Apparent tolerance of *Plasmodium falciparum* in infants in a highly endemic area

P. VOUNATSOU[1], T. SMITH[1]*, A. Y. KITUA[2]†, P. L. ALONSO[3] *and* M. TANNER[1]

[1] *Swiss Tropical Institute, Socinstrasse 57, PO Box 4002, Basel, Switzerland*
[2] *Ifakara Center, PO Box 53, Ifakara, Tanzania*
[3] *Hospital Clinic i Provincial, Villaroel 170, E-08036, Barcelona, Spain*

(*Received 16 March 1999; revised 12 July 1999; accepted 13 July 1999*)

SUMMARY

The incidence of fever among infants in the village of Idete in the Morogoro region of Tanzania was analysed in relation to densities of *Plasmodium falciparum* parasites in the peripheral blood. Parasite densities in both fever cases and in asymptomatic infants, were compared and a Bayesian non-parametric mixture decomposition algorithm was used to estimate the proportion of fevers attributable to malaria and hence the incidence of clinical malaria. Age group-specific densities of peripheral parasitaemia showed little seasonality, but the clinical malaria incidence showed a clear peak in the wet season in children aged less than 9 months. Estimates of the parasitaemia-specific incidence of clinical malaria were used to quantify apparent tolerance of parasites, and indicated that clinical episodes occurred on average at lower parasite densities during the wet season than in the dry season. These patterns could reflect differences in levels of anti-toxic immunity, but the nature of the seasonal differences supports the alternative explanation that the variation in apparent tolerance may be an effect of changes in the ratio of peripheral parasite densities to the sequestered mass.

Key words: malaria, Tanzania, attributable fraction, Markov Chain Monte Carlo, mixture resolution.

INTRODUCTION

Inhabitants of areas endemic for *Plasmodium falciparum* may be tolerant of the parasite, in the sense that they can be infected without symptoms of acute disease. It is likely that this tolerance is a result of acquired immunological protection against malaria toxins (Jakobsen *et al*. 1995). It can be quantified epidemiologically by estimating the peripheral parasite density at which symptoms begin (the pyrogenic threshold, see for example Rogier, Commenges & Trape, 1996) or the probability of acute illness as a function of parasite density (Smith *et al*. 1994*a*).

The importance of variation in tolerance among the various factors accounting for age differences in morbidity rates in the youngest children is unclear. Several epidemiological studies in highly endemic areas have indicated that infants can suffer clinical malaria attacks at lower parasite densities than do older children (Rooth & Bjorkman, 1992; Smith *et al*. 1994*b*; McGuinness *et al*. 1998), but although infants are protected against malaria morbidity for the first few months of life (Garnham, 1949; Bruce-Chwatt, 1952), this does not imply that new-born children have a high degree of tolerance. Protection at this stage involves both control of parasite densities and high rates of clearance of infection (Kitua *et al*. 1996; Smith *et al*. 1998*a*). In an area of moderate endemicity in Ghana, epidemiological analysis did not indicate any transiently high tolerance in new-born children (McGuinness *et al*. 1998).

In areas of the highest endemicity, both incidence and severity of clinical attacks is highest during the middle and latter part of the first year of life (Macdonald, 1950; Colbourne & Edington 1954; Baird *et al*. 1993). The subsequent decrease in morbidity rates could result from control of parasite densities, but alternatively it could be the result of acquisition of tolerance. It has long been suggested that in endemic areas immune protection against acute attacks develops before the host is able to control parasite densities (McGregor *et al*. 1956).

In our community studies in the village of Idete, in the Kilombero District of southern Tanzania, the highest incidence of malaria morbidity and highest parasite densities were in infants of 3–5 months of age (Kitua *et al*. 1996). In older children the incidence of clinical malaria gradually decreased with age (Kitua *et al*. 1996) at a rate related to cumulative exposure to asexual-stage parasites (Smith *et al*. 1998*b*).

We have now analysed how the risk of fever in the infants in Idete varies by age, season, and parasite density. We first modelled the risk of fever as a function of parasitaemia with a non-parametric

* Corresponding author: Department of Public Health and Epidemiology, Swiss Tropical Institute, Socinstrasse 57, CH-4002 Basel, Switzerland. Tel: +41 61 284 84 27. Fax: +41 61 271 79 51. E-mail: smith@ubaclu.unibas.ch
† Present address: National Institute of Medical Research, PO Box 9653, Dar es Salaam, Tanzania.

 © 2000 Cambridge University Press
https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

curve using a Markov chain Monte Carlo algorithm (Vounatsou *et al*. 1998). We then used this model to estimate malaria attributable fractions among the fever cases, and thus the incidence of malaria attributable fever as a function of parasite density for each age group and season. This enabled us to examine the rate at which tolerance appears to develop and to determine whether the protection in older infants resulted from control of parasite densities, or from acquisition of tolerance. It also allowed us to evaluate case definitions for clinical malaria among these children.

MATERIALS AND METHODS

Study area and population

The present work forms part of a study of the incidence of malaria infection, clinical malaria and natural immunity to malaria in infants carried out in the village of Idete, Kilombero district, Morogoro region, south-eastern Tanzania (Kitua *et al*. 1996, 1997; Charlwood *et al*. 1998; Smith *et al*. 1998*b*). Most of the population of this area depend on subsistence farming of rice and fishing and live in houses built from bamboo, earth and grass thatch (Tanner *et al*. 1991). There is a high prevalence of *P. falciparum* and high parasite densities all year round, although the intensity of malaria transmission is seasonal (Charlwood *et al*. 1998).

