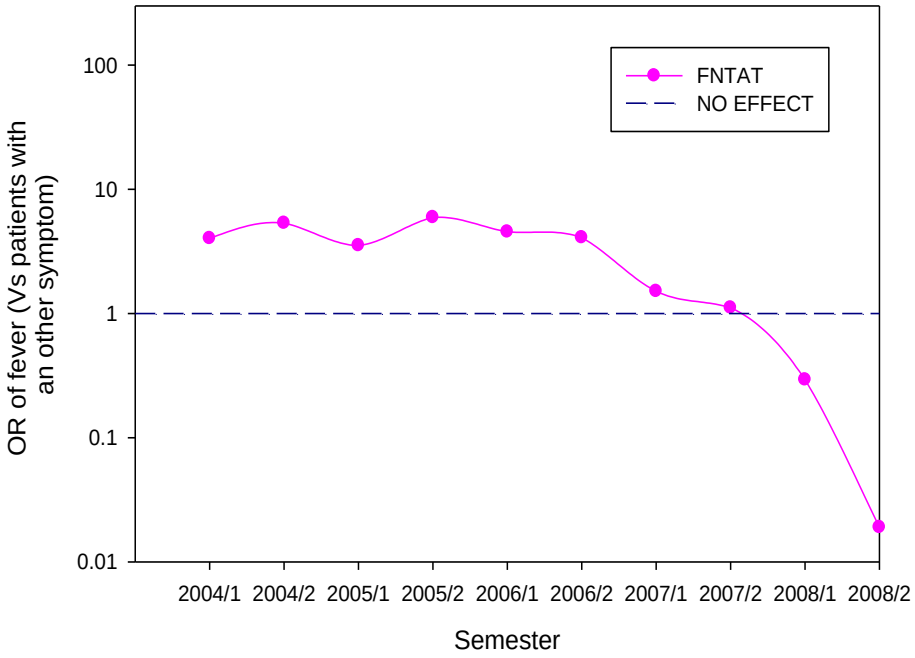
Taken together, these analyses show a relative increase in diagnosing non-malarious fevers and treating parasitologically-negative cases with non-malaria drugs, paralleled by a decrease in the practice of giving antimalarial treatment to parasitologically-negative cases. At the same time, there was no stable change for antimalarial treatment of confirmed malaria or in the practice of giving antimalarials without parasitological confirmation.

Elinkinde. The odds of being treated with an antimalarial drug on presumptive diagnosis of malaria (category 4, FNTAT) were ~10 times higher than being diagnosed and treated for fever other than malaria (category 5, FOS) during 2004-2006. The ratio then began to decrease and was 100 times lower in the second semester of 2008 (Figure 5a). Similarly, for the only two categories available at this site throughout the entire 2004-2008 period, compared to 2004, the situation did not change until the end of 2006, and then the odds decreased for category 4 and increased for category 5 (Figure 5b).

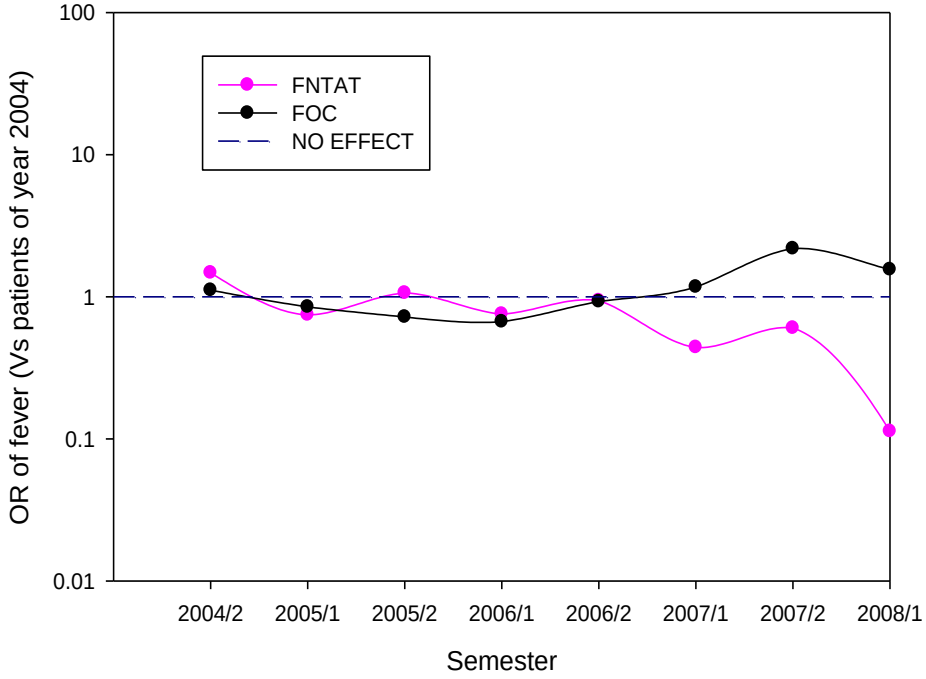
A sensitivity analysis was conducted to assess whether the results of the models could be related to seasonality. Two alternative models were used: (i) independent models for the first and second semesters; (ii) the inclusion in the original model of variable describing the first and second semester for both locations. The output of these analyses (not shown) confirms the result presented above.

Figure 5. Management of fever over time in Elinkinde. Odds of occurrence of each fever management category (a) antimalarial treatment given on clinical grounds without a parasitological test (FNTAT) referred to fever other than malaria (FOS) within each semester; (b) FNTAT and FOS with reference to itself in the first semester of 2004. Odd ratios are on a logarithmic scale. Horizontal dotted line at 1 = no difference

