RELATIONSHIP BETWEEN THE INTENSITY OF EXPOSURE TO MALARIA PARASITES AND INFECTION IN THE USAMBARA MOUNTAINS, TANZANIA

RENÉ BØDKER,* HAMISI A. MSANGENI, WILLIAM KISINZA, AND STEVE W. LINDSAY

Danish Bilharziasi Laboratory, Charlottenlund, Denmark; National Institute of Medical Research, Amani, Tanzania; School of Biological and Biomedical Sciences, Durham University, Durham, United Kingdom

Abstract. The relationship between exposure to Plasmodium falciparum malaria and parasite density and prevalence was studied in six communities along an altitude transect. Prevalence of parasitemia in children decreased by 5% for every 100 meter increase in altitude from 82% in the lowlands at 300 meters to 12% in the highlands at 1,700 meters. This decrease in prevalence corresponded to a 1,000-fold reduction in transmission intensity. The ability to suppress parasite density and prevalence with age increased proportionally with increasing transmission intensity when transmission rates were higher than 0.1 infective bites per year, but developed after 2–3 years of age, regardless of transmission intensity. However, at transmission rates less than 0.1 infective bites per year, prevalence remained similar in all age groups. We propose that both exposure-dependent acquired immunity and age-dependent acquired immunity regulate parasite prevalence and density and suggest that transmission control will not hinder the development of protective anti-parasite immunity.

INTRODUCTION

To assess the impact of malaria transmission intensity on human health and to predict the potential gains from reducing transmission, it is important to understand the relationship between intensity of transmission and disease. This is not a simple relationship and it has been suggested that reductions in transmission intensity might even have an adverse long-term impact on malaria morbidity and mortality by hindering the acquisition of protective immunity. Reduced levels of antibodies against sporozoites and variant surface antigen, the latter being important for acquired immunity, have been reported after introduction of bed nets. Similar studies have reported both increased and decreased levels of protective antibodies against merozoites antigens in children and pregnant women after bed nets were introduced, and suggested an effect of age. Others have argued that acquired immunity is largely age dependent and thus independent of the level of exposure, so that any reduction in transmission would be expected to be beneficial. Exposure-dependent acquired immunity can be interpreted as an increasing ability to recognize strain-specific Plasmodium falciparum antigens as the immune system is presented to different strains through repeated infections with time. If this is correct, the long-term effect of reducing transmission through vector control would reduce immunity to such an extent that prevalence of infection, or densities of parasites, in older age groups would increase compared with pre-control levels. In this scenario, one would expect an accumulated increase in crude prevalence rates, despite a decrease in young children.

Others have interpreted the age-specific prevalence rates as an effect of age itself rather than accumulated exposure. It is suggested that the mechanism responsible for age-dependent acquired immunity is a maturation of the immune system at older age groups, allowing a strain transcending immunity after a limited number of infections with P. falciparum. Thus, according to the hypothesis of an age-dependent acquired immunity, a reduction in transmission will not result in an increased prevalence in older age groups. Rather, it will postpone a proportion of the infections to older age groups with a more mature immune system.

To predict long-term effects of different types of interventions it is therefore important to understand the nature of acquired immunity to malaria. The greater the contribution of age-dependent acquired immunity, the greater the advantage of reducing transmission because exposure is shifted to older immune-competent individuals. Short-term intervention projects are not expected to show the potential long-term effect of reduced immunity because the immunity acquired before the intervention is expected to last for years under the reduced intensity of transmission. Thus, the immediate reductions in parasitologic parameters observed in short-term intervention trials may not be sustainable. It is therefore important to access the long-term effects of reduced transmission intensity. This can be achieved by prolonging intervention trials for years. Here we used an alternative ecologic approach and compared malaria indices in six communities differing only in malaria transmission intensity. Using this ecologic approach, we quantified the relationship between transmission intensity and infection rates, spleen enlargement, and density of infections in children and adults. It can be difficult to distinguish the effects of age and accumulated exposure because these are linked. However, by examining villages with different transmission intensities, we were able to analyze the separate effects of age and transmission because similar age groups will have different accumulated exposure in the different villages. Functional immunity is likely to consist of several different mechanisms working together to reduce disease and death by controlling and tolerating the different stages of the parasites and their interaction with the human body. However, this study focuses specifically on the ability to control parasitemia. The six study communities were situated along a transmission transect ranging from holoendemic malaria in the lowlands resulting from 91 infective bites per person per year to hypoendemic malaria caused by an estimated 0.03 infective bites per person per year in the highlands of the Usambara Mountains in Tanzania. These communities reflect the range of malaria endemicity experienced across much of the African continent. This field study appears to be the first to simultaneously investigate the relationship between transmission intensity, age, spleen rates, parasite rates, and parasite densities at all age...
groups over the full range of endemicity from holoendemic to hypoendemic transmission levels.