Passive case detection

Passive case detection at the village dispensary was carried out for the infants as well as for older children from Idete participating in the malaria vaccine trial of Alonso *et al*. (1994). At each visit to the dispensary, a morbidity questionnaire was completed, the axillary temperature was measured and thick and thin blood films were taken if the infant was found to be febrile ($\geqslant$ 37·5 °C). Films were also prepared if the parent or guardian reported that the child had suffered fever during the previous 24 h. Such illness episodes defined the cases for case-control analysis.

Blood films were stained using a standard Giemsa method described by Alonso *et al*. 1994. The malaria parasite species were identified and the parasites counted against 200 leucocytes. Parasite counts were converted to number of parasites/μl by assuming a standard of 8000 leucocytes/μl (Shute, 1988). Unpublished data from other studies in the area indicate that variation by age and season in leucocyte counts in infants is much too small to affect the conclusions from the present study.

Cross-sectional surveys

Field surveys of study participants were carried out every 2 weeks between July 1993 and October 1994. The infants included in these surveys were an age-stratified random sample of those under 1 year of age at the time of the surveys, whose parents gave consent for participation. In total 12 age cohorts (corresponding to ages 0–11 months) were defined initially, and 5 randomly sampled infants were enrolled from each cohort. Each month all children reaching 12 months of age and 1 additional child from each of the other cohorts were dropped. The former were replaced by 5 randomly selected newborn children and the latter by new children of the same age as the ones dropped out. Each infant was thus included for a maximum of 4 months. Further details of the study design have been given by Kitua *et al*. (1996).

At each household visit a morbidity questionnaire was completed, the axillary temperature was measured with an electronic thermometer (MBO, Munich) and thick and thin blood films were taken by finger or heel prick. These films were stained and read in the same way as those from the cases. Blood films from infants without fever (axillary temperature $<$ 37·5 °C) constituted the controls for the case-control analysis of fever risk in relation to parasite density.

Model for dependence of fever risk on parasite density

Case-control analysis of the relationship between the risk of fever and parasite density was carried out separately in each age group and for each season using the method of Vounatsou *et al*. (1998). Cases corresponded to fever episodes detected by passive case detection and control slides were those collected during the cross-sectional surveys.

The distribution of parasite densities in the cases was resolved into 2 components. One component corresponds to non-malaria fever episodes and the other to episodes of clinical malaria. Parasite densities from control samples were assumed to be a sample from the non-malaria fever component of the mixture. The parasite densities, X, of controls and cases were then divided into K ordered categories, $i = 1, 2, \ldots, K$. A simulation-based Bayesian approach was then used to estimate the mixing proportion, λ_i, among the cases in each category i, and hence the overall proportion, λ, of febrile children whose fever is attributable to malaria. Details of how this method was applied are given in the Appendix. Statistical hypotheses were tested by examining whether there were overlaps between interval estimates for different age and season groups derived from these models.

Operating characteristics of case definitions

In epidemiological studies clinical malaria is frequently defined to correspond to all febrile episodes

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

Table 1. Prevalences of *Plasmodium falciparum* positivity, fever (axillary temperature ⩾ 37·5 °C or reported fever the last 24 h) and presumptive malaria (fever and parasitaemia) in community surveys and dispensary attendances, by age group and season: July 93–October 94

		Cross-sectional surveys				Dispensary attendances			
Age in months	Season	No. of samples	*P. falciparum* (%)	Fever (%)*	Presumptive malaria (%)†	No. of samples	*P. falciparum* (%)	Fever (%)*	Presumptive malaria (%)†
0–2	Wet	203	31·0	15·3	7·4	70	58·6	81·4	51·4
	Dry	189	19·1	16·4	5·8	64	31·3	73·4	26·6
3–5	Wet	202	59·9	28·7	19·8	225	80·4	92·4	76·9
	Dry	209	51·7	23·9	15·8	126	60·3	88·1	54·8
6–8	Wet	140	65·0	34·3	25·0	191	79·6	91·6	75·9
	Dry	177	66·7	29·9	24·9	185	79·5	95·1	77·8
9–11	Wet	101	70·3	32·7	29·7	157	85·4	87·9	77·1
	Dry	135	73·3	29·6	23·0	214	76·2	88·3	67·3
Total		1356	52·1	25·4	8·8	1232	74·2	89·4	68·9

* Axillary temperature ⩾ 37·5 °C or reported fever the last 24 h.
† Parasitaemia and fever (measured or history of fever).

with parasite densities exceeding a given cut-off value (e.g. Trape *et al*. 1985; Greenwood *et al*. 1987; Snow *et al*. 1988). Following Smith *et al*. (1994*a*) the sensitivities of such case definitions were estimated by $(n_c\lambda_c)/(N\lambda)$ and the specificities by $1-n_c(1-\lambda_c)/(N(1-\lambda))$ where:

$$n_c = \sum_{i=c}^{K} n_i \quad \text{and} \quad \lambda_c = \left(\sum_{i=c}^{K} \lambda_i n_i\right)/n_c,$$

and n_i is the number of fever cases in the age group, season and parasite density category i, and c denotes the parasite density category of which the cut-off is the lower bound.

Incidence rates of clinical malaria

Age and season-specific incidence rates of fever episodes at the dispensary were calculated as the ratio of the number of attendances at the dispensary with fever (measured or reported) for each age and season group (N), $N = \Sigma n_i$, $i = 1, \ldots, K$, to the total person-years at risk of all infants in the village of that age group and season (T). The incidence of clinical malaria was estimated by $N\lambda/T$. Parasite density-specific times-at-risk, t_i were obtained by multiplying T by the proportion of cross-sectional survey attendances in that age group and season which fell within each parasite density class i. The parasite density-specific incidence of malaria attributable fever was then estimated by $\lambda_i n_i/t_i$.

