Available online at www.sciencedirect.com
SCIENCE DIRECT
Infection, Genetics and Evolution 6 (2006) 241–248
Infection, Genetics and Evolution
www.elsevier.com/locate/meegid

Malaria transmission intensity and the rate of spread of chloroquine resistant *Plasmodium falciparum*: Why have theoretical models generated conflicting results?

Ambrose O. Talisuna [a,b,*], Annette Erhart [b], Sagarika Samarasinghe [b], Chantal Van Overmeir [b], Niko Speybroeck [b], Umberto D'Alessandro [b]

[a] *Ministry of Health, Epidemiological Surveillance Division, PO Box 7272, Kampala, Uganda*
[b] *Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerp, Belgium*

Received 7 April 2005; received in revised form 22 June 2005; accepted 29 June 2005
Available online 22 August 2005

Abstract

The rate at which *falciparum* resistant malaria spreads in different transmission settings is still a controversial subject. We have assessed the spread of mutant *Plasmodium falciparum* parasites in six Ugandan populations with varying prevalence of chloroquine resistance (CQR), malaria transmission intensity, multiplicity of parasite clones and prevalence of CQ use. For each population, we have determined the wild and mutant allele frequency at codons 76 and 86 of the *pfcrt* and *pfmdr1* genes, respectively.

The highest frequency (median = 16.3%, range: 0.0–70.4%) of infections with two pure mutants (no wild genotype in either gene), adjusted for clone multiplicity, was observed at the extremes of malaria transmission intensity. The wild/mutant (*W/M*) allele ratio (an index for tracking the progression of CQR) was less than one in all sites (median = 0.51, range: 0.09–0.98) for the *pfcrt*-76 gene, while it was greater than one in two of six sites (median = 0.75, range: 0.4–1.6) for the *pfmdr1*-86 gene, suggesting that the *pfcrt*-76 mutants were the predominant parasites at all sites. Furthermore, the *pfmdr1*-86 *W/M* allele ratio was consistently higher than that of the *pfcrt*-76.

The spread of mutations linked to CQR in *P. falciparum* commences with the *pfcrt*-76 gene mutations, followed later by the *pfmdr1*-86 gene mutations that modulate higher CQR. Such spread occurs faster at the extremes of the transmission spectrum and could explain why mathematical models have previously generated conflicting results with respect to malaria transmission intensity and spread of CQR.

Keywords: Malaria drug resistance; *Pfcrt*; *Pfmdr1*

1. Introduction

Human malaria is caused by the eukaryote of the genus *Plasmodium*, which has an obligate sexual phase in the female anopheles mosquito, resulting in a diploid zygote (ookinete) that undergoes a meiotic cell division, during which genetic recombination and assortment takes place in a normal Mendelian pattern (Walliker et al., 1987). The latter is believed to be responsible for the generation or breakdown of novel parasite genotypes (diversity) and has important implications for the emergence and spread of drug resistance. Furthermore, the occurrence of a meiotic cell division in the first few hours of sexual fertilization ensures that *Plasmodium* is in an asexual haploid phase during most of its life cycle that largely occurs in the human hosts. This has important and practical implications for the molecular epidemiology and the population dynamics of malaria parasites (Greenwood, 2002).

The genetic basis for chloroquine resistance (CQR) has been previously linked to point mutations in the *Plasmodium falciparum* multi-drug resistance (*pfmdr1*) gene, encoding the Pgh1 glycoprotein homologue, thought to be involved in protein transport across the parasite digestive vacuole (Foote et al., 1990; Reed et al., 2000). However, recent studies have

* Corresponding author. Tel.: +32 3 247 63 54.
E-mail addresses: acesd@afsat.com, atalisuna@yahoo.com (A.O. Talisuna), udalessandro@itg.be (U. D'Alessandro).

1567-1348/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.meegid.2005.06.003

provided strong evidence that CQR is linked to mutations in the *pfcrt* gene that encodes the *P. falciparum* chloroquine resistance transporter (*PfCRT*) protein (Fidock et al., 2000; Sidhu et al., 2002).

It is still unclear what factors influence the rate of spread of anti-malarial drug resistance. Several theoretical models have been produced (Hastings, 1997; Hastings and Mackinnon, 1998; Cross and Singer, 1991). Besides drug selection pressure, models have generated conflicting predictions about other potential determinants. The question whether anti-malarial drug resistance spreads faster in areas of high or low transmission has been a controversial subject since Paul et al. (1995) reported that substantial inbreeding occurs in malaria parasite populations even where malaria transmission is intense. Currently, there are several contrasting theories as to whether malaria transmission intensity has any independent role on the spread of parasite resistance, predicting faster spread at high transmission (Mackinnon and Hastings, 1998), low transmission (White, 1999), at the extreme of transmission (Hastings, 1997; Hastings and D'Alessandro, 2000) or regardless of transmission, at least during the early stages (Hastings et al., 2002). These hypotheses depend on their own underlying assumptions on the population dynamics of *Plasmodium*. Predictions that resistance might spread faster in low transmission areas have important implications for interventions aiming at decreasing or interrupting malaria transmission (insecticide treated bed nets and residual indoor spraying) because drug resistance could spread faster as a consequence of the intervention itself.

In this report, we present data on the evolution of two mutations linked to CQR in six Ugandan communities with varying malaria transmission intensity and CQ use. Furthermore, we derive a conceptual model and discuss why previous mathematical models have generated conflicting results.