MATERIALS AND METHODS

Study area. Six villages were surveyed along an altitude transect in the West Usambara Mountains in northeastern Tanzania (Table 1), approximately 100 km inland from the port of Tanga. The villages are densely populated, ranging from 500 to more than 1,000 individuals, but the villages are often separated into a few well-defined clusters. Environmental and entomologic parameters and malaria parasite strains for the six villages have been described in detail previously.15–17

Selection of study households. A geographically defined cluster of houses containing up to 1,000 people was selected in each village. All households in each cluster were numbered approximately one year before the survey. Four to six months before blood sampling, all study households were visited by a member of the survey team, and family members interviewed in Swahili about their age and residence status. Householders were given a unique identification number, including people not living in the village during this registration period, but locally considered to be members of the household. All persons registered were invited for the survey.

Malarioriometric data. Registered households were given written invitations by a village assistant specifying the members of the households to be surveyed the following day during the survey in November and December 1996. Once identified, subjects were questioned about whether they had spent one or more nights outside the village in the previous month and if they had taken any antimalarials in the previous two weeks. Subjects were palpated in a horizontal position for presence of enlarged spleen by the same medical doctor throughout the survey. Blood was obtained from all subjects by finger prick, and thin and thick blood smears were prepared on glass slides. Slides were stained with Giemsa and 100 fields examined for asexual P. falciparum parasites at 1000× magnification. Positive slides were counted against 200 leukocytes.

Data analysis. For graphic presentations, the populations were grouped for age-specific calculations in the following nine age intervals adapted from the World Health Organization:18 children < 2 years of age, children 2–4 years of age, juveniles 5–9 years of age, adolescents 10–14 years of age, young adults 15–19 years of age, and four adult groups 20–29, 30–39, 40–59, and > 60 years of age. Densities were calculated as geometric mean densities of positive samples (positive samples in 100 fields examined) using the equation GM = antilog_{10}(\frac{\sum \log_{10}(x_i + 1)}{n}) − 1, where \( x_i \) = number of parasites per 200 leukocytes and \( n \) = is the number of slides. Prevalences were calculated as the ratio of positive samples (at least one parasite seen in 100 fields examined). Counts were converted to asexual parasites per microliter of blood assuming an average concentration of 8,000 leukocytes/μL of blood.

Prevalence of enlarged spleen defined as a Hacketts size ≥ 119 was calculated among palpated subjects. The relationship between altitude and infection rates in children < 10 years of age and the relationship between annual number of infective bites and infection rates in children < 10 years of age was assessed with linear regression.

Two logistic regression models were developed: one on prevalence of asexual P. falciparum parasites and one on prevalence of enlarged spleen. A linear relationship between the log transformed prevalence and the predictive variables were visually examined by plotting the relationship using the ksm smoothing procedure (Stata version 7.0; Stata Corporation, College Station, TX). Log_{10}(annual number of infective bites), the square root of age, the interaction term of the product of square roots of age, the log_{10}(annual number of infective bites), overnight stays, and antimalarial use were entered as predictive variables in both models. Data were clustered at the village level, and to avoid underestimating the variance, we used a random effect model, Proc NLMIXED (SAS System version 8; SAS Institute, Cary, NC) with villages as random effect, assuming the random effects to be normally distributed. Parameters were entered in the above order in a manual stepwise forward logistic procedure. A parameter was included in the model if it significantly (at the 5% level) reduced the deviance compared with a model without the parameter. The assumption of normally distributed random effects was visually confirmed in the final model by plotting the predicted random effects in a normal probability plot using the UNIVARIATE procedure (SAS Institute). To quantify the effect of age in each village, we used the two resulting models excluding both the log_{10}(annual number of infective bites) and the interaction term, and estimated the odds ratio of increasing age village by village. Confidence intervals (95%) for the odds ratios were calculated in all logistic models from the more robust, but less sensitive, Wald statistics. This means that the confidence interval of the beta estimate of a variable may include zero, although the variable is significantly improving the model according to the likelihood statistics (deviance).