RESULTS

Sample sizes

A total of 542 births were registered in Idete, during the period of July 1992 to September 1994. Of these infants 303 were visited in the cross-sectional surveys between July 1993 and October 1994, of whom 159 (52·5 %) were female. Each child attended on average 4·5 surveys giving rise to a total of 1356 cross-sectional attendances. In addition, 1232 passive case detection attendances were recorded during the above period, corresponding to 305 infants, of whom 238 were seen in the cross-sectional surveys. Out of the 305 infants, 148 (48·5 %) were females.

Age and season distribution of fever

Table 1 shows the age distributions of all the control samples and of those passive case detection attendances, with measured fever or a history of fever in the last 24 h. By design, the youngest children were sampled relatively more frequently than older children (Kitua *et al*. 1996) in the community surveys. On 344 (25·37 %) occasions, fever during the previous 24 h was reported or the axillary temperature was ⩾ 37·5 °C.

The sampling at the dispensary was not age biased and the attendance rate was around 3 times as high in infants older than 3 months than in those below this age. For children older than 3 months there is relatively little age dependence in the rate of attendance at the dispensary with fever (Smith *et al*. 1998*b*).

Figure 1 gives the incidence rates of morbidity (dispensary attendances) by age group and season. Data for the months February to July can be considered to correspond to the wet season and the other months to the dry season. There is considerable seasonality in malaria transmission in Idete (Charlwood *et al*. 1998) but a clear peak in morbidity in the wet season is only seen in children less than 6 months of age. There is a slight excess of morbidity in the wet season in the 6–8 months age group. There is no

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

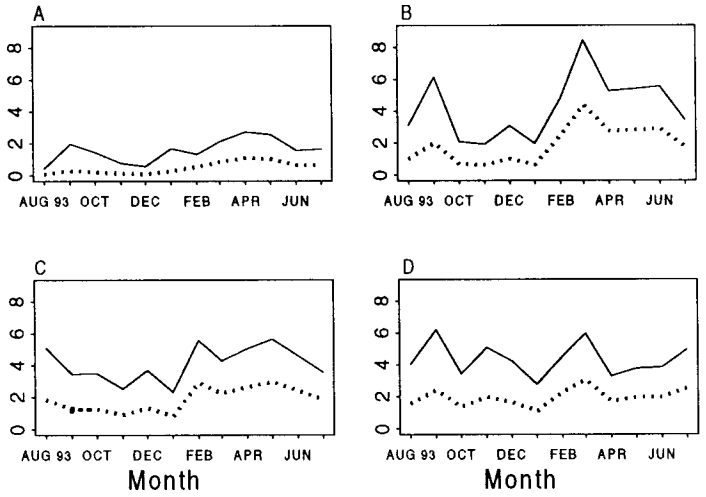

Fig. 1. Incidence rates (per child-year) for dispensary attendances (—) and *Plasmodium falciparum* (·····) attributable episodes by age group and season. (A) Age 0–2 months, (B) Age 3–5 months, (C) Age 6–8 months, (D) Age 9–11 months.

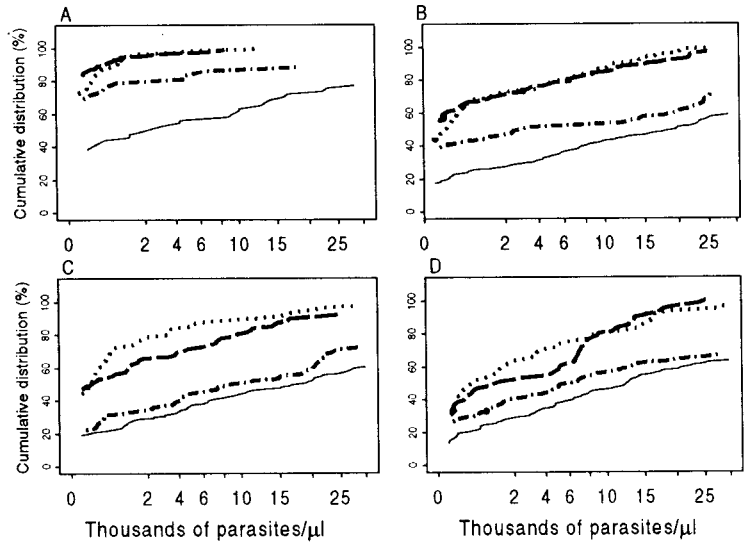

Fig. 2. Cumulative parasite density distributions by age group. Parasite densities are plotted on a square-root scale. (A) Age 0–2 months, (B) Age 3–5 months, (C) Age 6–8 months, (D) Age 9–11 months. Wet season: cases (—); controls (·····). Dry season: cases (–·–); controls (– –).

indication of an increase in morbidity rate associated with the wet season peak in malaria transmission, in the oldest age group, in agreement with analyses for older children (Smith *et al*. 1994*a*) and adults (Smith *et al*. 1993) in Kilombero.

Age and season distribution of parasitaemia

Of 1356 control slides 707 (52·1%) were *P. falciparum* positive with the prevalence of parasitaemia increasing gradually with age from 25% for the age group of 0–2 months old, to 72% for the 9–11 months old (see also Kitua *et al*. 1996). Average parasite densities in infected infants were lowest in the 0–2 month old children but showed little age trend in the older age groups (Kitua *et al*. 1996). In all age groups the parasite densities in febrile children were higher than those in controls (i.e. the lines for cases in Fig. 2 were below the lines for the corresponding controls). However, there was little seasonal variation in parasite densities in the controls. In infants less than 6 months old, fever cases at the dispensary, in the dry season had lower prevalence and densities than those in the wet season, but the difference was very small in children older than 6 months. The percentages of both wet and dry season cases with less than 25000 parasites/μl were similar. In all age groups, a substantial proportion of the clinical cases had very high parasite densities.

