EFFICACY OF PERMETHRIN-IMPREGNATED BED NETS ON MALARIA CONTROL IN A HYPERENDEMIC AREA IN IRIAN JAYA, INDONESIA: DIFFERENTIATION BETWEEN TWO AGE GROUPS

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Abstract. A malaria intervention trial was conducted for two years to evaluate the efficacy of permethrin-impregnated bed nets in reducing malaria infection and splenomegaly in two different age groups, *ie* below and over age of ten, in a hyperendemic area in Irian Jaya, Indonesia. Permethrin-impregnated or placebo-treated bed nets were provided to a treated and a control village, respectively. Immediately after periods with moderate rainfall in the first year, treated bed nets decreased *P. falciparum* and *P. vivax* density in the blood of children <10 years (group 1) but did not reduce the percentage of infection with either species. Children >10 and adults (group 2) showed significant reduction only in *P. falciparum* infection rates and density, whereas *P. vivax* was not influenced. After an excessive rainfall season in the second year, the risk for *P. falciparum* infections in both age groups using treated nets was less than half of that in the control village. *P. vivax* infection rates were significantly lower in the treated village at the beginning of and after these heavy rainfalls. In the treated village, spleen enlargement was markedly reduced in the younger age group during the second year.

INTRODUCTION

For nearly 2 decades, studies on the control of malaria using insecticide-impregnated bed nets have been conducted widely in many malarious countries (Rozendaal, 1989). Assessment of this community-wide intervention strategy has been considered complicated because of the influence of epidemiological, socio-economic and environmental peculiarities of each study site. Nevertheless, improvement of malariometric parameters was achieved in studies where, indeed, all inhabitants of treated villages had been provided with insecticide-impregnated bed nets (Snow *et al*, 1988; Luo *et al*, 1994). According to Lindsay *et al* (1989), this is due to the dramatic effect of insecticides in reducing vector biting rates. Reduction of mosquito density as reported in several studies (Li *et al*, 1989; Magesa *et al*, 1991; Jana-Kara *et al*, 1995; Kere *et al*, 1996) would presumably benefit the entire community, even near-by nonusers of bed nets (Binka *et al*, 1998).

Malaria often presents with seasonal fluctuations and varying age distribution depending on endemicity (Binka *et al*, 1994), likewise, the efficacy of impregnated bed nets must be appraised under these aspects. In hyperendemic areas, population differs in two age groups concerning immune status, adults who have acquired natural immunity and children, who have not yet developed full immunity.

A 2-year study was conducted on community-wide distribution of bed nets impregnated with 0.5g/m[2] permethrin in the south-central part of Irian Jaya, in a hyperendemic malaria area with perennial transmission and seasonal rainfall fluctuation (Sutanto *et al*, 1999). *Anopheles koliensis*, the primary vector in the study area requires fresh water for larval breeding sites (Lee *et al*, 1980), therefore local rainfall might influence malaria transmission in the study area. A decrease both in parasite prevalence and spleen enlargement among treated villagers was reported indicating successful application of impregnated bed nets (Sutanto *et al*, 1999). The aim of the present study is to discern efficacy of impregnated bed nets in two different age groups, children below the age of 10 *versus* children over 10 and adults.

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MATERIALS AND METHODS

Study area and its population

The study was accomplished from April 1993 until April 1995, in the southcentral lowland part of

Irian Jaya, East Mimika district, about 20 km west of Timika. After a preliminary baseline study in 6 villages around this town in September 1992 (Pribadi *et al*, 1998), two villages (Hiripau and Kaugapu) with comparable ethnology, tribal habits, environmental peculiarities, and levels of endemicity were chosen for the intervention study (Sutanto *et al*, 1999). The distance between the two villages is around two kilometers and surrounded by thick forest swamps and sago palms.

During the study, Hiripau had 657 villagers and 158 households with 237 children below the age of ten, whereas Kaugapu had 565 villagers and 141 households with 201 children under ten. Age determination and allocation to the groups of below (group 1) or over the age of ten (group 2) was based on interviews and judgement according to the physical appearance, since official records such as birth registration were not available.