Parasite densities were log transformed and analyzed with linear regression (Proc GLM, SAS) for each village with square root transformed age as predictor. Wald statistics was used to determine if the predictor variable was significantly improving the model at the 5% level.

Ethics. Informed consent was obtained from all adult participants and from the legal guardians of all minors. All subjects with malaria were offered appropriate treatment free of charge. In addition, all subjects were offered treatment of minor ailments or admitted to a health facility free of charge. Ethical approval for this study was provided by the Medical Co-ordinating Committee of the National Institute for Medical Research in Tanzania and registered at the Tanzania Commission for Science and Technology as No 95-217-CC, 96-264-ER-95-121 and 98-005-ER-95-21.

---

### TABLE 1
Geographic and entomologic characteristics of six study villages along an altitude transect in the Usambara Mountains, Tanzania

<table>
<thead>
<tr>
<th>Village</th>
<th>Altitude (meters)</th>
<th>Annual infective bites*</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kwameta</td>
<td>300</td>
<td>91</td>
<td>5° 06.6’ S, 38° 29.1’ E</td>
</tr>
<tr>
<td>Magundi</td>
<td>600</td>
<td>9.7</td>
<td>5° 05.3’ S, 38° 28.3’ E</td>
</tr>
<tr>
<td>Kwamhanya</td>
<td>800</td>
<td>1.7</td>
<td>5° 03.5’ S, 38° 27.6’ E</td>
</tr>
<tr>
<td>Bagamoyo</td>
<td>1,000</td>
<td>1.8</td>
<td>5° 04.0’ S, 38° 26.5’ E</td>
</tr>
<tr>
<td>Balangai</td>
<td>1,400</td>
<td>0.08</td>
<td>4° 55.6’ S, 38° 27.7’ E</td>
</tr>
<tr>
<td>Milungui</td>
<td>1,700</td>
<td>0.03</td>
<td>4° 45.3’ S, 38° 21.3’ E</td>
</tr>
</tbody>
</table>

* Bodker and others.15
RESULTS

A total of 3,833 (82.7%) of the 4,636 residents were examined during the survey, which included almost every resident present during the 3–4-day survey period. Some attending the survey refused either finger prick or palpation. Good slides and spleen recordings were obtained from 75.1% and 79.6%, respectively, of the resident populations.

We found very different patterns of malaria in the closely situated villages along the altitude transect from 300 meters to 1,700 meters. The prevalence of asexual *Plasmodium falciparum* parasitemia in children < 10 years of age decreased with increasing altitude in a linear fashion, with 5% fewer children infected for every 100-meter increase in altitude \((R^2 = 0.96, P < 0.001)\). This can be represented by the equation *P. falciparum* prevalence \((\%) = 99.1 - (0.05 \times \text{altitude in meters})\).

The relationship of prevalence of infection in children < 10 years of age and the estimated annual number of infective bites could conveniently be described as a log-linear relationship \((R^2 = 0.92, P = 0.002)\) by the equation \(*P. falciparum* prevalence \((\%) = 47.8 + (19.7 \times \log_{10} \text{number of infective bites per year})\).

This indicates that for every 10-fold increase in transmission intensity, an additional 20% of the children are infected with malaria, within the range of prevalence observed along the transect (Figure 1). However, these regression coefficients are dependent on age and therefore only apply to this specific age group. Infective mosquitoes were not collected in the two highest villages. The numbers of infective bites in these two villages are therefore estimates based on the observed human biting rate in the villages, and the proportion of infective mosquitoes observed at 800–1,000 meters.

