TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2002) 96, 91–95

The Lys-76-Thr mutation in *PfCRT* and chloroquine resistance in *Plasmodium falciparum* isolates from Uganda

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Abstract

Recent molecular studies of chloroquine (CQ) resistance of *Plasmodium falciparum* have demonstrated an association between a mutation in the *PfCRT* gene and CQ resistance. We identified wild type and mutant alleles of the *PfCRT* codon 76 in baseline pre-CQ treatment *P. falciparum* isolates collected during 1999 and investigated their relationship to CQ efficacy in 3 different sites with different levels of CQ parasite resistance in Uganda. Of 32 isolates from Mulago Hospital, all were mutant (100%), while of 45 isolates from Tororo, 5 (11%) were mixed wild type and mutant and 40 (89%) were mutants only. Of 41 isolates from Apac, 13 (32%) were mixed wild type and mutant whereas 28 (68%) were mutants only. The finding of 100% prevalence of the Thr-76 mutant allele in all isolates at the 3 sites was remarkable. We found no association between the presence of Thr-76 mutation and treatment outcome at all the sites. However, the prevalence of the wild-type Lys-76 allele was higher in Apac, an area with lower CQ parasite resistance, compared to Tororo and Mulago which have relatively higher CQ parasite resistance. The Thr-76 allele as a marker of CQ resistance is probably useful in regions where the allele frequency has not yet plateaued.

Keywords: *Plasmodium falciparum*, genetic analysis, gene mutation, *PfCRT*, drug resistance, chloroquine, Uganda

Introduction

In Uganda, malaria is the principal cause of death in children aged <5 years and is responsible for 25% of all hospital admissions. Malaria control is currently by chemotherapy, with chloroquine (CQ) and sulfadoxine–pyrimethamine (SP) being first- and second-line drugs, respectively. CQ is the cheapest antimalarial drug, which can be afforded by most poor rural communities. However, the efficacy of this antimalarial drug is seriously threatened by the emergence of drug-resistant *Plasmodium falciparum* strains in Uganda. The monitoring of CQ resistance in various parts of Uganda is therefore of critical importance for the Malaria Control Programme. At present, the monitoring of antimalarial drug resistance involves patient enrolment, drug administration and a 14-day follow-up to determine parasitological and clinical outcomes. In some cases, parasite culture *in vitro* is used to confirm the resistance *in vivo* observed in the field. These approaches are labour intensive, expensive, and might not be feasible in areas of unstable political climate. By contrast, molecular techniques offer several advantages over classical traditional microscopy and antimalarial drug efficacy studies *in vitro* and *in vivo*. These advantages include the ability to detect minority parasite strains which might be lost during susceptibility studies *in vitro*, the ability to distinguish between recrudescent and re-infection parasites, and the fact that in some circumstances (e.g., areas of unstable political climates), if validated, they might be the only way to determine the drug resistance picture promptly and expeditiously.

Resistance to aminoquinolines and aminoalcohols has been associated with lower drug accumulation, possibly due to an ATP-dependent drug efflux similar to that of cytotoxic drugs in multidrug-resistant (mdr) cancer cells (KROGSTAD *et al.*, 1987). Several point mutations in the *Pfmdr1* gene on chromosome 5 have been associated with CQ resistance in culture-adapted strains of *P. falciparum* from South-East Asia, Papua New Guinea, and South America, but studies with wild isolates from Thailand and the Sudan yielded contradictory findings (FOOTE *et al.*, 1990; AWAD-EL-KARIEM *et al.*, 1992; WILSON *et al.*, 1993). Despite these findings, new evidence suggests that *Pfmdr1* probably modulates CQ resistance (REED *et al.*, 2000). More recently, 2 new gene loci on chromosome 7 have been implicated in CQ resistance, *cg2* and *PfCRT* (SU *et al.*, 1997; FIDOCK *et al.*, 2000b). A protein encoded by *cg2* has size variations in 3 repeat regions (κ, γ, and ω); polymorphisms in these regions appear to be associated with all but one CQ resistant strains examined (SU *et al.*, 1997). There has been conflicting evidence about the usefulness of these polymorphisms as reliable molecular markers of CQ resistance (ADAGU & WARHURST, 1999; BASCO & RINGWALD, 1999; DURAND *et al.*, 1999; MCCUTCHEON *et al.*, 2000). DNA transfection and allelic exchange has provided unequivocal evidence that *cg2* and *cg1* play no role in CQ resistance (FIDOCK *et al.*, 2000a). There is strong evidence that point mutations in a novel transmembrane protein *PfCRT*, a putative chloroquine resistance related transporter (**CRT**) linked to *cg2*, are completely associated with verapamil-reversible CQ resistance in *P. falciparum* parasite lines from Africa, South America and Asia (FIDOCK *et al.*, 2000b).