Of dispensary attendances 74·2% were *P. falciparum* positive, with 89·4% reporting fever during the last 24 h or had an axillary temperature $\geqslant$ 37·5 °C and 68·9% had both fever and parasites.

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

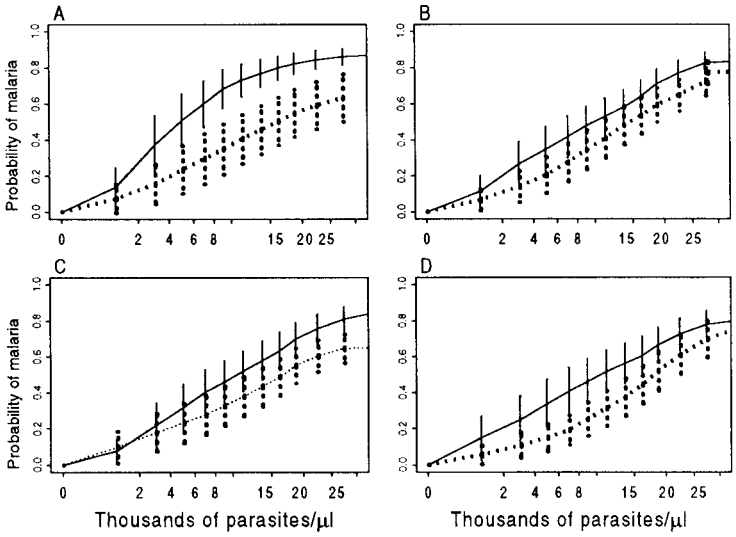

Fig. 3. Estimates of λ_i by parasite density. Parasite densities are plotted on a square-root scale. Error bars indicate ± 1 S.E. (A) Age 0–2 months, (B) Age 3–5 months, (C) Age 6–8 months, (D) Age 9–11 months. Wet season (—), Dry season (·····).

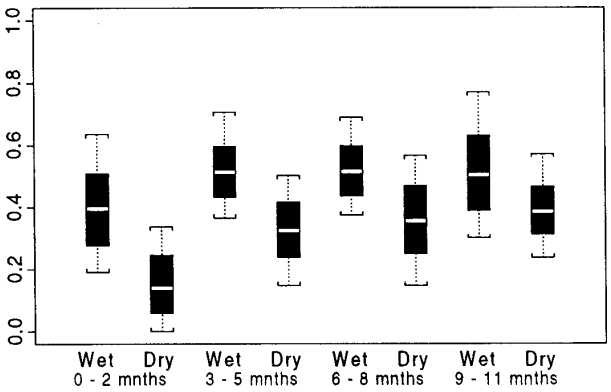

Fig. 4. Boxplots summaries of the samples drawn from the marginal posterior distributions of λ according to season and age group. The top, middle and bottom lines represent the 0·95, 0·50 and 0·05 quantiles of the samples, respectively.

Relationship of fever to parasitaemia

In all age groups studied, the probability that a fever case was malaria attributable at a given density of parasitaemia (λ_i) increased only gradually with density and was higher in the wet season than in the dry season (Fig. 3). These probabilities were remarkably similar across age groups, with the exception of the wet season data for the youngest age group, where λ_i increased more steeply with density than among older children.

Corresponding to these values of λ_i are the overall attributable fraction estimates, λ (Fig. 4), which indicate the proportion of the fever cases which are of malaria aetiology. In all age groups this proportion was substantially higher in the wet season than the dry season. Whilst λ was lower for children less than 3 months old, it showed little variation between the older age groups.

These patterns were reflected in the sensitivities and specificities of possible cut-offs for use in case definitions for clinical malaria (Fig. 5). In all age groups the sensitivity of all cut-offs (c) fell off only gradually as c was increased. In contrast to the sensitivities, specificities were highly age dependent. In the youngest children, a case definition of fever with any parasites was highly specific. The specificity fell as the children grew older, implying that a higher cutoff might be appropriate.

Incidence of malaria attributable fevers

The effects of seasonality on the incidence rates of malaria attributable fever at the dispensary also depended on the age group (Fig. 1). While the group 0–2 months old had the lowest incidence rates, it showed considerable seasonal variation in incidence (Fig. 1A). Infants between 3 and 5 months, who had the highest incidence rates, also showed seasonal variation (Fig. 1B), with the attendance rate with fever varying from 2 to 8 episodes per year and most of this variation due to malaria episodes. There was also some seasonality in morbidity rates of infants older than 6 months, but this seemed to become less as the children grew older (Fig. 1C, D).

When the incidence was estimated separately for each parasite density category there was only a little difference between the age groups in the wet season incidence at any given parasite density (Fig. 6). During the dry season, the incidence at each parasite density was less than in the wet season, but this difference became less as the children grew older. In the youngest children, dry season parasitaemia was unlikely to lead to fevers, even at high parasite densities.