a



b



Between-site comparison

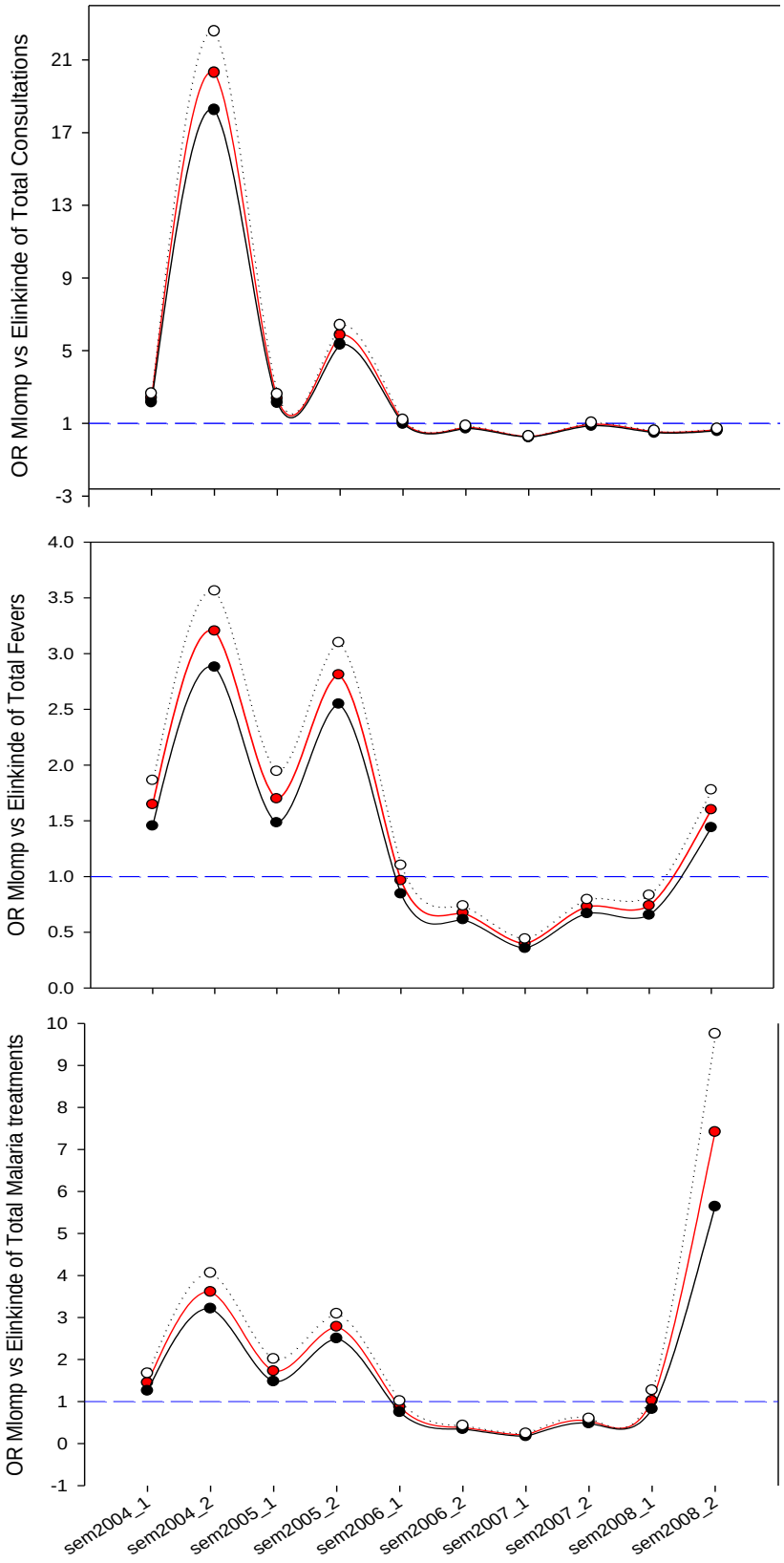
After correcting for the population in the villages, the comparison of the 6-month prevalence rates (Table 3) between Mlomp and Elinkinde with proc logistic shows: (i) that consultations, fevers and total antimalarial treatments (on any grounds) were significantly more frequent in Mlomp in 2004 and 2005 (with a climax in the second semester of 2004); (ii) an equipoise in the first semester of 2006 for all of the above parameters; (iii) the 6-months prevalence was higher in Elinkinde from the second semester of 2006 through 2008 for consultations (with an occasional equipoise in the second semester of 2007), until mid 2008 for fevers and until the end of 2007 for malaria treatments; (iv) in second semester 2008 the 6-month prevalence rates of fevers and malaria treatments were again higher in Mlomp (Figure 6.)

DISCUSSION

Implementing health policy changes in general and for malaria case management in particular is challenging. The implementation of the new policy (artesunate plus amodiaquine on parasitological confirmation, replacing quinine or chloroquine on clinical presentation) was staggered over several years at the different dispensaries of the district of Oussouye. We studied and compared two different cases. In Mlomp the new malaria case management policy was introduced in 2000 under research conditions at a pilot scale and then at full scale until 2006 when it became country policy and was implemented under routine conditions. Elinkinde followed the national policy (interim use of amodiaquine plus sulfadoxine-pyrimethamine in 2004-06; artesunate plus amodiaquine starting in 2006; rapid diagnostic tests from 2007) with no added research component.

The results of this study carry altogether good news, but show also some warning signs. In Mlomp there is a clear trend towards a reduction in the overall number of consultations and malaria treatments since 2000. For comparison, in the period preceding this study (2000-2003), the number of consultations decreased from 9361 to 6542; the corresponding number of antimalarial treatments were 5391 and 3243. Specifically within the study period (2004-08) consultations dropped further by ~20% (from 5566 to 4485) and antimalarial treatments by ~65% (from 2595 to 885). Compared to 2000, consultations and antimalarial treatments have dropped by 52% and 84%, respectively. Thus, it can be inferred that malaria prevalence and morbidity are declining in this village. However, these data require a considered interpretation.

Figure 6. Comparison of the 6-month prevalence of total consultations, consultations for fever and all malaria treatments (suspected or confirmed malaria). Odd Ratios between Mlomp and Elinkinde by semester (dry and rainy season). Red line: Odds Ratio (OR), Black and dotted lines: 95%Conficence Intervals (CI). Horizontal dotted line at 1 = no difference



The models applied show a relative increase of non-malaria diagnoses and treatments particularly since 2006 and a concomitant decline in antimalarial treatments initially, though with a subsequent inversion of the trend, particularly for antimalarials prescribed without parasitological diagnosis. There are therefore departures from the national policy and a tendency to revert to the habit of prescribing antimalarials on clinical suspicion (the past policy.) Parasitological diagnosis is not applied systematically, and even decreased from 75% to 46% of fevers between 2004 and 2008. Only 22% of the antimalarial treatments are for parasitologically-confirmed malaria. There was limited confidence in a negative test: 70% of the treatments given after parasitological diagnosis were for patients with a negative test. Quinine is still in use for uncomplicated malaria (55% of all antimalarial treatments overall) though it has decreased by ~66% in 2008 with respect to 2004. Artesunate plus amodiaquine (the country first-line treatment) represents on average 22% of all treatments but its use exceeds the number of confirmed malaria cases now. So, achievements made in the first part of the new policy implementation (2000-04) in terms of prescriber's compliance to policy are being eroded. One reason is that during the earlier implementation phase the level of supervision was higher and intervention studies were in place[Brasseur, P, *et al.* 2007]**Error! Bookmark not defined.**. Research activities had a positive impact on practice and favoured compliance to policy; apparently, these effects are waning away after studies ended.

In Elinkinde, personnel complied with the national treatment recommendations in terms of the drugs to be used (only 49 cases treated with quinine in 2008; introduction and withdrawal of amodiaquine plus sulfadoxine/pyrimethamine; introduction of artesunate plus amodiaquine) but not parasitological diagnosis (very few cases initially, increasing to approximately one third of fevers in 2008). The population served by the Elinkinde dispensary is 58% of that of Mlomp's; while the average number of consultations, fevers and malaria treatments per capita are very similar in the two dispensaries, trends over time differ. When comparing the 6-month prevalence rates of total consultations, consultations for fever and antimalarial treatments (whether for clinically suspected or parasitological confirmed malaria), there was an inversion in the Odd Ratios with an equipoise in the first semester of 2006; before all events where significantly more frequent in Mlomp, then they occurred more in Elinkinde but the trends changed again in the second semester of 2008. It must be noted that two concomitant events that occurred in 2006

(the activation of a new dispensary and the arrival of a new nurse) may have contributed to these changes.

The situation during the latter part of the study period (2006-08) was similar in both places in terms of infections other than malaria (58% of all fevers in 2007 in Mlomp; 44% and 57% in 2007 and 2008 respectively in Elinkinde), in particular respiratory tract infections. During the same period the use of antimalarials decreased in Mlomp and had a transient increase in Elinkinde which was proportionally lower than the fevers and overall consultations. Confirmed malaria in Mlomp was 27% in 2007, compared to an average 30% for 2004-08, which is lower than the average for 2000-03, (44% consistent with previous figures at the site[Agnamey, P, *et al.* 2005]), but in line with results of a PNLP study (27% in 2008 [Ministère de la Santé et de la Prévention Sociale du Sénégal. Programme National de Lutte Contre le Paludisme (PNLP) 2008]).

Overall 73% of suspected malaria fevers had a confirmatory test done in Mlomp, but the site performance is decreasing and only 46% had a test done in 2008. Only one-third of cases had an RDT done in Elinkinde.

These findings point to issues with compliance to policies. Practices may vary within the same district in spite of training programmes. The vast majority of antimalarial treatments are given on clinical presumption of malaria, and even in the presence of a negative test - a problem shared with other countries [Bisoffi, Z, *et al.* 2009, Olliaro, P 2009]. In Mlomp, whether a parasitological diagnosis was done and tested negative, or was not done, an antimalarial treatment was given in 83% and 89% of cases, respectively.