In the present paper, we report on the results of a study conducted to investigate the association between the Lys-76-Thr mutation in *PfCRT* in parasite isolates and parasitological and clinical failure in individual patients. The major aim was to ascertain whether the presence of the mutant codon 76 in individual parasite isolates was predictive of in-vivo and parasitological and clinical failure to CQ at the individual patient and community level.

Materials and Methods

Study populations and study design

The study was conducted at Aduku Health Centre (Apac District) and Nagongera Health Centre (Tororo District), 2 sentinel sites in Uganda chosen by the East Africa Network for Monitoring Antimalaria Treatment (EANMAT) and the Uganda National Malaria Control Programme (UNMCP) for surveillance of antimalarial drug efficacy; the study was also carried out at Mulago Hospital in Kampala. Cross-sectional surveys were conducted among patients enrolled for drug efficacy studies during 1999 at all 3 sites and parasite resistance *in vivo* and prevalence of mutant and wild-type alleles for codon 76 in *PfCRT* were measured.

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Measurement of parasite resistance in vivo

In-vivo drug efficacy tests were conducted at the 3 sites using the World Health Organization (WHO) protocol for in-vivo efficacy test for areas of intense transmission (WHO, 1996). In-vivo clinical failure for and parasitological resistance to CQ and SP were determined among children aged 6–59 months. Both clinical and parasitological classifications were used (WHO, 1973, 1996). Details of the conduct of these studies and the detailed results are under preparation for publication. In brief, symptomatic patients were enrolled and followed-up for 14 days. Clinical and parasitological measures were assessed on days 0, 3, 7 and 14. Parasitological response for each isolate was classified as sensitive (S) or resistant (RI, RII, and RIII) according to the WHO definitions (WHO, 1973). Treatment outcome for each patient was classified as adequate clinical response (ACR), early treatment failure (ETF), and late treatment failure (LTF) according to the WHO definitions (WHO, 1996). Approval for ethical considerations was obtained from the Uganda National Council for Science and Technology.

Collection of blood samples

Fingerprick blood samples were collected from children aged 6–59 months, before and 14 days after CQ treatment at Aduku Health Centre (Apac District) and Nagongera Health Centre (Tororo District). Pre- and post-treatment venous blood samples were obtained from out-patient adults attending Mulago Hospital for uncomplicated malaria. These adults were enrolled after informed consent, treated with CQ, and followed-up for 14 days. For Apac and Tororo *P. falciparum* isolates, blood was used to soak 4 strips of 3M Whatman filter paper for subsequent DNA isolation. The filter papers were allowed to dry, put in individual envelopes, and transported back to Kampala where they were stored at room temperature. For Mulago isolates, red blood cell pellets were obtained, transported on ice to the laboratory, and stored at −80°C until used for DNA extraction.