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

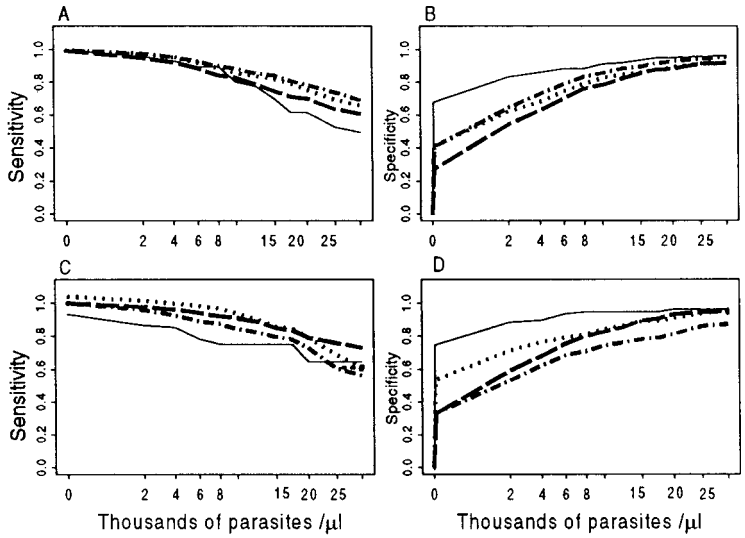

Fig. 5. Estimated sensitivities and specificities of parasitaemia cut-off values by age group and season. Parasite densities are plotted on a square-root scale. Wet season: (A) cases (—); (B) controls (·····). Dry season: (C) cases (–·–); (D) controls (– –).

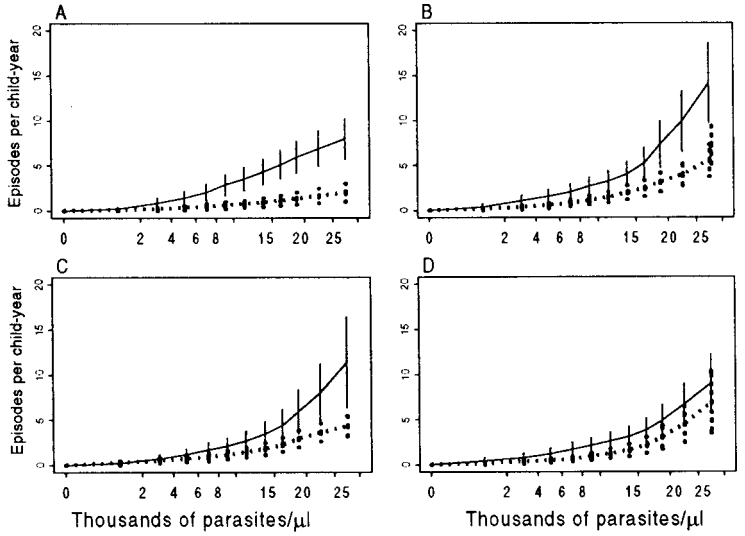

Fig. 6. Incidence of malaria fever (per child year), as reported to the dispensary, by age group, season and parasite density. Parasite densities are plotted on a square-root scale. Error bars indicate ± 1 S.E. (A) Age 0–2 months, (B) Age 3–5 months, (C) Age 6–8 months, (D) Age 9–11 months. Wet season (—), Dry season (·····).

DISCUSSION

In endemic areas, differences in the incidence of malaria fever can arise either because of variation in the frequency of high parasite densities, or because of differences in the degree of tolerance in different subsets of the host population. The present study suggests that both these factors contribute to variation in morbidity rates within the first year of life.

Other studies have shown that once the first year of life is over the threshold parasite density leading to fever declines with age (Miller, 1958; Smith *et al.* 1994*a*; Rogier *et al.* 1996). Children above 1 year of age, who in general have higher parasite densities when asymptomatic than do adolescents or adults, also tolerate higher densities. Adults have relatively low pyrogenic thresholds, but because their parasite densities are generally controlled at very low densities they nevertheless have lower incidence of clinical attacks than do younger individuals. Broadly speaking, therefore, the age distribution of tolerance is consequently similar to that of parasite densities, which is what would be expected if tolerance is the consequence of an immunological response with little memory, stimulated by toxins released during schizogony. Moreover, in an area of much lower transmission than Idete, where average parasite densities remained low throughout the first year of life, pyrogenic thresholds remained low too (McGuinness *et al.* 1998). This also supports the conclusion that tolerance reflects the parasitological load in the immediate past, possibly because the key

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

mediator of tolerance may be short-lived IgM antibody against phospholipid toxins (Jakobsen *et al*. 1993; Bate & Kwiatkowski, 1994).

For the first few months, the age trend in incidence of malaria fevers in Idete also closely tracks the age distribution of peripheral parasite densities in the community (Kitua *et al*. 1996) reaching a maximum associated with the high parasite densities in the middle of the first year of life. At first sight, the gradual decrease in incidence subsequently would seem to be a result of small decreases in the incidence associated with given parasite densities, i.e. acquisition of tolerance, in keeping with the observations in older individuals.

However, when our results are considered by season this straightforward interpretation cannot be sustained. Despite the dramatic age dependency in the parasite load to which the infants were exposed, apparent tolerance in the wet season is largely age independent, and the age trend in wet season morbidity is dominated by effects of variations in parasite densities. Two additional factors give rise to a contrasting pattern in the dry season. Firstly, in the very youngest infants (but less so as the children grow older) there is a lower parasite positivity rate in dry season fever cases than in the wet season ones, and hence a lower malaria attributable fraction. Secondly in the 3- to 5-month-old age group there is a lower incidence rate at given peripheral parasite densities in the dry season (in other words higher apparent tolerance) than in the wet.