Houses were made from wood and placed on stilt 0.5m above the ground. Mosquitos could easily enter houses from gaps and holes between the boards of floors and walls. Because there was no electricity in this area, adults normally sat outside the houses at night, particularly during the full moon, before they went to sleep around 22.00 hours.

General and study-independent intervention

General intervention of PT Freeport Indonesia and of the governmental primary health care station had been described and discussed, previously (Sutanto *et al*, 1999).

Rainfall measurements

Ambient temperature was 25°C - 30°C throughout the year, with high humidity of up to 90 - 100%. Rainfall was measured with a rain gauge device, placed in an open field in the control village to collect daily precipitation.

Intervention study

Bed nets were made of nylon in rectangular shape and family size (2 m x 2 m x 1.5 m) with 70 cm overlap. For impregnation 0.5 g/m^2 permethrin (Permanet® 100 EC, PT. Bina Guna Kimia, Jakarta) was used based on the method of Scherk and Self (1985). In April 1993, the treated village (Hiripau) was provided with 277 impregnated bed nets (approximately 1.7 per household), while 261 bed nets were distributed in the control village, Kaugapu. In both villages, on the average one bed net was used by 0.8 children below the age of ten or by 2.3 individuals in total. Every four months nets were replaced with new ones to provide all inhabitants with the same maximum physical barrier protection throughout the entire study period.

Malariometric surveys

In April 1993, prior to distribution of bed nets, a baseline (entrance) survey was carried out in both villages. Blood samples were taken for microscopy and enlarged palpable spleens examined to determine the level of endemicity. Then, every two to four months a mass survey was conducted until the end of the study in April 1995; finger-stick blood was collected and spleen enlargement examined from every one who presented to the surveys. Average coverage rate for the treated village was 54.5% (range 40.6 - 64.4%) and 46.5% (range 36.1 - 55.6%) for the control village. Coverage rate of children under ten was 36.4% (range 34.1 - 39.5%) in the treated and 41.8% (range 34.4 - 47.1%) in the control village out of the total number of examined inhabitants.

Blood films were stained with Giemsa and parasites were counted on 200 fields of a thick smear under a 1000x oil immersion lens before declared negative. Positive slides were determined to parasite species. All blood slides were reinvestigated by an experienced microscopist in Jakarta, who was not involved in the field study to provide objective blinded evaluation. *P. falciparum* and *P. vivax* were primarily found, whereas *P. malariae* accounted for less than 2% of all infections during the study.

Spleen enlargement was determined according to the Hackett grading system (Bruce-Chwatt, 1980). One experienced physician accomplished all spleen examinations during the whole study period except for April 1994. For further analysis, the April 1994 examination was not considered valid, because of high and discrepant values.

Entomological assessment

Bioassays were carried out each month based on 30 and 60 minutes contact time (WHO, 1975) showing effectiveness of 95 - 100% during the whole study period. *Anopheles koliensis* was determined as the primary vector and *An. punctulatus* as a secondary one. Biting activities of *An. koliensis* were found to occur from 18.00 hours until 06.00 hours with peak activity between 23.00 hours and 01.00 hour. Further entomological results will be published separately.

Statistical analysis

All data were analysed with SPSS-PC version

4.0 (SPSS, Inc, Chicago, USA) and EpiInfo5 (CDC, Atlanta, USA). Qualitative data on malariometric parameters were assessed by χ^2 or Fisher Exact tests, whereas quantitative data on geometric means of parasite density and AES (average enlarged spleen) were analyzed with the non-parametric Mann-Whitney test due to the abnormal data distribution. All statistics used $p = 0.05$ as the limit of significance and $p < 0.001$ for highly significant difference. Determination of parasite density was based on 1 µl blood containing 8,000 leukocytes: parasites were counted corresponding to 200 leukocytes and multiplied by 40. The geometric mean of parasite density per microliter was calculated as the antilogarithm of the quotient of the sum of all logarithmic values of parasites counted and divided by the total number of positive samples. The AES (Average enlarged spleen) index was calculated by multiplying the number of individuals in each class of enlarged spleen with the class of spleen and dividing the product by the total number of individuals with splenomegaly (Bruce-Chwatt, 1980).