2. Materials and methods

2.1. Data collection

The study was conducted at six Uganda sentinel sites currently used for monitoring anti-malaria drug efficacy. A detailed description of the sites is published elsewhere (Talisuna et al., 2002). Briefly, the sentinel sites were chosen by the East Africa Network for Monitoring Anti-malarial Treatment (EANMAT) and the Uganda National Malaria Control Programme (UNMCP) for the surveillance of anti-malarial drug efficacy. The sentinel sites were selected according to several criteria that included: the malaria transmission intensity (high, medium, low), moderate/high population density, easy accessibility/practicability, inclusion of at least a country border area, geographical representation and the presence of a stable population with limited in and out migration. Five of the study sites (Arua, Apac, Tororo, Kabarole, and Rukungiri) are rural and one (Jinja) is peri-urban.

The CQ in vivo efficacy tests were conducted over the period 1998–1999 using the standard WHO protocol (World Health Organisation, 1996) and a modified field manual developed by EANMAT (www.eanmat.org). Eligible patients were children aged 6–59 months presenting to the local health facility for fever (body temperature $\geq$37.5 °C) or history of fever within 24 h prior to the visit, with a *P. falciparum* mono-infection and a parasite density between 2000 and 100,000/μL of blood. Details of the drug efficacy studies such as inclusion and exclusion criteria have been presented elsewhere (Talisuna et al., 2002). An independent population-based survey was conducted in 1999 at each site on a sample of 250 people aged 1–45 years randomly selected from a census file. During the survey, the parasite prevalence (a proxy for malaria transmission) was measured, the prevalence of CQ use in the community was estimated using a dip stick test for urine (Eggelte, 1990), and blood samples on Whatmann filter paper number 3MM were taken for the analysis of mutations linked to CQ resistance (*pfcrt* codon 76 and *pfmdr1* codon 86) using previously published techniques (Djimde et al., 2001; von Seidlein et al., 1997). Moreover, the mean number of malaria parasite clones per individual was estimated using the polymorphic genes *msp1* and *msp2* as described previously (Ranford-Cartwright et al., 1997).

2.2. Statistical analysis

Data were double entered in Epi-Info 6 (Centres for Disease Control, CDC, Atlanta GA), range and consistency checks were done before statistical analysis with Stata Version 8.0 (StataCorp.2003, College Station, Texas, USA). The frequency of wild and mutant genotypes at codon 76 of *pfcrt* and codon 86 of the *pfmdr1* genes among infected individuals were compared amongst the different sites using the chi-square test. We have previously proposed the mutant to wild (*M/W*) allele ratio as a robust index for tracking drug resistance (Talisuna et al., 2002, 2003). We have extended this concept and have used the *W/M* allele ratio to measure the disappearance of sensitive parasites as drug resistance evolves. The relationship between the prevalence of wild and mutant alleles, the *W/M* allele ratio, and CQ use was determined by linear regression. Different indices such as the *W/M* allele ratio, the inverse of the wild allele of *pfcrt* (1/K76) and the ratio of the wild allele in *pfmdr1*-86 to the wild allele of *pfcrt*-76 (N86/K76) were used to determine the spread of drug resistance in each site. In addition, we have carried out a multinomial logistic regression to examine the effect of the presence of the *pfcrt*-76 mutation (explanatory variable) on the risk of having a *pfmdr*-86 mutation (dependent variable), taking into account the survey characteristics and using the sites as primary sampling units. We used a baseline-category model to relate the categorical outcome (*pfmdr1* mutation) to other risk factors:

Table 1
Prevalence of mutant (*M*), wild (*W*) and *W/M* ratios for *pfcrt*-76 and *pfmdr1*-86 at different sites in Uganda

Site	% CQ use (all ages)	Mean MPC (S.D.)	% CQ TTF	% CQ ETF	*N* (*pfcrt*)	*N* (*pfmdr1*)	% *pfcrt*-76 (*W*)	% *pfmdr*-86 (*W*)	% *pfcrt*-76 (*M*)	% *pfmdr1*-86 (*M*)	*pfcrt*-76 (*W/M*)	*pfmdr1*-86 (*W/M*)	*pfcrt*-76 (pure *M*)	*pfmdr1*-86 (pure *M*)	*pfmdr1* Y86 and *pfcrt* T76 (pure *M*[a])
Jinja (PR = 13)	82 (75–87)	2.1 (1.2)	28.0	28.0	12	9	9.1	33.3	100	77.8	0.09	0.4	91.7	66.7	70.4
Rukungiri (PR = 39)	68 (61–75)	2.5 (1.2)	10.0	10.0	59	29	23.3	37.9	93.6	89.2	0.25	0.4	76.3	62.1	38.5
Tororo (PR = 91)	41 (34–47)	2.9 (1.0)	42.4	20.3	43	29	32.3	75.9	93.6	93.1	0.35	0.8	62.8	24.1	18.3
Kabarole (PR = 67)	43 (36–51)	2.1 (0.9)	43.8	12.5	48	29	65.3	62.1	98.0	93.1	0.67	0.7	33.3	37.9	14.5
Arua (PR = 83)	33 (26–40)	2.5 (1.2)	21.1	8.8	73	65	80.8	84.6	86.3	64.6	0.94	1.3	19.2	15.4	6.2
Apac (PR = 79)	32 (26–38)	3.5 (1.3)	14.8	7.4	61	31	88.2	100.0	90.2	64.5	0.98	1.6	11.7	0.0	0.0

MPC: multiplicity of parasite clones, CQ: chloroquine, TTF: total treatment failure, ETF; early treatment failure, *M*: mutant allele, *W*: wild allele.
[a] Adjusted for number of clones.

this model leads to a system of simultaneous logistic regression models, where each outcome category is considered as one independent group and separately compared to the reference category. For this purpose, we used the ''svymlogit'' command in STATA.8, which fits baseline-category models for survey data and is able to take into account clustering by pseudo-likelihood estimation. The *pfmdr1*-86 mutation was categorised as follows: (1) presence of the pure mutant (*MM*); (2) mixed (*MW*); (3) the pure wild genotype (*WW*). A uni and multivariate-adjusted analysis has been performed in order to adjust the effect of the *pfcrt*-76 mutation for potential confounders such as sex, age, drug use, fever, anaemia and multiplicity of parasite clones. For clone multiplicity, we have used a binary variable defining for each infection if it was mono (0) or polyclonal (1).