Clinical presentation is misleading; infectious diseases other than malaria are frequent, and in some cases, differential diagnosis may be difficult. Here, lower tract respiratory infections followed the same seasonal trends as malaria. The use of clinical algorithms alone is not enough, particularly in areas of lower malaria endemicity [Chandramohan, D, *et al.* 2002]; the combination of clinical algorithm and microscopy reduced by ~1/3 malaria prescriptions in Zanzibar with no effect on the use of antibiotics [Ngasala, B, *et al.* 2008].

Overall in these two dispensaries in Senegal, 4196 cases of fever received an antimalarial despite a negative microscopy or RDT. Assuming that 60% of fevers considered to be malaria were not caused by malaria (a conservative estimate), some 7383 of the 12,306 antimalarial treatments given on clinical grounds at the two sites were not needed. This amounts to 11,579 episodes in five years which were unnecessarily treated with an an-

timalarial and possibly denied appropriate alternative treatment - although it was not possible to assess from the clinic registries the consequences of inappropriate case management on people's health.

Indeed cost of diagnosis must be considered. Currently the national programme purchases the HRP II-based test Paracheck® at 275 CFAs (0.55 US$ at an exchange rate of 0.002 US$/CFA) and provides it for free. Had an RDT be done on all 3358 fever cases considered to be malaria at the two sites in 2007-08 (when RDTs were made available) the PNLP would have incurred a cost of 1846 US$ for diagnosis.

However, judgment should not be based on purely economical terms. The guiding principles should be improving standard of care (specifically here management of fever) and optimising the lifespan of effective use of medications.

While malaria morbidity may be declining in these villages, the long-term prospects of current practice in terms of costs and benefits, sustainability and effects on disease epidemiology and general health should be considered. These results are relevant to other areas where the general trend is towards declining malaria endemicity [Guerra, CA, *et al.* 2008]. Parasitological confirmation becomes all more important with decreasing malaria incidence.

It is clear that corrective actions (more training and supervision) are needed to realign practice to policy; so is a better understanding of the reasons behind health providers' behaviour in order to build confidence in malaria tests. A study of clinical practice in hospitals in Tanzania [Chandler, CI, *et al.* 2008] identified various spheres of influence on malaria over-diagnosis explaining why mindlines are followed over guidelines. It also showed that for clinicians malaria is easier to diagnose than alternatives and that missing malaria is thought to be indefensible. Similar studies should be conducted with the personnel of peripheral health facilities to help understand what measures should be set in place to realign practice to policy.

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AUTHORS' CONTRIBUTIONS

Philippe Brasseur was the principal investigator of the study, contributed to the planning of the study, collected the data and contributed to the interpretation of the analysis and writing of the paper;

Malick Badiane and Moustafa Cisse were the district medial officers, organized the training and supervised the operations;

Cheikh Sokhna was a co-investigator and participated in data collection;

Jean-Marie Kindermans contributed to the planning of the study and data verification;

Patrice Agnamey was a co-investigator and participated in data collection;

Michel Vaillant contributed to the analytical protocol and conducted the analysis;

Pascal Millet contributed to the planning of the study and interpretation of the analysis

Piero Olliaro contributed to the planning of the study, conducted the analysis with M.Vaillant and was the primary writer of the paper

All authors have seen and accepted the final version submitted.

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Changing patterns of malaria between 1996-2009 in an area of moderate transmission in Southern Senegal

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INTRODUCTION

The Global Malaria Action Plan (RBM 2008) launched in 2008 aims for (i) providing universal coverage (prevention + case management) by 2010; (ii) reducing the malaria burden and deaths by 50% in 2010, 75% in 2015 as compared to 2000, (iii) eliminating malaria in 8-10 countries by 2015; and (iv) eradicating malaria in the long term.

The foundations of this plan are in the availability and deployment of interventions which, individually in the previous decade, proved beneficial [insecticide treated nets (ITN), insecticide residual spraying (IRS), artemisinin-based combination therapies (ACT)] and the possibility of scaling-up their combined use.

Around the time of the launch of the MAP, a Lancet commentary (Byass, 2008) found that in the previous 10 years some 80 papers had reported trends in malaria incidence (increased in 15%, not changed in 14% and decreased in 71%). The same issue of the Lancet published two papers reporting decreasing malaria burden in the Gambia and Kenya (Ceesay et al 2008, O'Meara et al 2008). These general trends towards a reduction of malaria were confirmed by the World Health Organization (WHO) in the Malaria Report 2009 and other papers (e.g. D'Acrémont et al 2010, Ceesay et al 2010).

A central question to both measuring achievements and targeting interventions is now whether malaria is receding, where and under what circumstances (and where it is not).

It is important to document trends across endemic areas, while being cognisant that long-term malaria trends are notoriously difficult to interpret (likely multi-factorial) and prone to bias (including reporting and publication biases).

This was a facility-based study of malaria incidence and risk over a period of 14 years during which malaria treatment policy changed from administering antimalarial monotherapy on clinical grounds to artemisinin combination for parasitologically confirmed cases.

MATERIAL AND METHODS

Study area. Mlomp is a village of ~6000 inhabitants in the district of Oussouye (~39,000 inhabitants), South-western Senegal. The malaria treatment policy in Senegal has been chloroquine or quinine on clinical grounds until 2004; following an interim policy of amodiaquine plus sulfodoxin/pyrimethamine, artesunate plus amodiaquine (AS-AQ) became the first-line treatment in 2006 and rapid diagnostic tests were made available from 2007 for parasitological diagnosis of malaria. After an initial clinical trial conducted in 1999 (Adjuik et al, 2002), AS-AQ was initially used for children under 10 in the rainy season and then extended to the whole population (Brasseur et al, 2007, Brasseur

et al, 2009). The implementation of the new policy (AS-AQ for parasitologically-confirmed malaria) and the previous practice (quinine or another drug on clinical grounds) have been going on in parallel during this period. The effects in terms of efficacy and safety of this combination have been documented (Agnamey et al, 2005; Brasseur et al, 2007).

The age structure of the malaria cases in this area has remained stable over several years (Brasseur et al, 2007) and consistent with other areas of similar endemicity in Senegal (Trape and Rogier, 1996), with the highest risk of malaria being in older children and young adolescents (57% of the patients are 6-15 years of age), that extends also later in life (8% were 21-30, 7% 30 and above).

Data. Data on overall consultations for fever, malaria treatments, parasitological test results and patients' age were extracted from the clinical registries of the dispensary of the village of Mlomp for the period 1996-2009. The rate of positive parasitological tests to all parasitological tests was applied to the overall number of treatments to obtain the projected malaria cases per year. The rates of treatments given after a positive, negative or no parasitological tests were calculated out of the number of treatments and the number of consultations for fever. The number of artesunate-amodiaquine treatments was also divided by the number of treatments to give the relevant rate.

Age (recorded as actual age in months or years) was analysed both as a continuous variable and by age categories (0-5 years, 6-10, 11-15, 16-20, 21-30 and >30). The distribution of age throughout the years between patients with positive and negative malaria tests was assessed with a general linear model. The parasitological tests were either a thin plus thick smear or a rapid diagnostic test (RDT) based on the histidine rich protein II (HRP-II).

Parasitological tests count model. A count data model was used to evaluate the effect of parasitological test results, age and time on the number of tests done in Mlomp. Count data were first tested for over-dispersion (a limitation of the Poisson model for count data) with the Lagrange multiplier test (Greene, 2002) for over-dispersion with the negative binomial model as the alternative. The model accounted for the patient's age when the test was done, the year, the interaction between age and year (the effect of age nested into the year), the parasitological test result, the interaction with years and the interaction with age and years. The relative risk (RR) of a lower or higher count of blood smears with a 95% confidence interval (95%CI) was also evaluated from the estimates of the model with the age category 0-5y, the year 1996 and a negative malaria test as the reference. The age was first introduced in the model as a continuous variable and subsequently as a categorical variable (the RR of a continuous variable is interpreted as an increase in the "risk" of more tests done for each increase of one unit, implying a linear relationship) with the group 0-5 years as the reference.