The extent of seasonality in malaria fever thus declines with age mainly because the difference between seasons in apparent tolerance diminishes as the children grow older not because of age-dependence in seasonality of parasite densities. Directly contrary to the idea that tolerance reflects recent parasite load, the apparent tolerance in these very young children is higher in the dry season, although exposure to blood stages (and toxins) during the dry season is similar to, or less than, exposure during the wet season.

We propose an alternative hypothesis for the variations in terms of the ratio of circulating: sequestered parasites (CS ratio). Pyrogen release occurs during schizont rupture, hence pyrogen concentrations are likely to reflect the number of sequestered parasites, not the numbers of trophozoites in the peripheral circulation. To quantify tolerance we would therefore ideally measure clinical malaria risks in relation to the sequestered parasite load, instead of the peripheral density.

To understand how the CS ratio might vary, consider the effect of different changes in the acquired immune response to blood stages. If the immune response to merozoites becomes more effective, this lessens the number of trophozoites arising from any given load of sequestered parasites. This in turn will appear to reduce the pyrogenic threshold estimated from peripheral parasitaemia data. On the other hand, if the host response selectively attacks antigens involved in cytoadhesion, this will reduce the proportion of trophozoites which sequester, and hence increase the CS ratio and the apparent pyrogenic threshold.

It follows that the seasonal variation in apparent tolerance in the youngest children could be explicable as a consequence of higher CS ratios during the dry season. Immunity to cytoadherence ligands (e.g. *Plasmodium falciparum* erythrocyte membrane protein-1, PfEMP-1) is probably acquired rather quickly (Bull *et al*. 1998), and during the dry season relatively few new inoculations occur, presumably exposing the infant to only a limited repertoire of PfEMP-1 genotypes. Anti-cytoadherence immunity is therefore likely to be relatively effective then, leading to a high CS ratio and high apparent tolerance. In contrast, during the wet season the child is continually exposed to novel PfEMP-1 genotypes, and hence is less able to develop protective responses against them all, leading to a lower CS ratio. As the child grows older, he or she will nevertheless acquire an adequate repertoire of anti-PfEMP-1 responses, leading to reduced morbidity and a decrease in seasonality in the incidence of malaria fevers. This need not lead to an increased pyrogenic threshold measured against peripheral parasite densities, since at the same time there is likely to be acquisition of immunity to merozoites.

It would be important to test this admittedly speculative explanation for the seasonal variations in apparent tolerance, since it has a direct bearing on the likely effectiveness of blood-stage vaccines during the first year of life. Moreover, age dependence in the CS ratio would imply that the epidemiological studies of apparent tolerance in older individuals (Smith *et al*. 1994*a*; Rogier *et al*. 1996) need to be reinterpreted. There is evidence that effective immunological responses against merozoite antigens are indeed acquired slowly (e.g. Al-Yaman *et al*. 1995, 1996). If the CS ratio gradually decreases with age after the first birthday, this would account for the parallel decrease in apparent tolerance. However, there is at present no practicable approach to assessing the number of sequestered *P. falciparum* parasites in the individual human host and hence to determine the CS ratio directly.

Our approach to optimization of case definitions for clinical malaria is not compromised by the question of whether peripheral parasitaemia accurately reflects total parasite load. However, it is important to remember that the sensitivity estimates from our analysis refer only to the proportion of true malaria cases among the infants with fever at the dispensary. Many malaria cases in the first year of life do not present with fever (Garnham, 1949; Smith *et al*. 1994*b*), and not all fever cases presented to the dispensary. The analyses implied that the

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

appropriate cut-off for use with these dispensary fever cases is somewhat age-dependent. In the youngest children, a specificity of almost 80 % can be obtained with a simple definition of fever plus parasitaemia, whilst in the older age groups a requirement for a parasite density of at least 10000/μl must be imposed to achieve this specificity.

The authors are grateful to Chris Newbold and Louis Molineaux for useful discussions, to the people of Idete village for their participation in the study, and to the field and laboratory workers of the Ifakara Centre. Penelope Vounatsou was supported by Swiss National Science Foundation grant 32-43527.95. Ethical clearance for the field study was provided by the Medical Research Coordinating Committee of the Tanzanian National Institute of Medical Research and research clearance was obtained from the Tanzanian Commission of Science and Technology (UTAFITI) as per ref. NSR/RCA 90. Written informed consent was obtained from every mother or guardian before an infant was enrolled in the study.

APPENDIX

Let n_i be the number of observations from the mixture (cases) that belong to category i, and m_i be the number of observations from the control sample that belong to the category i, $i = 1, 2, \ldots, K$. We assume that the data come from 2 Multinomial distributions, $(m_1, m_2, \ldots, m_K) \sim Mn(\Sigma m_i, \theta_1, \theta_2, \ldots, \theta_K)$ and $(n_1, n_2, \ldots, n_K) \sim Mn(\Sigma n_i, p_1, p_2, \ldots, p_K)$, specified by the parameters, $\theta_i = \Pr(X \in \text{category } i \mid P_1)$, $\phi_i = \Pr(X \in \text{category } i \mid P_2)$, $\lambda = \Pr(X \in P_2)$ and $p_i = (1-\lambda)\theta_i + \lambda\phi_i$, where P_1 and P_2 are the distribution functions of the component distributions of the mixture. The joint density of the data $\mathbf{m} = (m_1, m_2, \ldots, m_K)^T$ and $\mathbf{n} = (n_1, n_2, \ldots, n_K)^T$ is:

$$L(m, n \mid \boldsymbol{\theta}, \boldsymbol{\phi}, \lambda) \propto \prod_{i=1}^{K-1} \theta_i \left(1 - \sum_{i=1}^{K-1} \theta_i^{m_i}\right)^{m_K} \prod_{i=1}^{K-1} ((1-\lambda)\theta_i + \lambda\phi_i)^{n_i} \left((1-\lambda)\left(1 - \sum_{i=1}^{K-1} \theta_i\right) + \lambda\left(1 - \sum_{i=1}^{K-1} \phi_i\right)\right)^{n_K} \quad (1)$$

where

$$0 < \theta_i, \phi_i, \lambda < 1, \quad \sum_{i=1}^{K} \theta_i = 1 \quad \text{and} \quad \sum_{i=1}^{K} \phi_i = 1.$$