3. Results

3.1. Malaria transmission intensity, drug use and clone multiplicity

We have used the parasite prevalence (PR) in the age group 2–9 years as an index for malaria transmission intensity and endemicity (Metselaar & Thiel, 1959). Based on the PR, three sites (Arua, Apac and Tororo) have holo-endemic malaria (PR >75%), one site (Kabarole) has hyper-endemic malaria (PR 51–75%) and two sites (Jinja and Rukungiri) have meso-endemic malaria (PR, 11–50%) (Table 1). Chloroquine (CQ) use in the community varied significantly according to endemicity ($p < 0.001$, Table 1). Higher CQ use was observed in the meso-endemic sites compared to the holo- or hyper-endemic sites and was inversely correlated to the parasite prevalence ($r = -0.88$, $p = 0.01$). The mean multiplicity of parasite clones (MPC) was lower in the meso-endemic sites compared to sites with intermediate or high PR (Table 1).

3.2. Dynamics for the spread of the mutations linked to CQR within populations

The molecular analysis of the *pfcrt*-76 mutation was carried out on 302 blood samples and the presence of the wild (K76) or mutant (T76) genotype was determined for 296 isolates. Fewer isolates (192) for *pfmdr1* gene were analysed, the wild (N86) and mutant (Y86) genotypes were determined for all of them (Table 1). The prevalence of mutants in both genes varied significantly between sites according to transmission intensity and drug use ($p < 0.01$). The prevalence of the mutant (T76) *pfcrt* allele was always higher than that of the mutant (Y86) *pfmdr1* allele. Furthermore, the wild (N86) *pfmdr1* allele was also higher compared to the wild (*K* 76) *pfcrt* allele and consequently the *W/M* allele ratio (an index for the progression of CQR) was constantly higher for *pfmdr1*-86 compared to *pfcrt*-76.

Table 2
Multivariate adjusted analysis for the risk of *pfmdr1* (86) mutations

Genotype *mdr1* (86)	Risk factors	OR	95% CI (*p*-value)
Pure mutant (*MM*)[a]	*Pfcrt* (76)		
	MM	15.76	6.71; 37.06 ($p < 0.001$)
	MW	3.85	0.39; 38.14 ($p = 0.19$)
	WW	1	
Mixed (*MW*)[b]	*Pfcrt* (76)		
	MM	1	
	MW	3.59	1.24; 10.41 ($p = 0.027$)
	WW	7.56	0.73; 77.73 ($p = 0.08$)
	Age categories		
	0–15 years	1	
	>15 years	0.39	0.16; 0.97 ($p = 0.045$)

Using a multinimial logistic regrression model.
[a] Comparison group is pure wild *mdr1* (86) genotype.
[b] Comparison group is pure mutant *mdr1* (86) genotype.

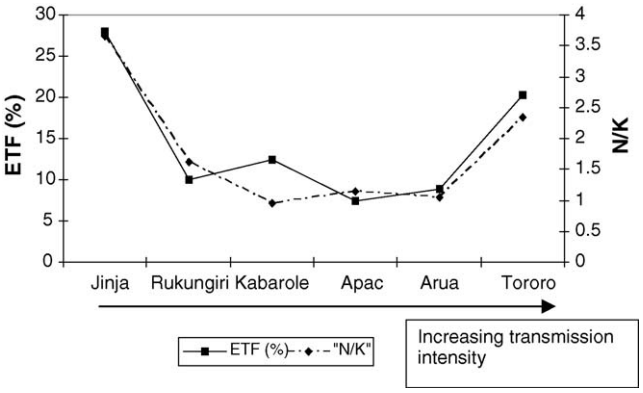

Fig. 1. ETF and *pfmdr1*/*pfcrt* wild ratio by site (ordered by transmission intensity), N/K: wild to wild allele ratio.

Indeed at one site the *pfmdr1*-86 (*MM*) genotype was absent, yet the prevalence of the pure *pfcrt*-76 mutant was 12% (Table 1).

The multinomial logistic regression analysis (multivariate-adjusted) showed that the *pfmdr1*-86 mutation was strongly and significantly associated with the *pfcrt*-76 mutation (Table 2). In the univariate analysis fever, age and sex were significantly associated with the risk of either pure *pfmdr1*-86 (*MM*) or mixed (*MW*) genotypes. In the multivariate-adjusted model, only age remained a significant risk fact for the presence of mixed (*MW*) *pfmdr1*-86 genotype. The risk of having the pure *pfmdr1*-86 (*MM*) mutant genotype, compared to the pure wild (*WW*) genotype, was 16 times higher, when the pure *pfcrt-76* (*MM*) genotype was present, but it was not significant when the mixed *pfcrt*-76 (*MW*) genotype was present. When compared to the *WW* genotype, the risk of having the mixed *pfmdr1*-86 (*MW*) genotype was not significant for any of the three *pfcrt*-76 genotypes. However, when compared to the pure *pfmdr1*-86 (*MM*) genotype, this risk was almost four times higher if the mixed *pfcrt*-76 (*MW*) genotype was present (OR = 3.59; $p = 0.027$) and almost seven times higher when the pure wild *pfcrt*-76 was present, though it did not reach statistical significance due to the small number of samples (only four). Young age was not a significant risk factor for the presence of pure *pfmdr1*-86 (*MM*) genotype. However, for the mixed (*MW*) *pfmdr1*-86 genotype, adults were significantly less at risk than children below 15 years old (OR = 0.39; $p < 0.05$).