Overall, the six villages showed an initial increase in both prevalence of *P. falciparum* infections and enlarged spleen with age in the first approximately two years of life, followed by a decrease with age (Figures 2 and 3). We analyzed the decrease in prevalence of infections and spleen enlargement with age in those more than two years old. Logistic regression, which allowed for clustering of the data at village level, showed a significant overall effect of transmission intensity and age and a significant interaction between transmission intensity and age for both prevalence of *P. falciparum* infections and prevalence of enlarged spleen (Table 2). The analysis of parasite rate and parasite density showed a log-linear relationship between transmission intensity and the probability of both infection and enlarged spleen. Both analyses identified a square root relationship between age and the probability of infection or enlarged spleen. We also identified a significant interaction between the square root transformed age and the log-transformed transmission intensity. This means that the reduction in prevalence with age is more rapid in younger age groups and levels off with age, and that the reduction in prevalence with age is more rapid at high transmission intensities than low ones. Since the identified interaction of age and transmission intensity is the product of age and exposure, we interpret the interaction as a measure for accumulated exposure. Thus, the analysis found that the probability of both infection and enlarged spleen was affected by the transmission intensity in the village, the accumulated exposure of the person, and the age of the person. The identified effect of age is adjusted for the effect of accumulated exposure in the analysis and therefore an effect of age itself. The odds ratios of age were remarkably similar in both models. Both analyses show that the impact of transmission intensity decreases with age, resulting in fairly similar prevalences in all villages in those 25–30 years of age, regardless of the transmission intensities. At no age group were the villagers better off at higher transmission intensities in terms of infections or enlarged spleens. We examined the effect of age on the probability of infection or enlarged spleen by analyzing the same data set village by village. Each village was analyzed with a simple logistic regression model adjusting for overnight stays outside the village and recent drug use. We identified

---

**Figure 1.** Relationship between annual number of infective mosquito bites per person and the prevalence of *Plasmodium falciparum* in children less than 10 years of age. A 10-fold increase in transmission intensity increases the prevalence of infections by 19.7% of the children. The transmission intensity is shown on a log scale. The six villages are connected with a linear regression line. This regression does not adjust for age and clustering at the village level.

**Figure 2.** Prevalence of asexual *Plasmodium falciparum* infection at different ages in six villages at different altitudes.
TABLE 2
Results of two logistic regression analysis with parasite prevalence (n = 3,256) and prevalence of enlarged spleen (n = 3,450) as outcome*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>P.f. Infection</th>
<th></th>
<th>Enlarged Spleen</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds ratio</td>
<td>Lower CI</td>
<td>Upper CI</td>
<td>df</td>
</tr>
<tr>
<td>Constant</td>
<td>1.65</td>
<td>1.03</td>
<td>2.64</td>
<td>5</td>
</tr>
<tr>
<td>Log_{10} (annual number of infective bites)</td>
<td>3.69</td>
<td>2.46</td>
<td>5.54</td>
<td>5</td>
</tr>
<tr>
<td>Square root of age</td>
<td>0.78</td>
<td>0.74</td>
<td>0.83</td>
<td>5</td>
</tr>
<tr>
<td>Interaction of log_{10} (annual infective bites) and</td>
<td>0.82</td>
<td>0.78</td>
<td>0.87</td>
<td>5</td>
</tr>
<tr>
<td>square roots of age</td>
<td>1.25</td>
<td>0.92</td>
<td>1.70</td>
<td>5</td>
</tr>
<tr>
<td>Staying outside village (Y/N)</td>
<td>0.85</td>
<td>0.62</td>
<td>1.18</td>
<td>5</td>
</tr>
<tr>
<td>Intake of antimalarial drugs (Y/N)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Random effects</td>
<td>5</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Subjects younger than two years were not included in analysis. Confidence intervals (CIs) are based on Wald statistics and P values are based on log-likelihood statistics. P.f. = Plasmodium falciparum; df = degrees of freedom; Y/N = yes/no.

DISCUSSION

The level of malaria endemicity along the six village altitudinal transect in the Usambara mountains decreased from holoendemicity in the lowland village to mesoendemicity or to hypoendemicity in the two highest villages depending on whether endemicity was classified according to parasite rates or to prevalence of enlarged spleens.15,21

The decreasing risk of infection with increasing altitude results in a simple linear relationship between altitude and prevalence in children less than 10 years of age, with 5% fewer of the children being infected for every 100-meter increase in altitude. The slope of the relationship is age-dependent and thus only valid for this specific age group.

According to estimates of the number of infective bites annually recorded in the previous year,15 holoendemic malaria was associated with 90 infective bites annually, hyperendemic malaria with 10–2, mesoendemic malaria with 2–0.1, and hypoendemic malaria with ≤ 0.1 infective bites per year.15,21 However, this is probably an oversimplification of the levels of exposure required for the different levels of malaria because the seasonality of transmission differs in villages at different altitudes and the highland areas have the potential for large inter-annual variation as a result of unstable short rains in the warm season.15,22

The relationship between transmission intensity and parasite prevalence in children less than 10 years of age was best described by a log-linear relationship, in which the proportion