Malaria risk model. The odds of having parasitologically-confirmed malaria were evaluated over the years by using a logistic model for a positive versus a negative malaria test, firstly with the year, and age considered independently, and then with the interaction between these two variables. The Odds Ratio (OR) of a malaria test outcome depending on age were assessed for each year., Since (i) there was a significant interaction between age and year in the model, and (ii) patients data are independent from one year to the next, single models for each year including age as continuous variable were ran in order to obtain the estimate of OR of malaria. The age as continuous variable implies a linear relationship in the logit. To test for a non-linear relationship, a quadratic form of age was evaluated in the model.

RESULTS

Malaria burden during 1996-2009

Overall trends. Between January 1996 and December 2009, a total of 99,758 patients consulted for fever and 45,801 of these (46%) received an antimalarial treatment (whether on clinical or parasitological confirmation); 26,156 (57% of total consultations) underwent parasitological confirmation (either thin/thick smear or RDT) and had age recorded; 10,391 of these (39.7%) tested positive for *P. falciparum*. When applying annual rates to the total number of treatments, the projected number of malaria cases over the entire period was 19,619.

Over the entire period under study, all these figures decreased steadily. Table 1 presents the yearly records of consultations for fever, antimalarial treatments dispensed (on either clinical or parasitological grounds) and projected real (parasitologically-confirmed) malaria cases in Mlomp during 1996-2009. The table presents also the total number of parasitological tests done each year and their outcome. Figure 1 displays graphically the monthly records over the same period for consultations for fever, all-causes antimalarial treatments and projected numbers of parasitologically-confirmed malaria cases.

The number of consultations for fever per-person year dropped from ~1.7 to ~0.7, so did the number of antimalarial treatments (from ~0.9 to ~0.13) and the risk of malaria (from ~0.4 to ~0.02 malaria episodes person-year) (Figure 2.)

Table 1. Yearly records of consultations for fever, antimalarial treatments dispensed (on either clinical or parasitological grounds) and projected real (parasitologically-confirmed) malaria cases in Mlomp during 1996-2009. The table presents also the total number of parasitological tests done each year and their outcome.

Year	*Consultations for fever*	*Antimalarial treatments*	*Projected Malaria Cases*	*Parasitological test result and age recorded*	*(% positive)*
1996	10270	4637	2219	2129	48.2%
1997	11274	5743	3063	2385	53.2%
1998	11006	5680	1893	2221	32.7%
1999	9807	5635	2220	2372	39.8%
2000	9361	5391	2123	2758	51.2%
2001	7910	4432	1702	2526	51.0%
2002	5596	2834	1150	1153	60.6%
2003	6542	3243	1271	2707	41.1%
2004	5558	2486	729	1799	31.3%
2005	5152	2677	656	1909	29.2%
2006	4130	1089	304	1343	23.2%
2007	4528	987	218	1535	12.6%
2008	4411	885	220	850	22.9%
2009	4213	82	16	469	19.6%
All	99758	45801	17784	26156	39.7%

Figure 1 . Monthly records of consultations for fever, antimalarial treatments dispensed (on either clinical or parasitological grounds) and projected real (parasitologically-confirmed) malaria cases in Mlomp during 1996-2009

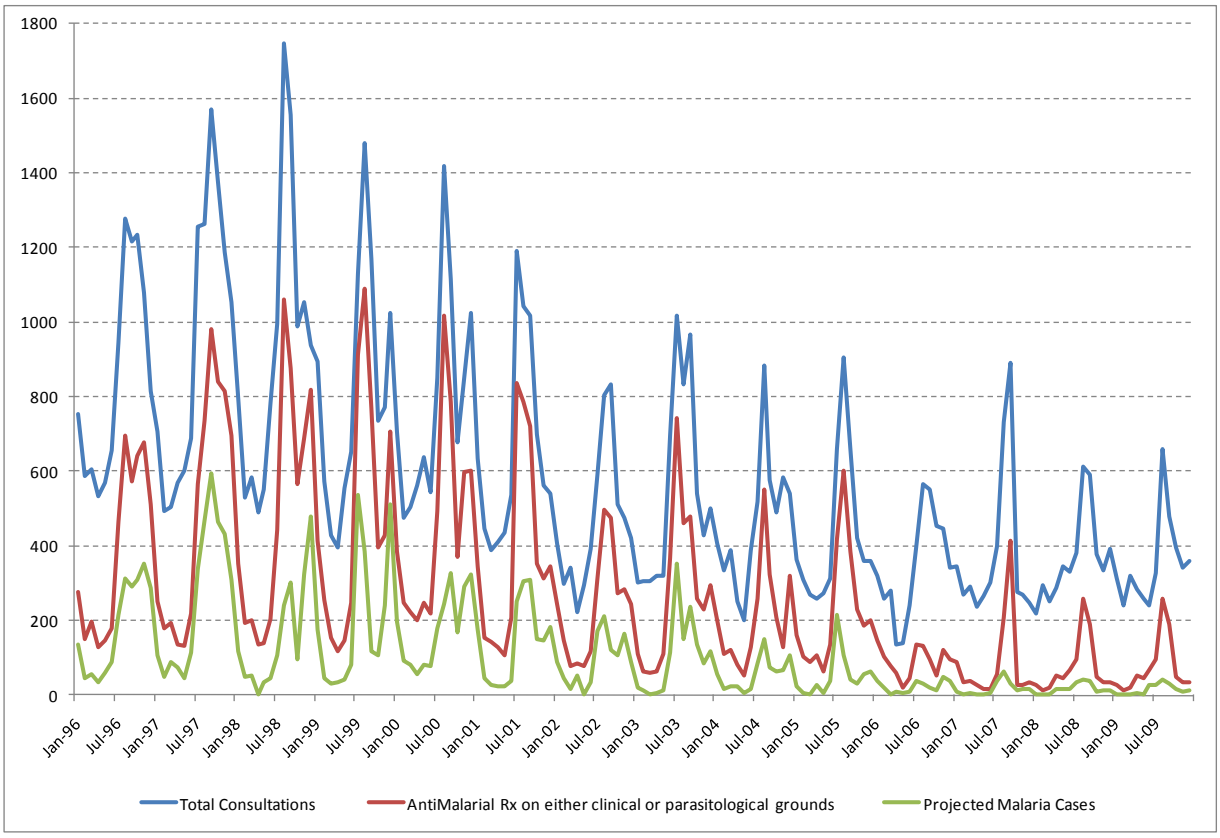
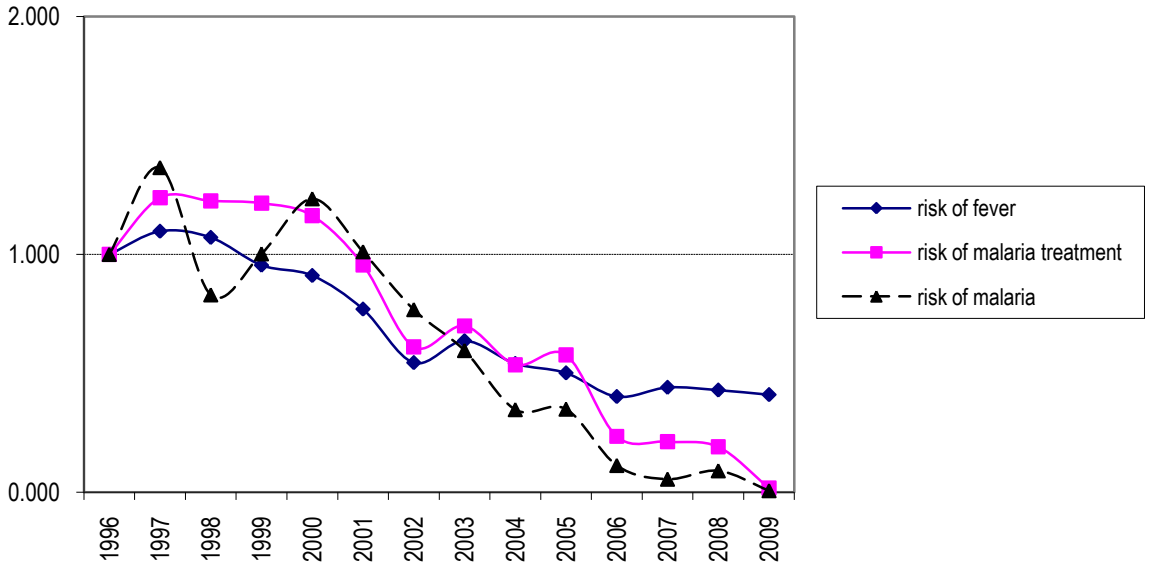