3.3. The pfmdr1-86 mutation and CQ use

The prevalence of the *pfmdr1*-86 (*MM*) ($r = 0.95$, $p = 0.004$) and that of both (*MM*) genotypes (no wild allele in either genes detected) ($r = 0.99$; $p < 0.001$) was positively correlated to CQ use, indicating the importance of drug pressure in the selection of the *pfmdr1* and *pfcrt* gene mutations. Moreover CQ use was significantly correlated with the disappearance of the wild *pfcrt*-76 (1/K76) allele ($r = 0.91$; $p = 0.01$), the latter index being also correlated to early treatment failure (ETF, Table 1).

3.4. Malaria transmission intensity and molecular indices for CQR

The N86/K76 index was higher for sites at the extremes of the transmission spectrum, the same sites where ETF was highest (Fig. 1). Indeed there was a strong positive correlation between the N86/K76 index and the prevalence of ETF ($r = 0.94$; $p = 0.005$). Furthermore, the product of the N86/K76 index and the *pfmdr1*-86 (Y86) mutant allele prevalence (NY/K index) was even more strongly correlated to ETF ($r = 0.95$; $p = 0.003$). No relationship between transmission intensity and the *pfcrt*-76 mutation prevalence was observed as this was almost 100% at all sites (Fig. 2). However, that of the *pfmdr1*-86 mutation was lower in sites of intermediate transmission as compared to those at the extreme of the transmission spectrum (the ''valley pattern''), while the prevalence of the wild alleles for both genes showed an opposite profile (the ''apex or reverse valley pattern'', Fig. 2). Similarly, when adjusting for clone multiplicity, the prevalence of the (*MM*) genotype for both

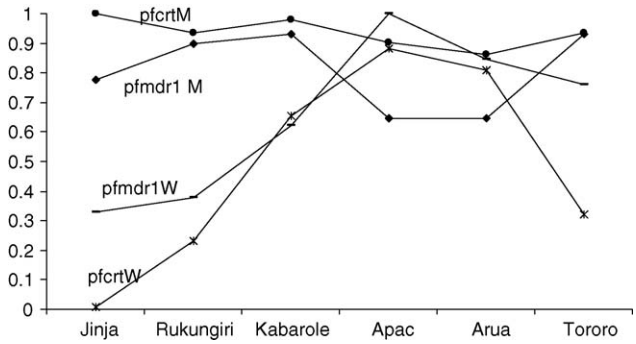

Fig. 2. Prevalence of the mutant and wild alleles by site ordered by increasing transmission intensity. *pfcrtW*: frequency wild *pfcrt*-76 allele, *pfcrt*M: frequency mutant *pfcrt*-76 allele, *pfmdr1W*: frequency wild *pfmdr1*-86 allele, *pfmdr1*M: frequency mutant *pfmdr1*-86 allele.

genes was lower in intermediate transmission sites and highest at the extremes of the transmission spectrum, and was strongly correlated with CQ use ($r = 0.97$; $p < 0.001$), suggesting that both mutations (T76 and Y86) are strongly linked to each other and vary according to transmission intensity and drug pressure.

4. Discussion

In these Ugandan sites the prevalence of the *pfcrt*-76 mutant allele was consistently higher than that of *pfmdr1*-86 mutant allele. Such an observation could be due several reasons; the progression of the selection of the mutations linked to CQR probably starts with the *pfcrt*-76 mutations followed later by the *pfmdr1*-86 mutations which might modulate higher levels of CQR. The latter observation probably explains why the relationship between the *pfmdr1*-86 mutations and CQ resistance has been inconsistent in previously published studies (Foote et al., 1990; Reed et al., 2000; Dorsey et al., 2001; Wellems and Plowe, 2001; Tinto et al., 2003). However, the same observation is compatible with the selection of the T76 *pfcrt* allele and the Y86 *pfmdr1* allele by mechanisms that are independent of each other at the molecular level but could be associated with another factor such as access to other anti-malaria drugs such as mefloquine that are linked to the *pfmdr1*-86 mutation. The latter explanation, though plausible is unlikely because mefloquine is not a recommended drug according to the Uganda national policy and is mainly used by non-immune visitors for chemoprophylaxis. The present study was probably conducted late in relation to the evolution of CQR in Uganda, the prevalence of the *pfcrt*-76 mutant allele was very high at all sites and did not vary between them. The lack of a clear pattern for the *pfcrt*-76 mutation at all sites is explained by the high CQR (range: 20–81%) (Talisuna et al., 2003). Therefore, the prevalence of the *pfcrt*-76 mutation alone could not predict CQR in such a setting of already high CQR as the prevalence of the *pfcrt*-76 mutant parasites had already reached a plateau. Instead, the disappearance of the *pfcrt*-76 wild alleles and the presence of the mutant *pfmdr1*-86 parasites were better markers of the actual level of CQR. Indeed the NY/K index, the product between the N86/K76 ratio and the prevalence of the *pfmdr1*-86 mutant allele, was the best predictor of the level of CQR as it was strongly correlated to ETF. This suggests that increasing and high CQR is due to the disappearance of parasites with the wild *pfcrt*-76 alleles and the accumulation of those with the *pfmdr1*-86 mutation.