We assume that observations from the first component are smaller than the ones from the second one, that is

$$\Pr(X \text{ from } P_2 \mid \text{category } 1) = 0 \quad (2)$$

and

$$\Pr(\text{X from } \mathrm{P}_2 \mid \text{category } \mathrm{i}-1) < \Pr(X \text{ from } P_2 \mid \text{category } i) \quad \text{for } i = 2, \ldots, K \quad (3)$$

We estimate the parameters $\boldsymbol{\theta}$, $\boldsymbol{\phi}$ and λ, by using a Markov Chain Monte Carlo simulation-based Bayesian approach with independent Uniform prior distributions in [0, 1] for the parameters, that is, $p(\boldsymbol{\theta}, \boldsymbol{\phi}, \lambda) \propto 1$, constrained to satisfy (1)–(3). Further details of the estimation have been given by Vounatsou *et al*. (1998).

In contrast to models used previously for the relationship of fever incidence to parasite density (Armstrong Schellenberg *et al*. 1994; Smith *et al*. 1994*a*; Rogier, Commenges & Trape, 1996), the approach of Vounatsou *et al*. (1998) provides interval estimates for the λ_i as well as for λ and makes no parametric assumptions about the shape of the relationship between parasite density and fever risk. Informal comparisons of the extent of overlap in these interval estimates were used in order to assess whether differences were likely to be due to chance.

REFERENCES

ALONSO, P. L., SMITH, T., ARMSTRONG SCHELLENBERG, J. R. M., MASANJA, H., MWANKUSYE, S., URASSA, H., BASTOS DE AZEVEDO, I., CHONGELA, J., KOBERO, S., MENENDEZ, C., HURT, N., THOMAS, T. C., LYIMO, E., WEISS, N. A., HAYES, R., KITUA, A. Y., LOPEZ, M. C., KILAMA, W. L., TEUSCHER, T. & TANNER, M. (1994). Randomised trial of efficacy of the SPf66 vaccine against *Plasmodium falciparum* malaria in children in southern Tanzania. *Lancet* **344**, 1175–1181.

ARMSTRONG SCHELLENBERG, J. R. M., GREENWOOD, B. M., GOMEZ, P., MENENDEZ, C. & ALONSO, P. L. (1994). Diurnal variation in body temperature of Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **88**, 429–431.

AL-YAMAN, F., GENTON, B., ANDERS, R., TARAIKA, J., GINNY, M., MELLOR, S. & ALPERS, M. P. (1995). Assessment of the role of the humoral response to *Plasmodium falciparum* MSP2 compared to RESA and SPf66 in protecting Papua New Guinean children from clinical malaria. *Parasite Immunology* **17**, 493–501.

AL-YAMAN, F., GENTON, B., KRAMER, K. J., CHANG, S. P., HUI, G. S., BAISOR, M. & ALPERS, M. P. (1996). Assessment of the role of naturally acquired antibody levels to *Plasmodium falciparum* merozoite surface protein-1 in protecting Papua New Guinean children from malaria morbidity. *American Journal of Tropical Medicine and Hygiene* **54**, 443–448.

BAIRD, J. K., PURNOMO, H. B., BANGS, M. J. *et al*. (1993). Age-specific prevalence of *Plasmodium falciparum* among six populations with limited histories of exposure to endemic malaria. *American Journal of Tropical Medicine and Hygiene* **49**, 707–719.

BATE, C. A. W. & KWIATKOWSKI, D. (1994). Inhibitory immunoglobulin M antibodies to tumor necrosis factor-inducing toxins in patients with malaria. *Infection and Immunity* **62**, 3086–3091.

BRUCE-CHWATT, L. (1952). Malaria in African infants and children in Southern Nigeria. *Annals of Tropical Medicine and Parasitology* **46**, 173–200.

BULL, P. C., LOWE, B. S., KORTOK, M., MOLYNEUX, C. S., NEWBOLD, C. I. & MARSH, K. (1998). Parasite antigens on the infected red cell surface are targets for naturally acquired immunity to malaria. *Nature Medicine* **4**, 358–360.

CHARLWOOD, J. D., SMITH, T., LYIMO, E., KITUA, A. Y., MASANJA, H., BOOTH, M., ALONSO, P. L. & TANNER, M. (1998). Incidence of *Plasmodium falciparum* infection in infants in relation to exposure to sporozoite infected anophelines. *American Journal of Tropical Medicine and Hygiene* **59**, 243–251.

COLBOURNE, M. J. & EDINGTON, G. M. (1954). Mortality from malaria in Accra. *Journal of Tropical Medicine and Hygiene* **57**, 203–210.

GARNHAM, P. C. C. (1949). Malarial immunity in Africans: effects in infancy and early childhood. *Annals of Tropical Medicine and Parasitology* **43**, 47–61.

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press

GREENWOOD, B. M., BRADLEY, A. K., GREENWOOD, A. M., BYASS, P., JAMMEH, K., MARSH, K., TULLOCH, S., OLDFIELD, F. S. J. & HAYES, R. (1987). Mortality and morbidity from malaria among children in a rural area of The Gambia, West Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **81**, 476–486.