RESULTS

Preintervention survey (April 1993)

In group 1, overall parasite prevalence and *P. falciparum* and *P. vivax* infection rates were significantly higher in the treated than the control village, whereas splenomegaly prevalence was similar in both villages. In group 2, overall parasite prevalence and *P. falciparum* infection were higher in the treated than in the control village, whereas *P. vivax* infection rate and splenomegaly prevalence were not significantly different.

Comparison between the two age groups in each village based on the above malariometric measurements showed significantly higher values in group 1 than in group 2.

Rainfall

The record showed a moderate rainfall period from the beginning of the study until September 1993 followed by an extremely dry season until February 1994. Excessive downpours were registered from March through August 1994 with a peak of 672mm in August and a subsequent period of moderate rainfall from September 1994 until the end of the study, with a small peak in March 1995 (see rainfall curve in Fig 1).

Overall parasite prevalence and geometric mean of parasite density

Shortly after the end of the first year's moderate rainy season in October 1993, parasite density was lower in the blood of both groups in the treated than in the control village (Figs 1, 2). These differences were highly significant. However, no significant difference was detected in the percentage of infections in both groups (parasite prevalence) after this moderate rainfall (Tables 1, 2).

By contrast, immediately after the period of heavy rain in 1994, *ie* in the surveys of August and October, although malaria infection rates rose in the whole study area, the risk of inhabitants who slept under permethrin impregnated nets to be infected was less than half that of control villagers (Tables 1, 2). Furthermore, the percentages of infected people in both age groups were significantly lower in the treated than in the control village in all surveys during the second year (Tables 1, 2).

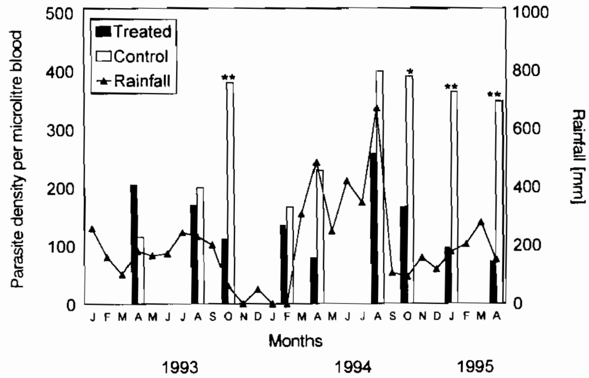

Fig 1–Geometric mean of parasite density in group 1 and rainfall.
* significantly different ($p < 0.05$)
** highly significant ($p < 0.001$)

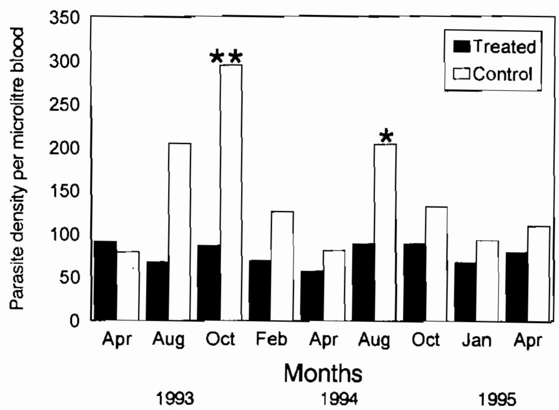

Fig 2–Geometric mean of parasite density in group 2.
* significantly different ($p < 0.05$)
** highly significant ($p < 0.001$)

Table 1
Parasite prevalence in group 1.

Survey month	Treated (%)	Control (%)	p-value	RR and 95% CI[a]
April 1993	52.7 (48/91)	22.2 (24/108)	0[c]	2.37 (1.59-3.55)
August 1993	19.8 (23/116)	18.1 (23/127)	0.7328	1.09 (0.65-1.84)
October 1993	28 (40/143)	28 (30/107)	0.9909	1.00 (0.67-1.49)
February 1994	21.5 (29/135)	23.5 (23/98)	0.7190	0.92 (0.57-1.48)
April 1994	6.1 (8/131)	30.4 (28/92)	0[c]	0.20 (0.10-0.42)
August 1994	26.5 (31/117)	58.7 (64/109)	0[c]	0.45 (0.32-0.63)
October 1994	24.1 (32/133)	61.9 (70/113)	0[c]	0.40 (0.29-0.56)
January 1995	12.1 (19/157)	21.5 (28/130)	0.0315[b]	0.56 (0.33-0.96)
April 1995	8.1 (12/149)	36.5 (35/96)	0[c]	0.22 (0.12-0.40)