The lower prevalence of *pfmdr1*-86 mutants in sites with intermediate transmission compared to either the low or high transmission sites and the higher prevalence of the wild alleles for both genes at sites with intermediate transmission was striking. Such pattern suggests that resistance spreads according to transmission intensity and drug pressure. Similarly, the observation that infections with pure mutations in both genes adjusted for clone multiplicity was lower in intermediate transmission and highest at the extremes of the transmission spectrum supports the role of transmission intensity modulating the spread of CQR.

The relationship between malaria transmission intensity and the spread of drug resistance is probably the result of its relationship with the multiplicity of parasite clones (MPC) (Babiker and Walliker, 1997). We have previously showed, using both *msp1* and *msp2* genes, that the mean number of clones increases as transmission intensity increases (Talisuna et al., 2003). Indeed, our observations have been recently replicated at these very sites using only the *msp2* gene (Slater et al., submitted). Populations exposed to low transmission and hence low multiplicity of parasite clones (MPC) (function of transmission and high drug use) are likely to have a faster spread of drug resistance if its genetic basis is modulated by two or more genes (Paul et al., 1995; Hill et al., 1995; Schmidt, 1995) because of the higher chance of inbreeding and transmitting the entire combination of mutated genes to the progeny. However, if the mechanism is monogenic, the low MPC would not particularly increase the rate of spread of mutant parasites and transmission intensity would not affect it.

In areas of high transmission, intra-host competition between co-infecting clones (intra-host dynamics) is probably an important factor (Hastings and D'Alessandro, 2000). The generalised immunity (GI) model of intra-host competition/dynamics predicts that resistance could spread faster in areas of high transmission (Hastings, 1997; Hastings and D'Alessandro, 2000). If intra-host dynamics play an important role, then the mutant parasite sub-population multiplies and replaces the susceptible one that is eliminated by chemotherapy and this process is favoured by a high MPC, which occurs in intense transmission areas. The intra-host dynamics hypothesis (Hastings and D'Alessandro, 2000) is indirectly supported by the present study and previous epidemiological observations in Uganda (Talisuna et al., 2002, 2003). The faster evolution of CQR at the extremes of the transmission spectrum is thus consistent with the interplay between intra-host dynamics and increased sexual recombination in the evolution of CQR.

Based on the present study, previous empirical studies and theoretical models (Talisuna et al., 2002, 2003; Hastings, 1997; Hastings, 2001; Hastings et al., 2002; Mackinnon and Hastings, 1998; White, 1999), we have developed two conceptual models that attempt to link the different determinants involved in the spread of drug resistance in scenarios of high and low transmission intensity (Figs. 3 and 4). The emergence of drug resistance is a function of the de novo emergence of spontaneous mutations, which is itself a function of the parasite biomass (White, 1999; White and Pongtavornpinyo, 2003). Parasite biomass is very attractive from a statistical point of view because mutations linked to drug resistance are more likely to occur in large parasite biomass infections. However, parasite biomass is very difficult to measure and does not