Figure 2. Risk of consulting for fever, receiving a malaria treatment and having a parasitologically-proven malaria attack in person-year relative to 1996



Malaria treatments and treatment practices. The number of malaria treatments was >4,400/year between 1996-2001 (peak in 1998 with 5680 treatments) and then started to decline steadily from 2002. Four drugs (quinine 82%, chloroquine 6%, artesunate+amodiaquine 9% and amodiaquine+ sulfadoxine/pyrimethamine 1%) account for ~98% of all drug use on either clinical or parasitological grounds over the 14-year period. Overall, 4,149 artesunate+amodiaquine treatments were delivered between 1999 and 2009 while 37,622 quinine treatments were delivered from 1996 to 2009. Quinine use was high (~80-95%) until 2003, then steadily decreasing to ~28% in 2009; chloroquine is no longer used since 2007; amodiaquine+ sulfadoxine/pyrimethamine was an interim recommendation of national malaria control programme (used here only in 2005-06); artesunate+amodiaquine is the current policy in Senegal and its use has been steadily extending to reach 72% in 2009. Indeed, in absolute terms, the number of treatments is decreasing sharply. (Table 2)

The policy of treating parasitologically confirmed cases with artesunate+amodiaquine was applied in 3,693 cases (55.2% of the parasitologically-confirmed treatments and 89% of all 4149 artesunate+amodiaquine treatments). The annual number and proportions of artesunate+amodiaquine treatments of the total consultations and treatment are presented in Figure 3.

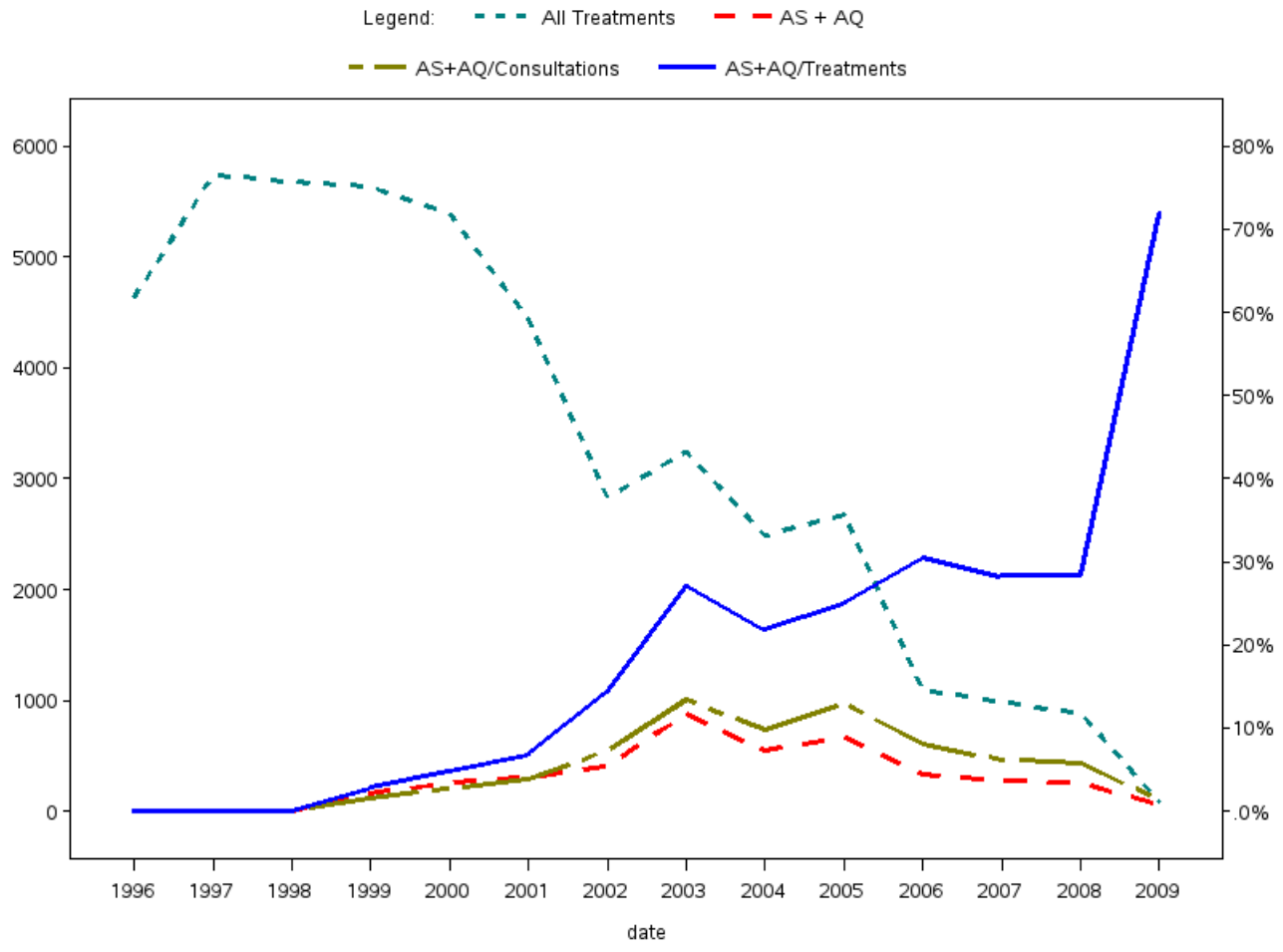
From the clinical registries and the malaria cases projections it can be derived that during the 14 years under study some 19,645 treatments dispensed (or ~63% of all malaria treatments) were not

supported by a positive parasitological test (i.e. were given without a parasitological done or with a negative test) .