Mutation-specific PCR

Parasite DNA was extracted from dried blood spots on filter paper using Chelex-100 and from red blood cell pellets using published procedures (FOLEY *et al.*, 1992; KAIN *et al.*, 1992). Parasite DNA was used as template in a polymerase chain reaction (PCR) to amplify the relevant portions of the *PfCRT* gene using nested PCR as described (DJIMDE *et al.*, 1999). During the first PCR run, flanking primers (TCRP1: 5′-CCGTTAATAATAAATACACGCAG-3′ and TC RP2: 5′-CGGATGTTACAAAACTATAGTTACC-3′) amplifying a 537-bp region encompassing the Lys-76-Thr mutation were employed in a reaction mixture containing 2·5 units *Taq* polymerase, 2·5 mM $MgCl_2$, 200 μM dNTPs and 1 μM concentration of TCRP1 and TCRP2. The cycling parameters consisted of an initial denaturation step at 94°C for 3 min and 45 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 30 s, and extension at 60°C for 1 min; in the last cycle, there was a final extension step at 60°C for 3 min. In addition to the DNA from field samples, DNA from the CQ-sensitive 3D7 strain and the CQ-resistant FCB-1 strain was used as positive controls; water was used as a negative control. The success of the PCR was assessed by running 10% of PCR products on an agarose gel, staining with ethidium bromide, and examining the gel under ultraviolet (UV) transillumination. The 537-bp outer PCR product was observed for the majority of the field isolates. For the nested PCR, 1–3 μL of the outer PCR product was amplified using a common inner primer (TCRP3: 5′-TGACGAGCG TTATAGAG-3′) and either a mutant-specific primer (TCRP4m: 5′-GTTCTTTTAGCAAAAATTG-3′) for the mutant codon or a wild type-specific primer (TCRPw: 5′-GTTCTTTTAGCAAAAATCT-3′) for the wild-type codon. The cycling conditions were as above except that the annealing and extension temperatures were 47 and 64°C, respectively. The positive control for the mutant and wild-type codon PCR was FCB-1 DNA and 3D7 DNA, respectively; again water served as a negative control. In successful mutant and wild-type nested PCR amplicons a band of 366 bp was observed after electrophoresis on 2% agarose gels, ethidium bromide staining, and UV transillumination.

Results

Predominance of the mutant Thr-76 allele in Uganda pre-treatment isolates

In the majority of isolates typed at codon 76 of *PfCRT*, the outer PCR product of 537-bp was observed (data not shown). When this PCR product was used as template for the second nested mutation- or wild type-specific PCR an amplicon of 366 bp was observed. Of 32 isolates from Mulago Hospital in Kampala, none was wild type and all were mutant (100%); there were no mixed infections. Of 45 isolates from Nagongera Health Centre in Tororo, 5 (11·1%) were both wild type and mutants and 40 (89%) were mutants only. Of 41 isolates from Aduku Health Centre, 13 (31·7%) were both wild type and mutant whereas 28 (68·3%) were mutants only. A representative illustration of the paucity of wild-type Lys-76 allele and the predominance of the mutant Thr-76 allele in isolates from Tororo is shown in the Figure.

Correlation between PCR results and CQ treatment outcome

Since the baseline prevalence of the mutant Thr-76 was 100%, we decided that genotyping studies on post-treatment isolates would be superfluous. We were more interested therefore in correlating the frequency of the