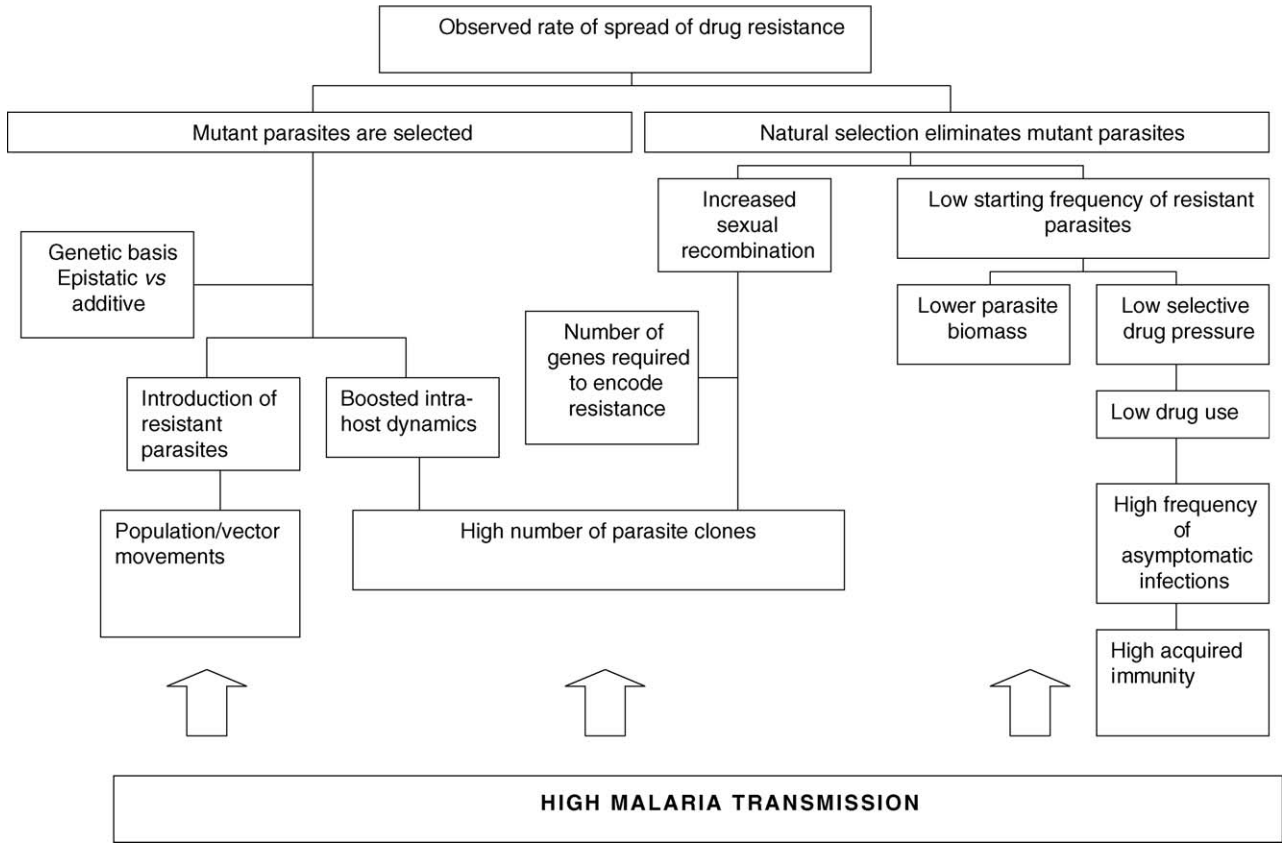

Fig. 3. High malaria transmission.

correlate with peripheral parasite density. The lack of correlation between parasite biomass and peripheral parasite density is best observed in pregnancy, where pregnant mothers can have malaria parasites sequestered in the placenta with a negative peripheral blood film. Large parasite biomass infections could occur even when the peripheral parasite density is low, with most of the parasites sequestered in organs such as the spleen, liver and for pregnant women in the placenta. Based on the parasite biomass theory, the emergence of drug resistance is likely to occur faster in areas of low transmission because large parasite biomass infections are more likely to occur in these areas compared to high transmission areas where immunity develops readily and regulates parasite biomass (White, 1999). The mutant parasites that emerge are then selected by drug pressure or eliminated by natural selection (White, 1999, Hastings, 2001). However, mutant parasites could also be introduced by human and vector movements and could then be selected by drug pressure (Roper et al., 2003). Transmission intensity affects directly parasite diversity and sexual recombination as well as intra-host dynamics, the effects of which seem antagonistic. Low parasite diversity in areas of low transmission facilitates faster spread of drug resistance because of the lower probability of breaking down mutant combinations during sexual recombination, while the high parasite diversity in areas of high transmission increases the probability of breakdown of mutant combinations, slowing the spread of drug resistance. However, high parasite diversity and drug pressure boost the effects of intra-host dynamics (Hastings, 1997, 2003) favouring faster spread of resistance in high transmission areas. Furthermore, malaria transmission intensity indirectly modifies the frequency of drug use. Although the overall number of clinical episodes is likely to be more in the high compared to the medium or low transmission areas, a higher proportion of infections in low transmission areas would progress to clinical disease and would be treated (Rogier et al., 1999) increasing the probability of drug exposure and possibly selection of resistant parasites. Though population movements are not influenced by transmission intensity, introduced mutant parasites might replace susceptible ones depending on the frequency of sexual recombination, intra-host competition and drug pressure. Finally, the type of malaria vectors could favour resistant parasite infections (Wernsdorfer and Payne, 1991).

The relationship between CQ resistance and transmission intensity is complex, but some explanations are possible. A conclusive causal relationship between transmission intensity and spread of drug resistance could be determined if more extensive studies (probably involving 30 or more sites in different countries) are conducted and a better index for transmission intensity is established. Nevertheless, our studies suggest that malaria transmission intensity is a critical determinant for the spread and evolution of drug resistance.