Table 2. Antimalarial treatments provided

YEAR	All Treatments	AS + AQ	Q	CQ	AQ + SP	Other Treatments
1996	4637	0	4132	504	0	1
1997	5743	0	5438	303	0	2
1998	5680	0	5475	203	0	2
1999	5635	160	4930	379	0	166
2000	5391	259	4794	284	0	54
2001	4432	305	3981	99	0	47
2002	2834	408	2191	234	0	1
2003	3243	879	2005	356	0	3
2004	2486	544	1565	355	0	22
2005	2677	671	1341	80	358	227
2006	1089	332	574	9	30	144
2007	987	280	697	1	0	9
2008	885	252	476	0	0	157
2009	82	59	23	0	0	0
All	45801	4149	37622	2807	388	835

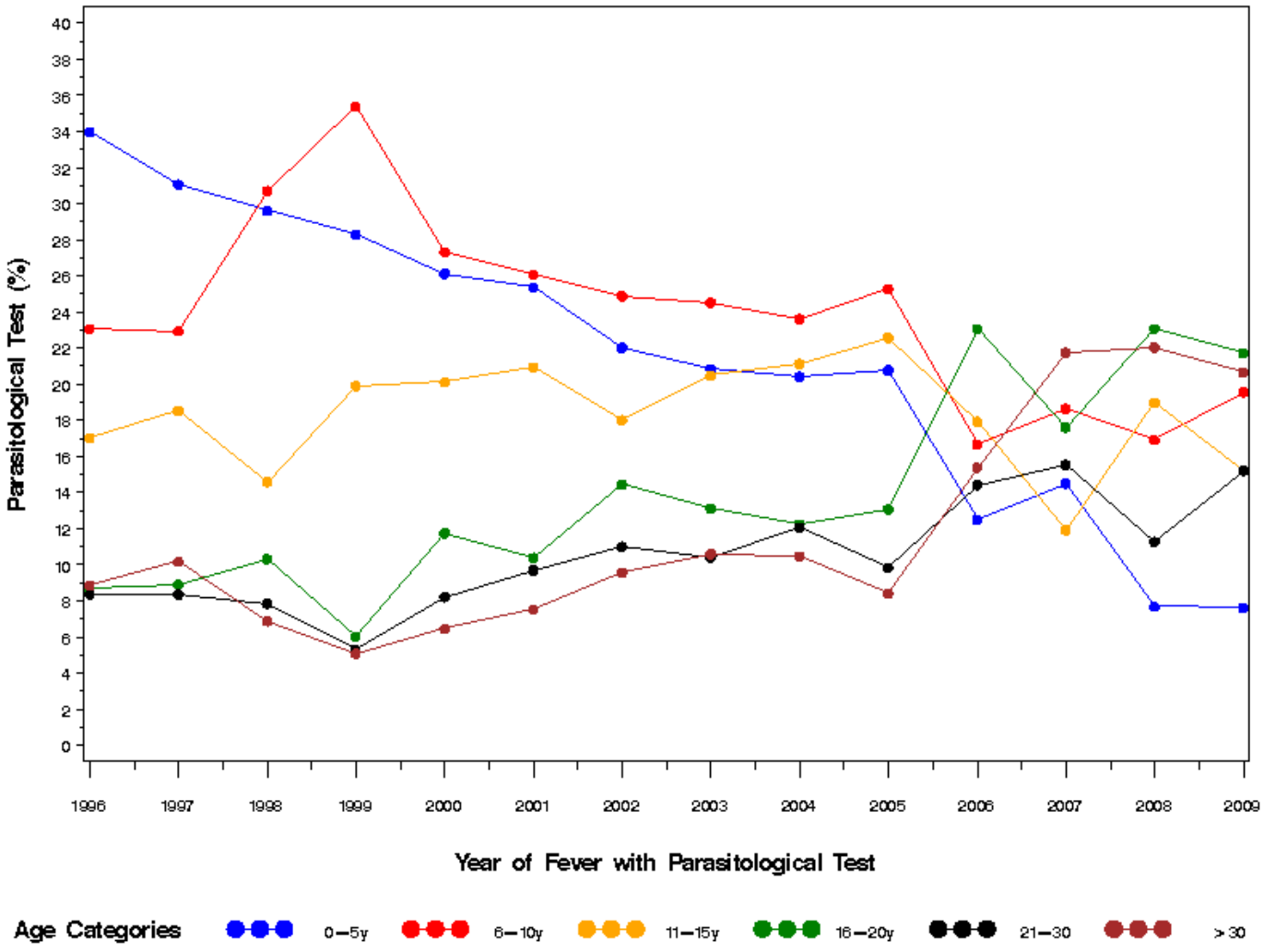
Figure 3. Antimalarial treatment dispensed in Mlomp



Age-dependent risk of malaria during 1996-2009

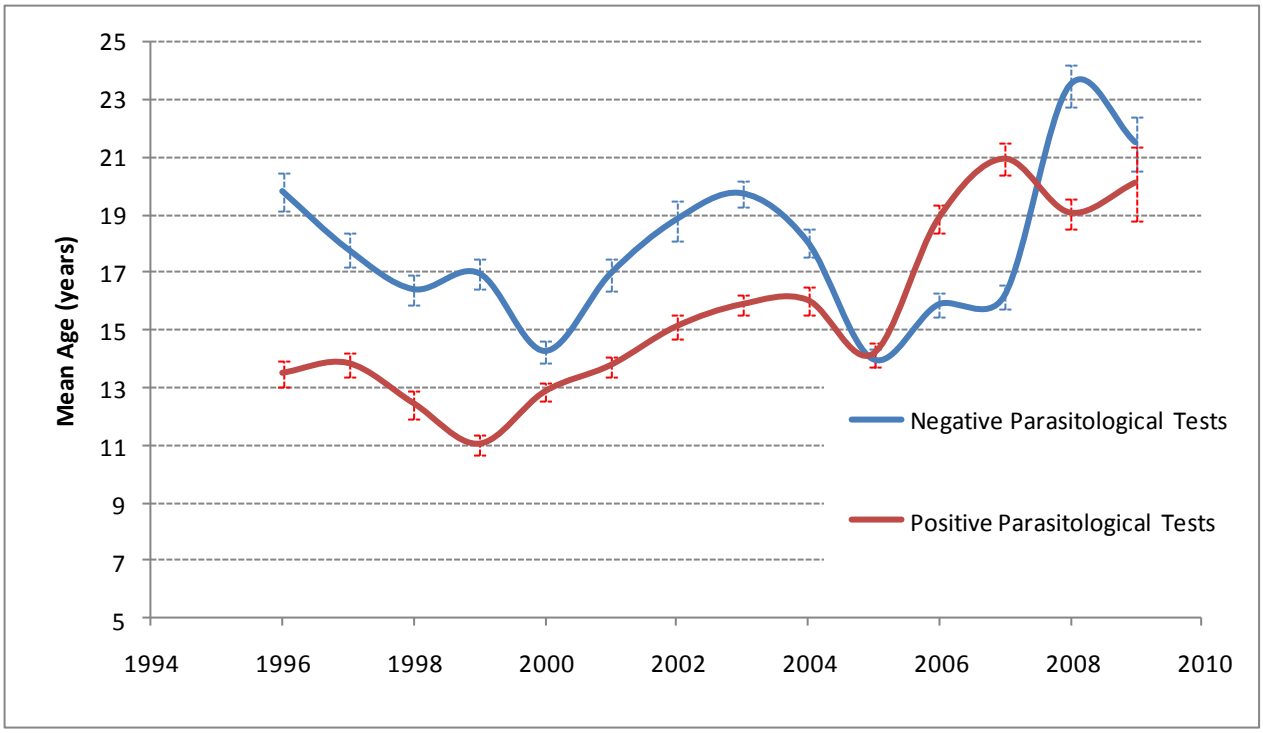
Figure 4 shows the proportion of parasitological positive cases of fever by age-group over the period 1996-2009. The age group 0-5y declined steadily throughout the entire study period; the 6-10y declined slightly; the 11-15y remained stable; the 16-20y, 21-30y and >30y all increased.

Figure 4. Proportion of the yearly parasitologically-positive malaria cases by age classes over the period 1996-2009



The mean age of the malaria-positive patients increased steadily from 13.4 years in 1996 to 20.1 in 2009, while it fluctuated over time for the negatives, with values in 2009 (21.5) similar to those in 1996 (19.8 years) (Figure 5). The general linear model used for age (considered as independent continuous variable, allowing for test result, year and the interaction between the two) was statistically significant - meaning that there was a significant difference in the evolution of age during 1996-2009 between the malaria-positive and -negative subjects.