FIGURE 3. Mean ratio of enlarged spleen at different ages in six villages at different altitudes.
of children infected increased 20% for every 10-fold increase in transmission intensity. This log-linear relationship should not be extrapolated beyond the transmission range defined by the highest and lowest village. However, the range covered by these six villages is considerable, covers most malaria-endemic areas in sub-Saharan Africa, and resembles transmission prevalence relationships found in meta-analysis.23–25

The relationship between exposure to malaria parasites and prevalence of parasitemia in young children has two important implications for malaria control. First, a reduction in transmission intensity will result in a reduction in infection where the pattern and intensity of transmission is similar to that experienced in the Usambaras. However, it should be noted that the reduction in prevalence will be proportionately smaller than the reduction in transmission. For example, it required a more than 1,000-fold reduction in transmission intensity to reduce parasite prevalence from 87% to 13% in 2–4-year-old children. Second, our findings suggest that almost all children will be infected if they receive more than 90 infective bites each year; this has also been found in meta-analysis.24,25 In such villages, transmission must be reduced sharply to have any impact on prevalence of infections in children.

Although there are large differences in parasite prevalence between children in the different villages, the prevalence in adults from the different villages is fairly similar, regardless of the large differences in transmission intensity. The age-prevalence relationships found in the present study closely resemble theoretical age-incidence relationships previously reported.26

Logistic regression models identified an inverse relationship between age and prevalence of both infections and enlarged spleens, with prevalences decreasing with age. There were two different age-prevalence patterns seen in adults and children more than three years of age. In the four villages with greatest transmission, prevalence decreased with age after the initial peak in the 2–3-year-old age group, and the higher the initial prevalence, the steeper the decrease in prevalence with age. In contrast, in the two villages with lowest transmission, prevalence was similar in all age groups.

Similar relationships were found when looking at changes in parasite densities with age and exposure intensity. When transmission increased above two infective bites annually, the highest parasite densities were found in the 2–3-year-old group, followed by a dramatic decrease with age, but there was no significant change in densities with age at lower inoculation rates.

We found no evidence of a long-term negative impact of reduced transmission. At any age between 2 and 70 years, people living in low-transmission villages were either better off or the same compared with people in neighboring villages with higher transmission with regard to prevalence of infection, density of infections, and prevalence of enlarged spleen. The data therefore suggest that the benefits in infection rates

### Table 3

<table>
<thead>
<tr>
<th>Village altitude (meters)</th>
<th>Annual infective bites</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>df</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>91</td>
<td>0.51</td>
<td>0.44–0.60</td>
<td>389</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>600</td>
<td>9.7</td>
<td>0.65</td>
<td>0.58–0.72</td>
<td>524</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>800</td>
<td>1.7</td>
<td>0.67</td>
<td>0.59–0.77</td>
<td>336</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1,000</td>
<td>1.0</td>
<td>0.77</td>
<td>0.71–0.85</td>
<td>587</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>1,400</td>
<td>0.08</td>
<td>1.00</td>
<td>0.92–1.10</td>
<td>736</td>
<td>1</td>
</tr>
<tr>
<td>1,700</td>
<td>0.03</td>
<td>0.99</td>
<td>0.88–1.12</td>
<td>624</td>
<td>0.75</td>
</tr>
</tbody>
</table>

*The impact of age is significant and decreasing with age in villages with transmission more than 2 infective bites annually. Parasite prevalence or prevalence of enlarged spleen showed no correlation with age in two low-transmission villages. Subjects less than two years of age was not included in the analysis. Confidence intervals (CIs) are based on Wald statistics and P values are based on log-likelihood statistics. For definitions of abbreviations, see Table 2.*

### Table 4

<table>
<thead>
<tr>
<th>Village altitude (meters)</th>
<th>Annual infective bites</th>
<th>Slope</th>
<th>95% CI</th>
<th>df</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>91</td>
<td>–0.16</td>
<td>–0.24–0.09</td>
<td>4.14</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>600</td>
<td>9.7</td>
<td>–0.21</td>
<td>–0.28–0.14</td>
<td>6.14</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>800</td>
<td>1.7</td>
<td>–0.26</td>
<td>–0.33–0.19</td>
<td>7.03</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>1,000</td>
<td>1.8</td>
<td>–0.20</td>
<td>–0.27–0.14</td>
<td>5.95</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>1,400</td>
<td>0.08</td>
<td>–0.04</td>
<td>–0.14–0.05</td>
<td>0.94</td>
<td>0.35</td>
<td></td>
</tr>
<tr>
<td>1,700</td>
<td>0.03</td>
<td>–0.02</td>
<td>–0.10–0.10</td>
<td>0.38</td>
<td>0.71</td>
<td></td>
</tr>
</tbody>
</table>