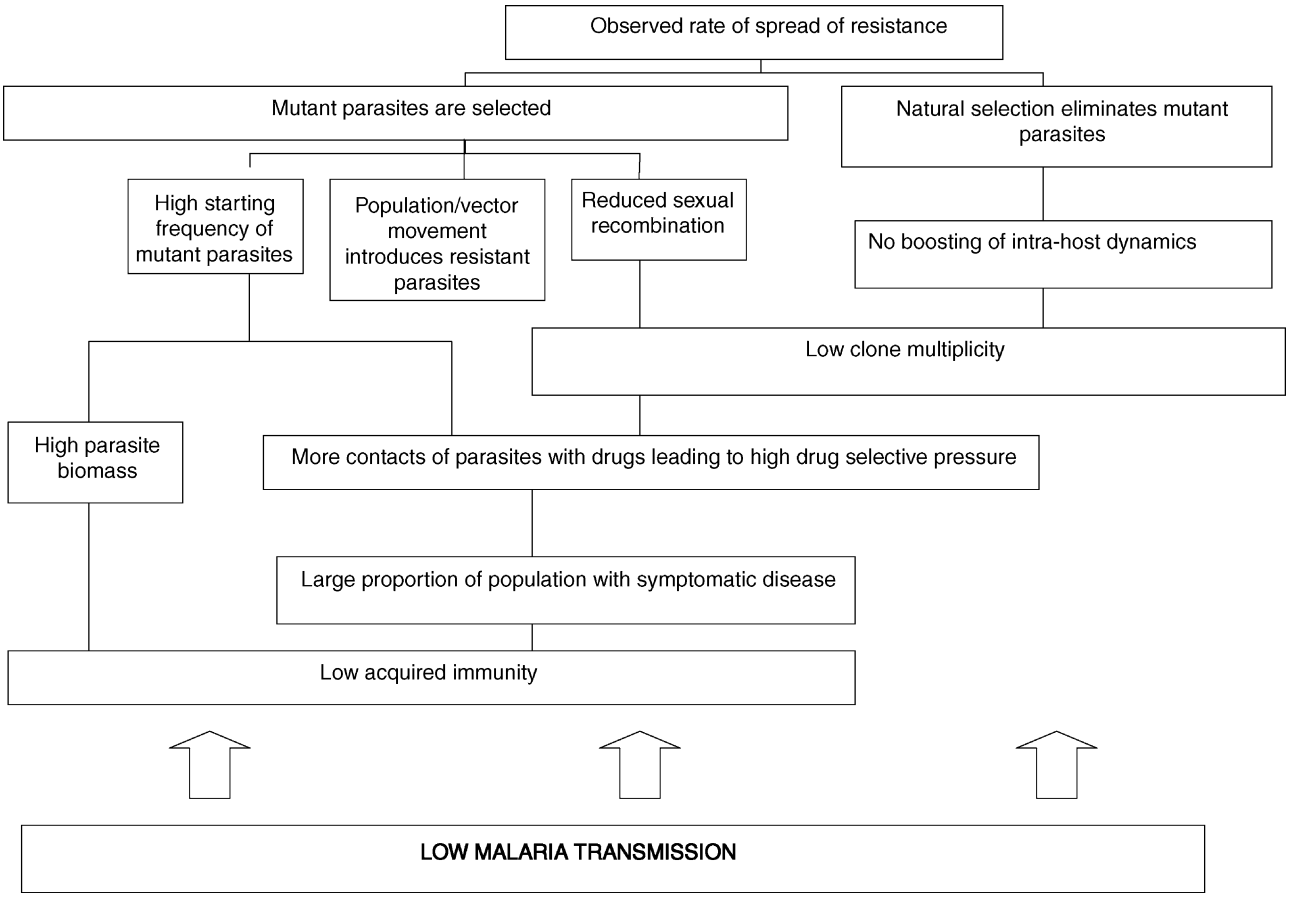

Fig. 4. Low malaria transmission.

The conflicting theoretical predictions about the effect of transmission intensity on the spread of drug resistance can be explained by the genetic basis of resistance and the interplay between intra-host dynamics and sexual recombination.

Acknowledgement

We thank all members of the survey team, namely Dr. Mashiale, Dorothy Kirunda, Nelson Budaka, Martin Waliwulya and Nuhu Kibampawo. We would also like to thank Prof. Marc Coosemans for useful discussions. We are grateful to the Uganda Ministry of Health leadership namely Prof. F.G. Omaswa (Director General of Health Services) and Dr. S.I. Okware (Commissioner of Health Services) and Dr. Peter Langi (Principal Medical Officer) for their technical and moral support. This study was supported by the Belgian Development Cooperation in collaboration with the Institute of Tropical Medicine, Antwerp (ITM).

References

Babiker, A., Walliker, D., 1997. Current views on the population structure of *Plasmodium falciparum*: implications for control. Parasitol. Today 13, 262–267.

Cross, A.P., Singer, B., 1991. Modelling the development of resistance of *Plasmodium falciparum* to anti-malarial drugs. Trans. R. Soc. Trop. Med. Hyg. 85, 349–355.

Djimde, A., Doumbo, O.K., Cortese, J.F., Kayentao, K., Doumbo, S., Diourte, Y., et al., 2001. A molecular marker for chloroquine-resistant *falciparum* malaria. N. Engl. J. Med. 344, 257–263.

Dorsey, G., Kamya, M.R., Singh, A., Rosenthal, P.J., 2001. Polymorphisms in the *Plasmodium falciparum pfcrt* and *pfmdr-1* genes and clinical response to chloroquine in Kampala. Uganda J. Infect. Dis. 183, 1417–1420.

Eggelte, T.A., 1990. Production of monoclonal antibodies against anti-malarial drugs for use in immuno assays. In: Navaratnam, V., Payne, D. (Eds.), The Validation of Chemical and Immunological Tests for Anti-malarials in Body Fluids, Centre for drug Research International Monograph Series 3, University Sains Malaysia, Penang, pp. 35–63.

Fidock, D.A., Nomura, T., Talley, A.K., Cooper, R.A., Dzekunov, S.M., Ferdig, M.T., et al., 2000. Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol. Cell 6, 861–871.

Foote, S.J., Kyle, D.E., Martin, R.K., Oduola, A.M., Forsyth, K., Kemp, D.J., et al., 1990. Several alleles of the multidrug-resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. Nature 345, 255–258.

Greenwood, B., 2002. The molecular epidemiology of malaria. Trop. Med. Int. Health 7, 1012–1021.

Hastings, I.M., D'Alessandro, U., 2000. Modelling a predictable disaster: the rise and spread of drug-resistant malaria. Parasitol. Today 16, 340–347.

Hastings, IM, 1997. A model for the origins and spread of drug-resistant malaria. Parasitology 115, 133–141.

Hastings, I.M., 2001. Modelling parasite drug resistance: lessons for management and control strategies. Trop. Med. Int. Health 6, 883–890.

Hastings, I.M., Watkins, W.M., White, N.J., 2002. The evolution of drug-resistant malaria: the role of drug elimination half-life. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 357, 505–519.