% : number of infected persons/total number of people examined
[a] : RR = risk ratio with 95% confidence intervals
[b] : significantly different ($p < 0.05$)
[c] : highly significant ($p < 0.001$)

Table 2
Parasite prevalence in group 2.

Survey month	Treated (%)	Control (%)	p-value	RR and 95% CI[a]
April 1993	14.8 (26/176)	5.3 (11/206)	0.0018[b]	2.77 (1.41-5.44
August 1993	10.3 (20/194)	5 (8/159)	0.0679	2.05 (0.93-4.53)
October 1993	9.6 (27/280)	12.1 (20/165)	0.4112	0.80 (0.46-1.37)
February 1994	10 (22/220)	11.6 (20/172)	0.6050	0.86 (0.49-1.52)
April 1994	3.8 (9/240)	11.5 (15/131)	0.0039[b]	0.33 (0.15-0.73)
August 1994	20.1 (36/179)	40.8 (51/125)	0[c]	0.49 (0.34-0.71)
October 1994	15.7 (38/242)	44.9 (70/156)	0[c]	0.35 (0.25-0.49)
January 1995	2.8 (7/247)	9.3 (15/162)	0.0048[b]	0.31 (0.13-0.73)
April 1995	5.9 (16/273)	16.7 (19/108)	0.0003[c]	0.33 (0.18-0.62)

% : number of infected persons/total number of people examined
[a] : RR = risk ratio with 95% confidence intervals
[b] : significantly different ($p < 0.05$)
[c] : highly significant ($p < 0.001$)

In group 2, parasite density of the control village had also become significantly higher than in the treated village in August 1994 (Fig 2). In the same month, an increase in parasite density was observed in group 1 with no significant difference between both villages. However, in the treated village, this increase was transient and density of parasites decreased rapidly and constantly from October 1994 to April 1995. By contrast, in the control village high density of parasites was persistent with significant differences *vs* the treated village, until the end of the study (Fig 1).

Plasmodium falciparum infection rate and geometric mean of *P. falciparum* density

Efficacy of permethrin-treated nets towards this species appeared slightly different in the two age groups. In the August 1993 survey, before the end of the moderate rainy season group 2 in the treated village showed evidence of reduced *P. falciparum* density in their blood (treated *vs* control village: 100 *vs* 562 parasites/µl blood; Mann-Whitney, $p = 0.0371$). Later, in October 1993, also the percentage of infections in group 2 decreased in the treated village with highly significant difference *vs* the control village (RR 0.21, 95% CI 0.08 – 0.57, $p = 0.0007$). Group 1 showed significantly reduced density of *P. falciparum* in the treated village *vs* control only in October 1993 (Fig 3) and the decrease in percentage of infections was not significant at all (not shown).

In August and October 1994, *P. falciparum* infection increased considerably in both villages. However, control villagers in both age groups showed a three-fold higher risk of infection than inhabitants in the treated village (group 1: RR 0.27 - 0.36, 95% CI 0.16 - 0.56, p < 0.0001; group 2: RR 0.35 - 0.44, 95% CI 0.23 - 0.67, p < 0.0001). In all surveys during the second year, percentage of *P. falciparum* infections in both groups was significantly lower in the treated than in the control village with greatest differences in August and October 1994, shortly after the peak of heavy rainfall.

In group 2, the density of *P. falciparum* in the control village increased temporarily in August 1994. This value was significantly different from *P. falciparum* density in the treated village where values were constantly low throughout the second year (not shown). In group 1, density of *P. falciparum* increased in both villages at the end of the high rainy season in 1994 and remained high in the control village (> 600 parasites/µl blood) until April 1995. Differences in these quantitative data between control and treated villages were significant in the final two surveys, in January and April 1995 (Fig 3).