*The impact of age is significant and similar in the villages with transmission more than two infective bites annually. Parasite densities showed no correlation with age in the two low-transmission villages. CI = confidence interval.*

![Figure 4](image-url)  
**Figure 4.** Geometric mean density of *Plasmodium falciparum* infections at different ages in six villages at different altitudes.
reported from many transmission control studies will be sus-
tainable throughout life. Persistent reductions in parasitemia
have been reported after four to six years in prolonged bed
net intervention studies in high-transmission areas in Tanza-
nia and Kenya, with sustainable reduction in morbidity and
mortality in children.12–14

Density of infection is likely to be relevant to disease. Our
data indicate that as transmission increases the geometric
mean density of parasitemia increases to a plateau, with any
further increase in transmission failing to increase density of
parasitemia. This threshold occurs at approximately 0.1–2 in-
fected bites per year. Such a plateau has been suggested pre-
viously for the relationship between transmission intensity
and malaria morbidity and mortality.1,27,28

More malaria was found in children in highland areas than
expected from the low-transmission intensity. It is difficult
to explain how 0.08 and 0.03 estimated infective bites annually
could maintain prevalences in 2–4-year-old children of 27%
and 14% in the two highest villages. However, similar high
prevalences have been reported in other studies.24,25 Even if
all infective bites were to result in human infections, which is
unlikely,29–31 it would take a mean duration of infection of
more than 2.5 years at 1,400 meters and 4 years at 1,700
meters, assuming prevalence to be the product of the number
of infective bites and the duration of infection. However, few
infections are expected to last for more than a year.32–36 The
highland transmission rates are estimations based on entomo-
logic data, and the confidence intervals of this kind of esti-
mates are likely to be wide.37 It is also possible that prior to
the collection of transmission data there was a period of
higher transmission intensity that resulted in a new, albeit
decreasing, number of cases as the prevalence regressed to a
new equilibrium that was not reached at the time of the sur-
vey.

It is tempting to suggest that all infections in the highest
villages originated during brief trips to the highly malarious
lowlands, but we believe that this is not the case. We are
confident that malaria transmission does occur in the highest
villages because parents of infected children often told us that
the children had never left the village. Two surveys 12 months
apart of a total of 138 children at the slightly higher village of
Longo (2,000 meters) failed to identify a single infection,
suggesting relatively little importance of imported cases in the
highland villages.22 We therefore suggest that movement of
children to high-risk areas must be of minor or no importance
in the highland villages. Nonetheless, some adult villagers do
travel to the lowlands, where the force of transmission is for-
midable. The risk of inoculation during one night in the low-
lands may be equivalent to more than three years accumu-
lated risk at 1,700 meters,15 and we detected a significant
overall increase in the odds of infection in people with a
recent history of staying overnight outside their village. Mi-
igration has also been shown to be relatively important in
Kayanza Province in the highlands of Burundi where trans-
mision appears to be very low.38

The reduced parasitologic prevalence and parasite density
in adults compared with children are the results of immunity
developing as the villagers get older. To what extent the de-
veloping immunity is the result of accumulated exposure or a
result of age itself is an important question.1,2,7 If immunity is
the result of accumulated exposure, the long-term effect of
transmission control may hinder the development of protec-
tive immunity by reducing exposure to parasite antigens.9
However, if immunity is the result of a maturation of the
immune system and thus largely an effect of age itself, trans-
mission control will not prevent the development of protec-
tive immunity.10,11