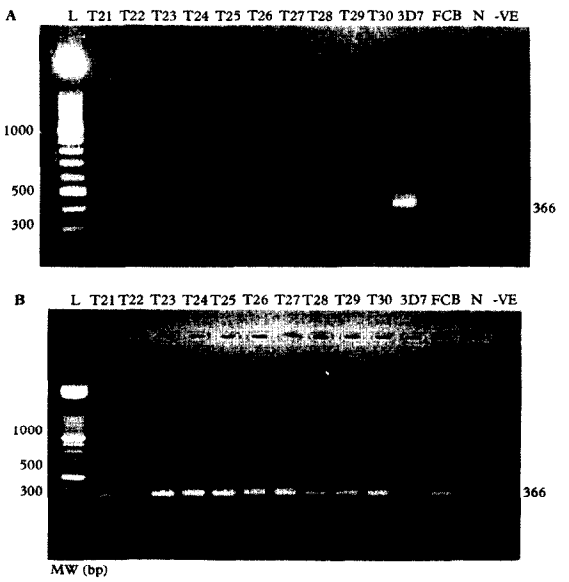

Figure. Mutation-specific nested PCR amplification of a 366-bp amplicon from DNA extracted from Tororo *P. falciparum* field isolates T21–T30 and the parasite lines 3D7 and FCB-1. (**A**) Results for the wild-type allele containing Lys-76. Note that the only positive signal is from the positive control DNA from 3D7 which is chloroquine-sensitive; the amplicon is not observed for DNA from all patient isolates and neither is it observed for FCB-1 which is chloroquine-resistant and for the water negative controls designated N and −VE. (**B**) Results for the mutant allele containing Thr-76. The positive signal is observed from all patient isolates and the positive control DNA from FCB-1; no amplicon is observed for DNA from 3D7 and the negative controls.

baseline wild-type Lys-76 allele and the overall parasitological and in-vivo chloroquine treatment outcome at the 3 study sites. The results for Apac, Tororo, and Kampala isolates for which we had both PCR results and treatment outcome data are striking in this regard (Table 1). There was a direct correlation between the prevalence of the putatively sensitive Lys-76 and the parasitologically sensitive (S) phenotype and ACR. Conversely, there was an inverse relationship between the prevalence of the wild-type allele and the frequency of RII/RIII or ETF/LTF in all districts.

When the genotype of each isolate was compared with the parasitological and in-vivo treatment outcome for individual patients, there was no correlation between the PCR results and the in-vivo treatment outcome for 23 out of 73 patients. PCR results and treatment outcomes for individual patient isolates from Apac District are shown in Table 2.

Discussion

There have been several published studies of CQ resistance in Uganda over the past 6 years. In Kabarole District in western Uganda, CQ sensitivity in symptomatic children was 42% (KAMUGISHA *et al.*, 1994). A study of schoolchildren in Hoima District in western Uganda found that CQ sensitivity in rural and urban areas was 76% and 42%, respectively (NDYOMUGYENI & MAGNUSSEN, 1997). CQ resistance at RII and RIII levels was 18% and 16%, respectively, in rural areas and 46% and 13%, respectively, in urban areas. In another study in which several sites were investigated, CQ sensitivity on days 7 and 14 after treatment was 74% (61–97%) and 45% (37–53%), respectively (NEVILL *et al.*, 1995). In Kampala, one study documented that 27% of 268 patients attending a clinic in 1994–97 had CQ-resistant *P. falciparum* strains (MUTANDA, 1999). However, there has been a paucity of molecular studies of CQ resistance in Uganda. One study documented a correlation between the MAD20 allele family of MSP1 in pre-treatment isolates and a decrease in antimalarial drug resistance (JELINEK *et al.*, 1999), and another study investigated the association between *Pfmdr1* mutations and CQ resistance (FLÜECK *et al.*, 2000). In the latter study, there was no clear association between CQ resistance and any one single mutation but 90% of the clinically resistant samples did present a mutation. These studies highlight the increasing problem of CQ resistance in Uganda over the past 6 years. Our study in a subset of individuals from Apac, Tororo and Kampala Districts in the northern, eastern, and central parts of Uganda, respectively, has confirmed the high level of CQ resistance in Uganda. In addition to demonstrating differences in CQ efficacy at the different study sites, the study also represents one of the first investigations of the *PfCRT* Lys-76-Thr mutation in Uganda.