In October 1994, differences in gametocyte rates of this species in group 1 became highly significant between the treated and the control villages (data not shown).

Plasmodium vivax infection rate and geometric mean of *P. vivax* density

In the first year of the study, the effect of insecticide-treated nets on reducing the density of *P. vivax* in group 1 was apparent only after a peak of rainfall in October 1993 (treated *vs* control village: 83 *vs* 166 parasites/µl blood; Mann-Whitney, p = 0.035). In group 2, neither qualitative nor quantitative differences were observed in group 2 during the same period of time.

In the second year, in October 1994, the risk of *P. vivax* infection was three times higher in group 2 of the control village than in those who slept under permethrin- treated bed nets (RR 0.34, 95% CI 0.15 - 0.79, p = 0.008). Moreover, the density of *P. vivax* in group 2 was significantly higher in the control village (treated *vs* control village: 49 *vs* 83 parasites per µl blood; Mann-Whitney, p = 0.019). Group 1 in both villages did not show significant distinction in infection rates (Fig 4) or in density of *P. vivax* in their blood.

Efficacy of impregnated bed nets in preventing *P. vivax* infection in group 1 was evident in the surveys of April 1994 and April 1995 when the risk of infection in the control village was significantly (four times) higher than in the treated village (Fig 4). In group 2, similar effects on *P. vivax* infection

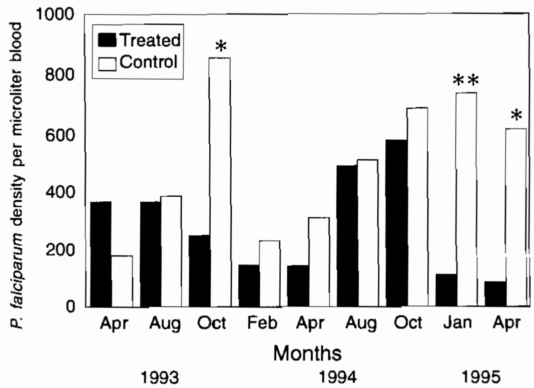

Fig 3–Geometric mean of *P. falciparum* density in group 1.
* significantly different
** highly significant (p < 0.001)

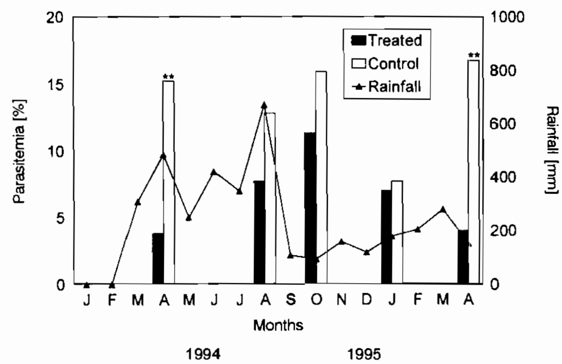

Fig 4–*P. vivax* infection rate in group 1 in the second year of the study and rainfall.
** highly significant (p < 0.001)

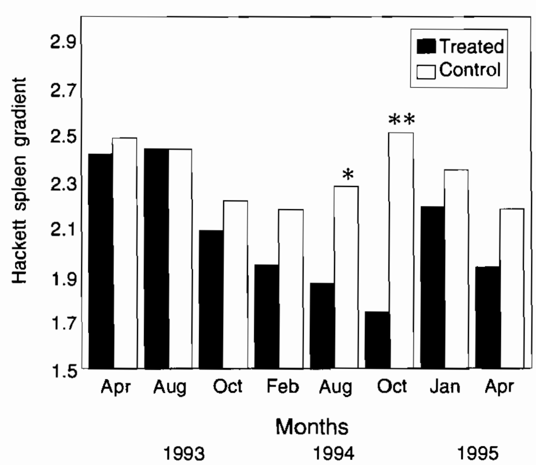

Fig 5–AES (average enlarged spleen) in group 1.
* significantly different (p < 0.05)
** highly significant (p < 0.001)

rates were observed, however, less pronounced than in group 1.