The lack of a simple linear relation between transmission
intensity and prevalence suggest an immune mechanism. The
log-linear relationship between transmission intensity and
prevalence of infections may occur for two reasons. As the
number of infective bites increases, fewer infective bites may
result in a patent infection or the duration of infection is
shortened. Both explanations are examples of density-
dependent effects reducing the reproductive success of spe-
cific parasite strains. These mechanisms may be explained by
acquired immunity increasing with intensity of transmission.
A similar log-linear relationship between exposure and child
prevalences has been predicted by mathematical models as-
suming exposure-dependent immunity.29 The log-linear rela-
tionship between transmission intensity and prevalence of
both infections and enlarged spleens thus support the exis-
tence of exposure-dependent acquired immunity. However,
other studies have reported that the proportion of infective
bites giving rise to infections decreases with increasing trans-
mission.31,40–42 The proportion of inoculations giving rise to
infections in infants decreased in the high-transmission season
compared with the low-transmission season in a holoendemic
village. In nearby East Usambaras, the proportion of inocu-
lations leading to a patent infection was eight times higher in
low-transmission villages compared with high-transmission
villages.31,40 Similarly, a disproportionately small reduction in
re-infection rates compared with reduction in transmission
intensity was observed in the Usambara lowlands with the
introduction of impregnated bed nets.41,42 Thus, a non-
immune mechanism may be involved in this phenomenon.

The logistic regression model of parasite prevalence sug-
gested that the prevalence was significantly related to the
interaction between age and the annual inoculation rate. This
interaction between age and exposure intensity causes the
prevalence to decrease faster in villages with high transmis-
sion and decrease faster in the youngest age groups. We in-
terpret this interaction as a measure of accumulated expo-
sure. The analysis was adjusted for the effect of transmission
intensity, and we therefore suggest that the developing im-
munity in the four lowest villages is partly caused by an in-
dependent effect of accumulated exposure separate from the
effect of transmission intensity. The logistic regression model
also suggested that parasite prevalence was significantly re-
lated to an effect of age alone. This effect of age can be
observed in the age-prevalence curves, which in the four low-
est villages peaks at the same age group and levels out at the
same age group, despite the accumulated exposure being 50-
fold greater in the lowest village. Thus, the accumulated ex-
posure in the lowland at two years of age is equivalent to that
accumulated after 100 years at 1,000 meters. A sub-sample
from each village also showed that super infections are re-
lated to both age and transmission intensity, but that the di-
versity of parasite strains in the six villages was similar.17 The
proportionally few high-density parasitaemias in adults in all
villages strongly suggests that the acquired immunity protect-
ing against high-density infections is related more to age itself
than to accumulated exposure. The regression model would
thus suggest that non-immune children suddenly exposed to
malaria transmission would develop anti-parasite immunity much more slowly than non-immune older age groups exposed to malaria transmission. Such an effect of age has been observed in Indonesia in people moving from malaria-free islands to malaria-endemic areas.\textsuperscript{10,11}

The data reported were collected during cross-sectional surveys in November–December. Thus, these findings apply to the low-transmission season, and the degree to which this season reflects the entire year remains to be explored. It should be appreciated that the findings presented in this study are parasitologic and not clinical. There may be antitoxic immune mechanisms that result in high-density infections without morbidity, but immunity against infection and high infection density discussed here is likely to contribute to clinical immunity.

The findings from the present study suggest that both exposure and age-dependent acquired immune mechanisms are involved in anti-parasite immunity. The relative impact of these two mechanisms has important implications for the effectiveness of different types of control strategies, for example, transmission control, mass drug administration, and vaccination.\textsuperscript{9,13}

Received February 2, 2005. Accepted for publication September 21, 2005.

Acknowledgments: We thank D. Shayo, L. N. Malle, Z. X. N. Savel, and W. Chambo (Amani Medical Research Centre), and G. Kitua and J. Raphael for invaluable help during the surveys. We are also grateful to the collaboration of all villagers and for the excellent work done by the six village assistants. We also thank Tom Smith for the helpful comments on an earlier version of the manuscript.

Financial support: This study was supported by the Danish Council for Development Research and the Danish Bilharziasis Laboratory.

Authors’ addresses: René Bødker, Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, 2860 Søborg, Denmark, Telephone: 45-72-34-70-99, Fax: 45-72-34-70-28, E-mail: reb@dfv.dk. Hamisi A. Msangeni and Willia, Kisinza, National Institute of Medical Research, PO Box 4, Amani Tanga, Tanzania, E-mails: hmsangeni@hotmail.com and kisina@hotmail.com. Steve W. Lindsay, School of Biological and Biomedical Sciences, Durham University, South Road, Durham DH1 3LE, United Kingdom, Telephone: 44-191-334-1349, Fax: 44-191-334-1289, E-mail: s.w.lindsay@durham.ac.uk.

Reprint requests: René Bødker, Danish Institute for Food and Veterinary Research, Mørkhøj Bygade 19, 2860 Søborg, Denmark.

REFERENCES