Figure 5. Mean age of Subjects with Negative and Positive parasitological tests during 1996-2009



Parasitological test count model. The interaction between test results and age showed that, overall, there was a higher risk of having a negative than a positive test all the time except in 2002 when the risk was equal; the risk of a negative parasitological test result was highest in 2007 (blue line in Figure 6). When considering the interaction between test results and year, for negative tests after an initial decrease in the RR until 2000 there was a general trend towards a decrease in the risk of positive parasitological tests, with a RR below 1 consistently from 2002 till 2009 (green line in **Error! Reference source not found.**). Negative tests followed a similar pattern with the risk being around 1 in 2001-2002 and below 1 from 2003 (red line in Figure 6). Overall, the models concur to show changes starting to occur around the year 2002.

When adding the interaction between year and age the results obtained for each age category (**Error! Reference source not found.**) were similar to the overall trends described above (see appendix for more details).

Figure 6**Error! Reference source not found.** shows the RR of a positive parasitological for each year of the period 1996-2009 for the different age categories referred to the age group 0-5y and calculated with the year as the main variable and the interaction coefficients of year by age. The result of the test is considered as positive. The frequency of parasitological tests showed overall a similar

trend for the 6-10y and 11-15y age categories over the years with occasionally significant RRs (see appendix for details) around the identity value (=1) with only a small increase in 2008-2009

Table 1 Relative risk (RR) of positive malaria test by age classes during the period of 1997-2009 with respect to 1996 using a negative binomial model

	0-5y		6-10y		11-15y		16-20y		21-30y		>30y	
Year	RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI	RR	95%CI
1997	1.14	[0.90; 1.46]	1.35	[0.76; 2.39]	1.24	[0.70; 2.22]	1.35	[0.74; 2.48]	1.33	[0.73; 2.41]	1.35	[0.76; 2.39]
1998	0.68	[0.53; 0.87]	0.92	[0.52; 1.65]	0.65	[0.36; 1.18]	0.77	[0.42; 1.43]	0.62	[0.34; 1.15]	0.92	[0.52; 1.65]
1999	0.79	[0.62; 1.01]	1.28	[0.72; 2.27]	0.89	[0.50; 1.60]	0.80	[0.43; 1.48]	0.70	[0.38; 1.28]	1.28	[0.72; 2.27]
2000	1.23	[0.96; 1.56]	1.63	[0.92; 2.88]	1.29	[0.72; 2.30]	2.15	[1.19; 3.89]	1.65	[0.92; 2.98]	1.63	[0.92; 2.88]
2001	1.25	[0.98; 1.60]	1.44	[0.81; 2.56]	1.23	[0.69; 2.20]	1.37	[0.75; 2.51]	1.25	[0.69; 2.27]	1.44	[0.81; 2.56]
2002	0.61	[0.47; 0.80]	0.76	[0.41; 1.41]	0.65	[0.35; 1.22]	0.90	[0.47; 1.73]	0.82	[0.43; 1.57]	0.76	[0.41; 1.41]
2003	0.85	[0.66; 1.09]	1.16	[0.65; 2.06]	1.11	[0.62; 1.99]	1.45	[0.80; 2.66]	1.30	[0.71; 2.36]	1.16	[0.65; 2.06]
2004	0.50	[0.38; 0.65]	0.58	[0.32; 1.06]	0.55	[0.30; 1.01]	0.67	[0.36; 1.27]	0.66	[0.35; 1.23]	0.58	[0.32; 1.06]
2005	0.50	[0.39; 0.65]	0.62	[0.34; 1.13]	0.56	[0.30; 1.02]	0.80	[0.43; 1.50]	0.52	[0.28; 0.98]	0.62	[0.34; 1.13]
2006	0.25	[0.19; 0.33]	0.26	[0.13; 0.49]	0.26	[0.14; 0.51]	0.62	[0.32; 1.20]	0.38	[0.20; 0.74]	0.26	[0.13; 0.49]
2007	0.14	[0.11; 0.19]	0.22	[0.12; 0.43]	0.13	[0.07; 0.26]	0.31	[0.16; 0.62]	0.27	[0.14; 0.54]	0.22	[0.12; 0.43]
2008	0.10	[0.07; 0.14]	0.20	[0.10; 0.40]	0.16	[0.08; 0.33]	0.33	[0.16; 0.69]	0.26	[0.13; 0.54]	0.20	[0.10; 0.40]
2009	0.05	[0.04; 0.07]	0.10	[0.05; 0.23]	0.09	[0.04; 0.20]	0.14	[0.06; 0.33]	0.11	[0.05; 0.26]	0.10	[0.05; 0.23]

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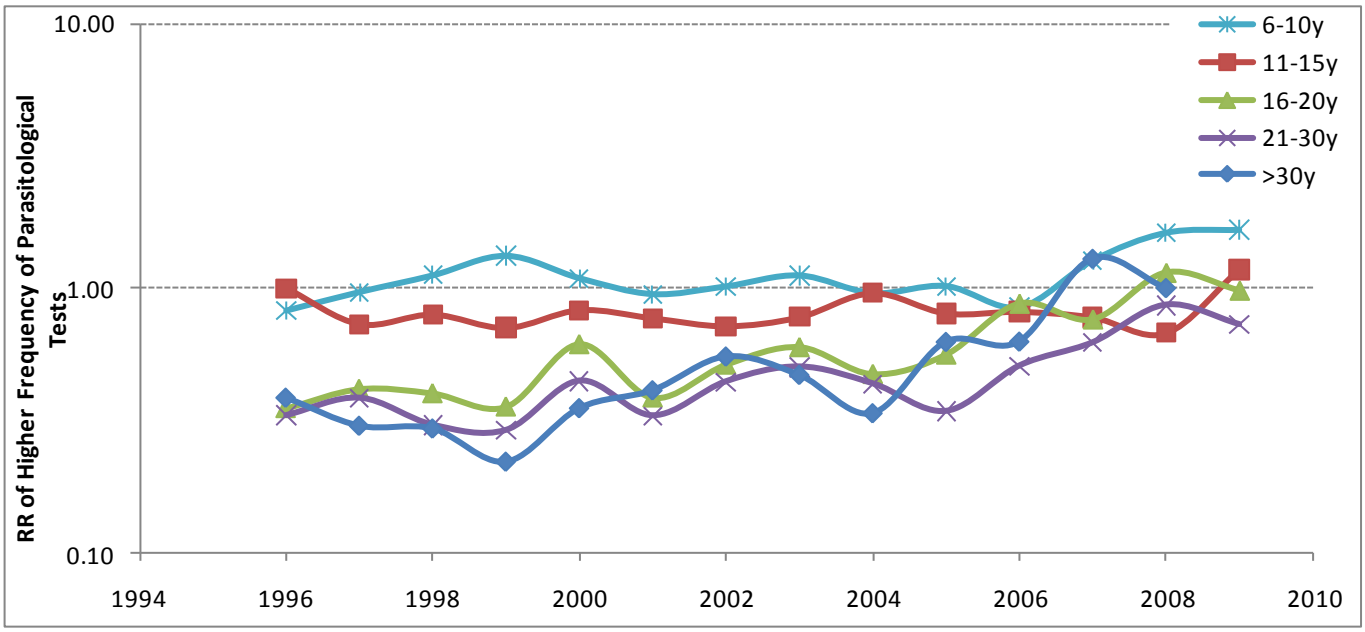
The three others age categories showed a constant increase from 2001 to become >1 for the 16-20y and >30y age categories. Overall, these results show that, while in the past the ages ≥16 years had a lower risk for a positive malaria test, more recently they caught up with the others and the risk is very similar across all ages.

Figure 6. Relative Risk of positive parasitological test by using the 0-5y age category as reference in the negative binomial model of counts of parasitological tests.



Malaria risk model. The logistic model of the risk of a positive parasitological test included the age of the subjects and the year of examination. The interaction was significant in the model. The addition of a quadratic term of age in the model was not significant, leading to a linear age relationship with the probability of positive parasitological test.