We investigated the usefulness of mutation-specific PCR which identifies *P. falciparum* isolates containing the mutant allele of the *PfCRT* codon 76 for predicting CQ treatment outcome. The most striking finding was the 100% prevalence of the mutant allele in 118 baseline pre-treatment isolates in 3 different regions in Uganda where CQ has been used. The mutant allele of the *PfCRT* codon 76 might therefore be a marker of CQ use. There was no clear-cut correlation between having a mutant isolate in pre-treatment blood samples and treatment outcome for individual subjects. PCR genotyping of *P. falciparum* cannot therefore be used for individual patient management. However, our data clearly suggest that monitoring the frequency of the wild-type Lys-76 allele might be a useful predictor of the CQ sensitivity of the parasites and in-vivo treatment outcome for the whole community in the 3 districts.

The finding of the mutant allele in all isolates, and the fact that the prevalence of the wild-type allele varies in regions with different levels of CQ resistance in Uganda, merits a re-assessment of the use of codon 76 mutation-specific PCR for the detection of CQ resistance in some regions. It is possible that the Thr-76 allele in Uganda was selected very early during the evolution of CQ resistance and its frequency rapidly increased with increasing CQ use over the years, as is probably the case in other East African countries. As the level of phenotypic CQ resistance increased, the frequency of the mutant allele plateaued as seen in Kampala isolates. The predictive value of the Thr-76 allele in regions like Kampala is therefore compromised. We speculate that Thr-76 allele might be a useful epidemiological marker for CQ resistance in regions where CQ use is low and the mutant allele frequency has not yet plateaued. We propose to confirm this hypothesis by determining the frequency of Thr-76 and Lys-76 alleles in isolates from regions with various levels of CQ resistance in Uganda.

In conclusion, this preliminary investigation of the prevalence of *PfCRT* codon 76 in Ugandan isolates has provided the following insights. First, there is no straightforward relationship between the mutant *PfCRT* codon 76 (Thr-76) in individual isolates and the outcome of CQ treatment because there are probably other host factors such as immunity which must be taken into consideration. Furthermore, in addition to *PfCRT*, there might be other genes such as *Pgh1* which modulate CQ resistance (REED *et al.*, 2000). Secondly, at the community level, an assessment of the frequency of the wild-type allele (Lys-76) might be a better predictor of CQ efficacy in regions where, as a result of many years of CQ pressure, the frequency of Thr-76 has already plateaued.

Table 1. The frequency of baseline *PfCRT* genotypes and chloroquine treatment outcome in Apac, Tororo and Kampala districts, Uganda (1999)[a]

	Genotypes[b]				Treatment outcome[c]		
District	K-76	T-76	K-76/T-76	Total T-76	S	ACR	TTF
Apac (41)	0	68·3	31·7	100·0	48·6	83·8	16·2
Tororo (45)	0	88·9	11·1	100·0	14·3	45·7	54·3
Kampala (32)	0	100·0	0·0	100·0	0·0	36·8	63·2

[a]Parasitological and treatment outcomes were determined according to WHO definitions. The figures in parenthesis refer to the number of isolates per district on which PCR genotyping was successfully carried out.
[b]Mutation-specific PCR was carried out on pre-treatment parasite isolates. The single letter code is used to indicate the wild-type (K-76), mutant (T-76), and mixed (K-76/T-76) alleles of *PfCRT* codon 76.
[c]Overall treatment outcome by district. These values were determined from a larger sample set ($n = 60$ per site). S, sensitive; ACR, adequate clinical response; TTF, overall total treatment failure (ETF + LTF); the treatment outcomes for Kampala (Mulago) are for children aged <5 years.