Hastings, I.M., 2003. Malaria control and evolution of drug resistance: an intriguing link. Trends Parasitol. 19, 70–73.

Hastings, I.M., Mackinnon, M.J., 1998. The emergence of drug-resistant malaria. Parasitology 117, 411–417.

Hill, W.G., Babiker, H.A., Ranford-Cartwright, L.C., Walliker, D., 1995. Estimation of inbreeding coefficients from genotypic data on multiple alleles, and application to estimation of clonality in malaria parasites. Genet. Res. 65, 53–61.

Mackinnon, M.J., Hastings, I.M., 1998. The evolution of multiple drug resistance in malaria parasites. Trans. R. Soc. Trop. Med. Hyg. 92, 188–195.

Paul, R.E.L., Packer, M.J., Walmsley, M., Lagog, M., Randford-Cartwright, L.C., Paru, R., Day, K.P., 1995. Mating patterns in malaria parasite populations of Papua New Guinea. Science 269, 1709–1711.

Ranford-Cartwright, L.C., Taylor, J., Umasunthar, T., Taylor, L.H., Babiker, H.A., Lell, B., Schmidt-Ott, J.R., Lehman, L.G., Walliker, D., Kremsner, P.G., 1997. Molecular analysis of recrudescent parasites in a *Plasmodium falciparum* drug efficacy trial in Gabon. Trans. R. Soc. Trop. Med. Hyg. 91, 719–724.

Reed, M.B., Saliba, K.J., Caruana, S.R., Kirk, K., Cowman, A.F., 2000. Pgh1 modulates sensitivity and resistance to multiple anti-malarials in *Plasmodium falciparum*. Nature 403, 906–909.

Rogier, C., Diagne, N., Fontenille, D., Spiegel, A., Trape, J.F., 1999. *Plasmodium falciparum* clinical malaria: lessons from longitudinal studies in Senegal. Parasitologia 41, 255–259.

Roper, C., Pearce, R., Bredenkamp, B., Gumede, J., Dralkely, C., Mosha, F., Chandramohan, D., Sharp, B, 2003. Antifolate resistance in southeast Africa: a population-based analysis. Lancet 361, 1174–1181.

Schmidt, KF, 1995. Inbred parasites may spur resistance. Science 269, 1670.

Sidhu, A.B., Verdier-Pinard, D., Fidock, D.A., 2002. Chloroquine resistance in *Plasmodium falciparum* malaria parasites conferred by *pfcrt* mutations. Science 298, 210–213.

Slater, M., Kiggundu, M., Dokomajilar, C., Kamya, M.R., Bakyaita, N., Talisuna, A., Rosenthal, P.J., Dorsey, G., 2005. Distinguishing recrudescence from new infections in antimalarial clinical trials: interpretation of genotyping results has a major impact on estimates of drug efficacy, submitted.

Talisuna, A.O., Langi, P., Mutabingwa, T.K., Van Marck, E., Speybroeck, N., Egwang, T.G., Watkins, W.M., Hastings, I.M., D'Alessandro, U., 2003. Intensity of transmission and spread of gene mutations linked to chloroquine and sulphadoxine-pyrimethamine resistance in *falciparum* malaria. Int. J. Parasitol. 33, 1051–1058.

Talisuna, O.A., Langi, P., Bakyaita, N., Egwang, T., Mutabingwa, T.K., Watkins, W., Van Marck, E., D'Alessandro, U., 2002. Intensity of malaria transmission, anti-malarial drug use and resistance in Uganda: what is the relationship between these three factors? Trans. R. Soc. Trop. Med. Hyg. 96, 310–317.

Tinto, H., Ouedraogo, J.B., Erhart, A., Van Overmeir, C., Dujardin, J.C., Van Marck, E., Guiguemde, T.R., D'Alessandro, U., 2003. Relationship between the *Pfcrt* T76 and the *Pfmdr-1* Y86 mutations in *Plasmodium falciparum* and in vitro/in vivo chloroquine resistance in Burkina Faso, West Africa. Infect. Genet. Evol. 3, 287–292.

von Seidlein, L., Duraisingh, M.T., Drakeley, C.J., Bailey, R., Greenwood, B.M., Pinder, M., 1997. Polymorphism of the *Pfmdr1* gene and chloroquine resistance in *Plasmodium falciparum* in The Gambia. Trans. R. Soc. Trop. Med. Hyg. 9, 450–453.

Walliker, D., Quakyi, I.C., Wellems, T.E., McCutchan, T.F., Szarfman, A., 1987. In: London, W.T., Corcoran, L.M., Burkot, T.R., Carter, R. (Eds.) Genetic Analysis of the Human Malaria Parasite *Plasmodium falciparum*. Science 236, 661–666.

Wellems, T.E., Plowe, C.V., 2001. Chloroquine-resistant malaria. J. Infect. Dis. 184, 770–776.

Wernsdorfer, W.H., Payne, D., 1991. The dynamics of drug resistance in *Plasmodium falciparum*. Pharmacol. Ther. 50, 95–121.

White, N., 1999. Anti-malarial drug resistance and combination chemotherapy. Philos.Trans.R. Soc. Lond. B: Biol.Sci 354, 739–749.

White, N.J., Pongtavornpinyo, W., 2003. The de novo selection of drug resistant malaria parasites. Philos. Trans. R. Soc. Lond. B: Biol. Sci. 270, 545–554.

WHO/CTD, 1996. Assessment of therapeutic efficacy of anti-malarial drugs for uncomplicated malaria in areas with intense transmission. World Health Organisation, WHO/MAL/96.1077.