The models estimated the Odds Ratio of positive parasitological test from 1 year aged to 35 year aged by a 1 year increment and then to 70 year aged by a 5 year increment. It was then possible to observe the evolution of the Odds Ratio with increasing age.

The models showed a significant age effect from 1996 to 2003. The ORs were all decreasing regularly from 1y to 70y expressing a decreasing risk of positive parasitological test with increasing age. Between 2004 and 2009 with the exception of the year 2007 (risk increasing with age), age was not significant in the models. Overall, these results concur to show that in the past few years, the risk of malaria has become the same throughout life.

DISCUSSION

The conclusions of this study are based on over 26,000 cases of suspected malaria and 14 years of work at a rural dispensary in an area of previously of stable malaria with moderate transmission intensity. This paper documents the decrease of burden of malaria and fevers between 1996 and 2009 and the concurrent increase in the age of malaria patients. Together, these variations indicate a reduction of malaria transmission.

The burden of malaria decreased approximately 16 times from ~0.4 to ~0.02 episodes person-year. Concomitantly, consultations for fever also decreased 2.4 times (from ~1.7 to 0.7 visits person-year) and antimalarial treatments decreased 7 times (from ~0.9 to 0.13 treatments person-year). This was paralleled by changes in the age profile of malaria patients so that the risk of malaria is now almost uniformly distributed throughout life, while in the past malaria used to concern more children under 16 years of age.

The age-dependent risk of malaria and its clinical presentation (uncomplicated and or severe) varies with transmission intensity. There are marked differences in the number of malaria attacks at different ages in varying epidemiological settings (reviewed in Lalloo et al, 2006). Obviously intensity of transmission and number of challenges over time determine the susceptibility to malaria infection and disease, in parallel with the speed at which immunity is built. Even in areas of stable malaria, intensity of transmission shapes up very different age profiles of malaria cases (Trape & Rogier 1996). While trends towards decreased malaria transmission, morbidity and mortality are being reported (WHO malaria report 2009, O'Meara et al, 2008, Ceesay et al 2008 and 2010, D'Acremont et al, 2010), there is very little information on how these changes affect different ages. O'Meary et al (2008) report that while the age of non-malaria fevers seen at a paediatric word in Kilifi, Kenya has remained unchanged between 1990-2007 (2.15 to 2.18 years), that of slide-positive patients increased from 2.3 to 3.59 years.

The mean age of the malaria patients increased steadily from 13.4 years in 1996 to 20.1 in 2009, while no trend is observed for non-malaria fevers (19.8 years in 2007 and 21.5 in 2009). While overall in agreement with the trends presented by O'Meara et al (2008), the situation here is clearly different from coastal Kenya where intensity of transmission is higher, age of patients lower, and where the curves of the malaria-positive and -negative hospital admissions start diverging already in the 1990s.

The challenge with these data was to translate an observation into a statistically robust analysis of trends over time, hence the use of models allowing for interaction between variables. All these ana-

lyses converge to show that there has been a progressive shift in the age-dependent risk of malaria and the risk has been increasing for adolescents and adults.

Indeed the yearly sample size has been decreasing with the falling malaria risk in the population. Patients with suspected malaria presenting at the clinic today are more likely to be submitted to a parasitological test. Specifically, adolescents and adults (16 years and older) and in particular adults >30 year old are now being assessed more. The question is whether this is introducing a bias or rather reflect, as we tend to believe, the changing risk of malaria as also appreciated by health workers.

Between 1996 and 2009 significant changes have been introduced to malaria policies and practices here like elsewhere. In Senegal, the official policy change from chloroquine or quinine given on clinical grounds to artesunate plus amodiaquine on parasitological confirmation was made in 2006 but rapid diagnostic tests were made available starting in 2007. In Mlomp, parasitological diagnosis with microscopy was applied occasionally in the 1990s and then more systematically from 2000 when piloting the implementation of the new recommendations of the World Health Organization (WHO) to use artemisinin-based combinations for parasitologically-confirmed malaria. In this sense, Mlomp may be different from other settings in the country and the changes seen here not be obvious elsewhere, at least as yet. However, they indicate what changes may be expected in other settings which have been implementing these measures for a shorter period of time.

The analysis of the overall changes in presentations with fever, suspected and confirmed malaria at this site (not in this paper) shows a decline over time of all these parameters. This is largely contributed to by suspected malaria. The interpretation of these changes is beyond the scope of this paper. What specifically matters here is that the reduced malaria risk is accompanied by a redistribution of the risk across ages, and that a similar change is not observed for the non-malaria fevers.

Competing interest

The authors declare no conflict of interest.

Authors' contributions:

All authors read and approved the final manuscript.

- P.Olliaro contributed to the concept of the project, design of the protocol and analyses, reporting of the study, and prepared the manuscript.
- M.Cisse and M. Badiane participated in the planning and supervised the implementation of the study
- M.Vaillant designed and conducted the analyses, and prepared the manuscript.
- P.Brasseur was the Principal Investigator of the study. He contributed to the concept, protocol, analysis and reporting of the study, and contributed to the preparation of the manuscript. He personally contributed to the treatment, follow-up of patients and quality control of the study.

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Disclaimer

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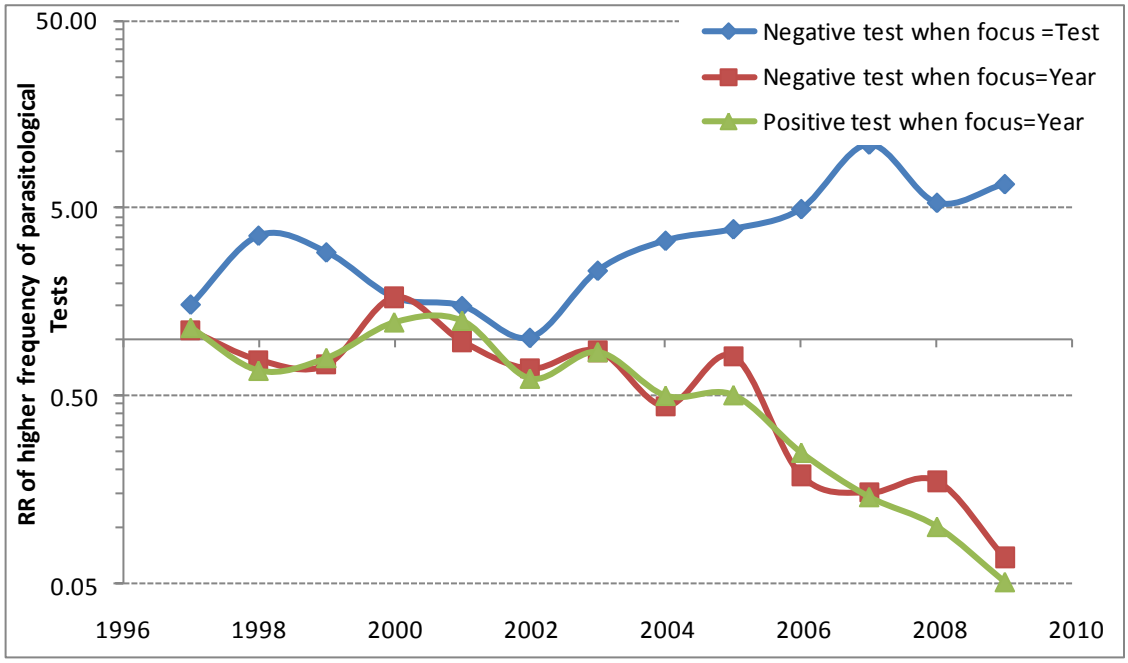
Supplemental Figure 1. Relative Risk of Positive or Negative parasitological test by using the Positive test as reference (blue line) or the year 1996 (red and green lines) in the negative binomial model of counts of parasitological tests.



Supplemental Figure3. Odds Ratio of positive parasitological test per age and year in Mlomp