KITUA, A. Y., SMITH, T., ALONSO, P. L., MASANJA, H., URASSA, H., MENENDEZ, C., KIMARIO, J. & TANNER, M. (1996). *Plasmodium falciparum* malaria in the first year of life in an area of intense and perennial transmission. *Tropical Medicine and International Health* **1**, 475–484.

KITUA, A. Y., SMITH, T., ALONSO, P. L., URASSA, H., MASANJA, H., KIMARIO, J. & TANNER, M. (1997). The role of low level *Plasmodium falciparum* parasitaemia in anaemia among infants living in an area of intense and perennial transmission. *Tropical Medicine and International Health* **2**, 325–333.

JAKOBSEN, P. H., MORRIS-JONES, S., HVIID, L., THEANDER, T. G. & HOIER-MADSEN, M. (1993). Anti-phospholipid antibodies in patients with *Plasmodium falciparum* malaria. *Immunology* **79**, 653–657.

JAKOBSEN, P. H., BATE, C. A., TAVERNE, J. & PLAYFAIR, J. H. (1995). Malaria: toxins, cytokines and disease. *Parasite Immunology* **17**, 223–231.

MACDONALD, G. (1950). The analysis of malaria parasite rates in infants. *Tropical Diseases Bulletin* **47**, 915–937.

McGREGOR, I. A., GILLES, H. M., WALTERS, J. H., DAVIES, A. H. & PEARSON, F. A. (1956). Effects of heavy and repeated malarial infections on Gambian infants and children. Effect of erythrocytic parasitization. *British Medical Journal* **ii**, 686–692.

McGUINNESS, D., KORAM, K., BENNETT, S., WAGNER, G., NKRUMAH, F. & RILEY, E. (1998). Clinical case definitions for malaria: clinical malaria associated with very low parasite densities in African infants. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **92**, 527–531.

MILLER, M. J. (1958). Observations on the natural history of malaria in the semi-resistant West African. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **52**, 152–168.

ROGIER, C., COMMENGES, D. & TRAPE, J. F. (1996). Evidence for an age-dependent threshold effect of malaria parasite density on the occurrence of fever suggesting an inverse relationship between anti-parasite and anti-toxic immunity. *American Journal of Tropical Medicine and Hygiene* **54**, 613–619.

ROOTH, I. & BJORKMAN, A. (1992). Fever episodes in a holoendemic malaria area of Tanzania: parasitological and clinical findings and diagnostic aspects related to malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **86**, 479–482.

SHUTE, G. T. (1988). The microscopic diagnosis of malaria. In *Malaria, Principles and Practice of Malariology* (ed. Wernsdorfer, W. H. & McGregor, I.), pp. 781–814. Churchill Livingstone, Edinburgh.

SMITH, T., CHARLWOOD, J. D., KIHONDA, J., MWANKUSYE, S., BILLINGSLEY, P., MEUWISSEN, J., LYIMO, E., TAKKEN, W., TEUSCHER, T. & TANNER, M. (1993). Absence of seasonal variation in malaria parasitaemia in an area of intense seasonal transmission. *Acta Tropica* **54**, 55–72.

SMITH, T., SCHELLENBERG, J. A. & HAYES, R. (1994*a*). Attributable fraction estimates and case definitions for malaria in endemic areas. *Statistics in Medicine* **13**, 2345–2358.

SMITH, T., GENTON, B., BAEA, K., GIBSON, N., TAIME, J., NARARA, A., AL-YAMAN, F., BECK, H. P., HII, J. & ALPERS, M. (1994*b*). Relationships between *Plasmodium falciparum* infection and morbidity in a highly endemic area. *Parasitology* **109**, 539–549.

SMITH, T., FELGER, I., KITUA, A. Y., TANNER, M. & BECK, H. P. (1998*a*). Dynamics of multiple *Plasmodium falciparum* infections in infants in a highly endemic area of Tanzania. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **93**, Suppl. 1, S1/35–S1/39.

SMITH, T., CHARLWOOD, J. D., KITUA, A. Y., MASANJA, H., MWANKUSYE, S., ALONSO, P. L. & TANNER, M. (1998*b*). Relationships of malaria morbidity with exposure to *Plasmodium falciparum* in young children in a highly endemic area. *American Journal of Tropical Medicine and Hygiene* **59**, 252–257.

SNOW, R. W., LINDSAY, S. W., HAYES, R. J. & GREENWOOD, B. M. (1988). Permethrin-treated bed nets (mosquito nets) prevent malaria in Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **82**, 838–842.

TANNER, M., DE SAVIGNY, D., MAYOMBASA, C., HATZ, C., BURNIER, E., TAYARI, S. & DEGRÉMONT, A. (1991). Morbidity and mortality at Kilombero, Tanzania, 1982–88. In *Disease and Mortality in Sub-Saharan Africa* (ed. Feachem, R. G. & Jamison, D. T.), pp. 286–305. Oxford University Press, Oxford.

TRAPE, J. F., PEELMAN, P. & MOZAULT-PEELMAN, B. (1985). Criteria for diagnosing clinical malaria among a semi-immune population exposed to intense and perennial transmission. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79**, 435–442.

VOUNATSOU, P., SMITH, T. & SMITH, A. F. M. (1998). Bayesian analysis of two-component mixture distributions applied to estimating malaria attributable fractions. *Applied Statistics* **47**, 575–587.

https://doi.org/10.1017/S0031182099005211 Published online by Cambridge University Press