Splenomegaly prevalence and AES (average enlarged spleen)

During the first year, the effect of impregnated bed nets on these malariometric parameters was observed only in group 2, but not in group 1. The survey in August 1993 revealed a slightly significant difference in splenomegaly prevalence between the treated and the control village (group 2: 16.5% *vs* 26.4%, χ^2 = 1.80, p = 0.023, RR 0.62, 95% CI 0.41 – 0.94).

Throughout the second year, splenomegaly in both groups was less prevalent in the treated than in the control village. Moreover, during the highest rainy season in August 1994 and at its end in October 1994, the size of spleens (AES) in group 1 in the treated village was reduced as compared to controls (Fig 5).

DISCUSSION

Our study observed significant differences in malariometric parameters (overall parasite and splenomegaly prevalences) between both age groups in either village. This indicates that the older group in the study area has already acquired natural immunity. Hence, for immune adults, even if mosquito bites are more extensive due to their ourdoor activities at night, the natural acquired immunity in addition to impregnated bed nets may attenuate infection. Reduced *P. falciparum* infection in the older age group was seen qualitatively (percentage of infected persons) and quantitatively (parasite density/μl) in both rainy seasons (particularly after a peak of downpour). However, in the younger group, significant differences were observed either in the percentage (high rainy season) or in the density (moderate rainy season). Therefore, in highly endemic areas, children become the primary target groups for intervention with impregnated bed nets.

In The Gambia, where malaria transmission occurred only several months in the year (rainfall ± 750 mm/year), Snow *et al* (1988) observed a decrease in percentage of children with heavy infection, but no reduction in overall parasite rates. However, in a later study in The Gambia, in a different area, Alonso *et al* (1993) reported a 50% reduction in heavy infection and a 40% decline in parasite prevalence in children who slept under permethrin impregnated bed nets. The different results in these two studies were suggested to be due to heavier rainfall of ± 850 mm/year and hence higher transmission in the second study. However, studies in different areas can not well be compared because of many other varying factors.

In a study in Burkina Faso, the efficacy of permethrin impregnated curtains showed seasonal variation over two years (Procacci *et al*, 1991), reducing most effectively parasite rates and density in low transmission seasons.

Impregnated bed nets in our study showed little effect towards *P. vivax* in the first year, particularly in the group of over ten years, which was presumably due to relapses of liver stages (Graves *et al*, 1987). Nevertheless, during the second year, reduction of infection rate was pronounced in both age groups. A similar situation was also observed with splenomegaly prevalence. For these two malariometric parameters, a longer period of time may be required to fully evaluate the effect of an intervention.

The continuation of rainfall at a moderate level, after the highest peak in August 1994, is perhaps important in supporting high persistence of parasite density in children from the control village in January 1995 (*P. falciparum* and *P. vivax*) and April 1995 (*P. falciparum*). Considering the time interval of 3 – 6 months, these events were probably due to new infections, rather than relapses of August and October 1994 infections.

Impregnated bed nets were effective in reducing malaria infection throughout the seasons of high rainfall (in the second year of the study) in both age groups. However, seasonal trends were shown for *P. falciparum* and *P. vivax*. In the younger group, bed nets were mostly effective in reducing *P. vivax* infection in the beginning of rainy seasons, *ie* in April 1994 and April 1995, when protective efficacy reached 75%. By contrast, *P. falciparum* infection was mostly reduced shortly after the highest peak of rain, in August and October 1994 with protective efficacy around 70%. Rowland *et al* (1996) reported seasonal trends of incidence rates of these two species in refugees from Afghanistan who slept under permethrin-impregnated bed nets. In the same season of malaria transmission, bed net efficacy was earlier towards *P. vivax* than towards *P. falciparum*.

Our intervention study indicates a sustained efficacy of permethrin impregnated bed nets over two years, reduction of the hyperendemic level of the treated village to low mesoendemicity. Nevertheless, rebound effects were not observed, neither in the younger nor older age groups until the end of the study regarding parasite and splenomegaly prevalences or parasite density and AES. However, the positive results achieved in this study still need clarification for intervention periods of more than two years. Lowering malaria transmission by using impregnated bed nets for a longer period of time might postpone development of immunity in children or diminish natural immunity in adults. This has still to be investigated.

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