Table 2. The results of *PfCRT* codon 76 mutation-specific PCR and the parasitological and the outcome *in vivo* of chloroquine treatment in individual children in Apac, Uganda (1999)[a]

	Mutation-specific PCR[b]		Treatment outcome[c]	
Patient no.	K-76	T-76	Parasitological	*In vivo*
A001	+	+	RI	ACR
A002	+	+	S	ACR
A003	−	+	RII	LTF
A004	+	+	RII	LTF
A006	−	+	S	ACR
A007	−	+	RIII	ETF
A008	+	+	S	ACR
A009	−	+	S	ACR
A011	+	+	S	ACR
A012	+	+	S	ACR
A013	+	+	S	ACR
A014	+	+	S	ACR
A015	−	+	S	ACR
A016	+	+	S	ACR
A017	−	+	NA	ETF
A018	+	+	RII	ACR
A020	−	+	RII	ACR
A022	−	+	RII	ACR
A023	−	+	RI	LTF
A024	−	+	RIII	ETF
A025	−	+	RI	ACR
A027	−	+	RII	ACR
A028	−	+	RII	ACR
A029	−	+	S	ACR
A030	−	+	RI	ACR
A031	−	+	RII	ACR
A032	+	+	RI	ACR
A033	−	+	RII	ACR
A034	+	+	RI	ACR
A035	−	+	RIII	ETF
A036	−	+	RII	ACR
A037	+	+	RI	LTF
A038	−	+	RII	ACR
A039	−	+	RI	ACR
A040	−	+	S	ACR
A041	−	+	RI	ACR
A042	−	+	RI	ACR

[a]Parasitological treatment outcomes [resistant (RI, RII, RIII), and sensitive (S)] and in-vivo treatment outcome for individual subjects [adequate clinical response (ACR), early treatment failure (ETF), and late treatment failure (LTF)] were according to the WHO definitions. NA, results not available.
[b]Mutation-specific PCR was carried out on pre-treatment parasite isolates. The single letter code is used to indicate the wild-type (K-76) and mutant (T-76) alleles of *PfCRT* codon 76.
[c]Parasitological and treatment outcomes were determined according to WHO definitions.

Acknowledgements

The molecular analyses in this study received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), WHO, Geneva (TDR Project 970722) and the International Atomic Energy Agency, Vienna, Austria, through a Technical Co-operation agreement with the Government of Uganda (RAF UGA 6012). The *P. falciparum* isolates from Tororo and Apac were collected as part of a larger field study conducted in collaboration with the Ministry of Health within the framework of the East African Network for Monitoring Antimalarial Treatment (EANMAT) which is supported by DfID, UK. In this regard we are grateful to Dr Peter Langi, Malaria Control Programme, Ministry of Health, for providing access to the field samples.

References

Adagu, I. S. & Warhurst, D. C. (1999). Association of *cg2* and *Pfmdr1* genotype with chloroquine resistance in field samples of *Plasmodium falciparum* from Nigeria. *Parasitology*, **119**, 343–348.

Awad-El-Kariem, F. M., Miles, M. A. & Warhurst, D. C. (1992). Chloroquine-resistant *Plasmodium falciparum* isolates from the Sudan lack two mutations in the *pfmdr1* gene thought to be associated with chloroquine resistance. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, **86**, 587–589.

Basco, L. K. & Ringwald, P. (1999). Molecular epidemiology of malaria in Yaounde, Cameroon V. Analysis of the omega repetitive region of the *Plasmodium falciparum* cg2 gene and chloroquine resistance. *American Journal of Tropical Medicine and Hygiene*, **61**, 807–813.

Djimde, A., Doumbo, O., Kayentao, K., Doumbo, S., Diourte, Y., Coulibaly, D., Cortese, J., Su, X., Fidock, D., Nomura, T., Wellems, T. & Plowe, C. (1999). In vivo chloroquine responses and PfCRT polymorphisms in Bandiagara, Mali [Abstract]. *American Journal of Tropical Medicine and Hygiene*, **61**, supplement, 335–336.

Durand, R., Gabbett, E., Di Piazza, J. P., Delabre, J. F. & Le Bras, J. (1999). Analysis of kappa and omega repeats of the *cg2* gene and chloroquine susceptibility in isolates of *Plasmodium falciparum* from sub-Saharan Africa. *Molecular and Biochemical Parasitology*, **101**, 185–197.

Fidock, D. A., Nomura, T., Cooper, R. A., Su, X.-Z., Talley, A. K. & Wellems, T. E. (2000a). Allelic modifications of the *cg2* and *cg1* genes do not alter the chloroquine response of drug resistant *Plasmodium falciparum*. *Molecular and Biochemical Parasitology*, **110**, 1–10.

Fidock, D. A., Nomura, T., Talley, A. K., Cooper, R. A., Dzekunov, S. M., Ferdig, M. T., Ursos, L. M. B., Sidhu, A. S., Naude, B., Deitsch, K. W., Su, X.-Z., Wootton, J. C., Roepe, P. D. & Wellems, T. E. (2000b). Mutations in the *P. falciparum* digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Molecular Cell*, **6**, 861–871.

Flüeck, T. P. F., Jelinek, T., Kilian, A. H. D., Adagu, I. S., Kabagambe, G., Sonnenburg, F. & Warhurst, D. C. (2000). Correlation of *in vivo*-resistance to chloroquine and allelic polymorphisms in *Plasmodium falciparum* isolates from Uganda. *Tropical Medicine and International Health*, **5**, 174–178.

Foley, M., Ranford-Cartwright, L. C., & Babiker, H. A. (1992). Rapid and simple method for isolating malaria DNA from fingerprick samples of blood. *Molecular and Biochemical Parasitology*, **53**, 241–244.

Foote, S. J., Kyle, D. E., Martin, R. K., Oduola, A. M. J., Forthsyth, K., Kemp, D. J. & Cowman, A. F. (1990). Several alleles of the multidrug resistance gene are closely linked to chloroquine resistance in *Plasmodium falciparum*. *Nature*, **345**, 255–258.

Jelinek, T., Kilian, A. H. D., Westermeier, A., Pröll, S., Kabagambe, G., Nothdurft, H. D., von Sonnenburg, F. & Löscher, T. (1999). Population structure of recrudescent *Plasmodium falciparum* isolates from western Uganda. *Tropical Medicine and International Health*, **4**, 476–480.

Kain, K. C., Wirtz, R. A., Fernandez, I., Franke, E. D., Rodriguez, M. H. & Lanar, D. E. (1992). Serologic and genetic characterisation of *Plasmodium vivax* from whole blood-impregnated filter paper discs. *American Journal of Tropical Medicine and Hygiene*, **46**, 473–479.

Kamugisha, J., Kipp, W. & Burnham, G. (1994). *In vivo* sensitivity of *Plasmodium falciparum* to chloroquine, amodiaquine and sulfadoxine–pyrimethamine in western Uganda. *Tropical and Geographical Medicine*, **46**, 364–365.

Krogstad, D. J., Gluzman, I. Y., Kyle, D. E., Oduola, A. M., Martin, S. K., Milhous, W. K. & Sclesinger, P. H. (1987). Efflux of chloroquine from *Plasmodium falciparum*: mechanism of chloroquine resistance. *Science*, **238**, 1283–1285.

McCutcheon, K. R. G., Freese, J. A., Frean, J. A., Veale, R. B., Sharp, B. L. & Markus, M. B. (2000). Chloroquine-resistant isolates of *Plasmodium falciparum* with alternative *cg2* ω repeat length polymorphisms. *American Journal of Tropical Medicine and Hygiene*, **62**, 190–192.

Mutanda, L. N. (1999). Assessment of drug resistance to the malaria parasite in residents of Kampala, Uganda. *East African Medical Journal*, **76**, 421–424.

Ndyomugyeni, R. & Magnussen, P. (1997). *In vivo* sensitivity of *Plasmodium falciparum* to chloroquine and sulfadoxine–pyrimethamine in school children in Hoima district, western Uganda. *Acta Tropica*, **66**, 137–143.

Nevill, C. G., Ochen, K., Munafu, C. G., Bekobita, D. & Sezi, C. L. (1995). Response of *Plasmodium falciparum* to chloroquine and Fansidar *in vivo* and chloroquine and amodiaquine *in vitro* in Uganda. *East African Medical Journal*, 72, 349–354.

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

Su, X., Kirkman, L. A., Fujioka, H. & Wellems, T. (1997). Complex polymorphisms in an ~330 kDa protein are linked to chloroquine-resistant *P. falciparum* in southeast Asia and Africa. *Cell*, **91**, 593–603.

WHO (1973). *Chemotherapy of Malaria and Resistance to Antimalarials*. Geneva: World Health Organization, Technical Report Series, no. 850.

WHO (1996). *Assessment of therapeutic efficacy of antimalarial drugs for uncomplicated falciparum malaria in areas with intense transmission*. Geneva: World Health Organization. WHO/MAL/96.1077.

Wilson, C. M., Volkman, S. K., Thaithong, S., Martin, R. K., Kyle, D. E., Milhous, W. K. & Wirth, D. F. (1993). Amplification of *Pfmdr 1* associated with mefloquine and halofantrine resistance in *Plasmodium falciparum* from Thailand. *Molecular and Biochemical Parasitology*, **57**, 151–160.

Received 26 January 2001; accepted for publication 29 June 2001

TRANSACTIONS OF THE ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (2002) **96**, 95

Book Review

Mosquito: the Story of Man's Deadliest Foe. A. Spielman & M. D'Antonio. London: Faber and Faber, 2001. xix+247pp. Price £10.99. ISBN 0-571-20980-7.

What a potentially promising topic for a best-seller! The authors (the senior a 'scientist' and the latter a journalist) have written for a 'broad audience' presumably based in the USA. They have divided their text into 3 parts (each containing 3 chapters): (1) 'Magnificent enemy', (2) 'The mosquito and disease', and (3) 'The balance'. The book, however, opens with a preface—a short lay account emphasizing the importance of the mosquito in a world context.

The first part contains a great deal of entomology (for the beginner), written I hasten to add in a journalistic style. The second section is mainly historical (and is undoubtedly the most valuable section of the book). There is great emphasis on yellow fever and the 'encephalitis viruses'. But several of the examples of infectious diseases given are simply *not* mosquito-borne!

The authors again make the oft-quoted mistake (page 86) that Manson and Ross (who were supposedly discussing the mode of transmission of *Plasmodium* spp.) strolled from '... Queen Anne's Street down Oxford Street to Seamen's Hospital'. The Albert Dock (Seamen's) Hospital was, however, situated in east London—some 10 miles distant!

The first part focuses on the modern management of mosquito-borne diseases; our present-day lack of success in taming this 'hardy, clever and relentless' foe of mankind is highlighted. Reference is made (page 161) to a conference held at the Royal Society of Tropical Medicine and Hygiene, held at Manson House—'a brick row building ... named for [sic] Sir Patrick Manson'! The relative failure of DDT strategies is well related. But this section too is dominated by the 'West Nile virus'.

This small volume is completed with 4 Figures. The first attempts to illustrate the major differences between *Anopheles* spp., *Aedes aegypti* and *Culex pipiens* (from egg to adult), and this is followed by 3 world maps showing the distribution of *Plasmodium* spp.-associated disease, dengue, and West Nile fever, respectively. The index (12·5 pages) is satisfactory.

Does the book then come up to one's initial expectation? I must admit, I found it extremely disappointing and I venture to suggest that the majority of *Transactions* readers would feel likewise. Although it might be a good read for many lay individuals (although far less successful I suspect than a similar-sized volume: Dava Sobel's *Longitude*) it will be of little or no value to the practitioner of either tropical medicine or 'medicine in the tropics'.

G. C. Cook
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