Tropical Medicine and International Health

VOLUME 1 NO. 6 PP 824–835 DECEMBER 1996

Deltamethrin-impregnated bednets as an operational tool for malaria control in a hyper-endemic region of Burundi: impact on vector population and malaria morbidity

W. Van Bortel[1,3], **C. Delacollette**[2,3], **M. Barutwanayo**[3] **and M. Coosemans**[1]

[1] *Laboratory of Medical Entomology, Prince Leopold Institute of Tropical Medicine, Antwerpen, Belgium*
[2] *World Health Organization, Division CTD, Geneva, Switzerland*
[3] *Programme National de Lutte Antipaludique, Bujumbura, Burundi*

Summary Within the framework of the National Malaria Control Programme Burundi, impregnated bednets were promoted through health care facilities, schools and local administration in Nyanza Lac district. The decision to buy a bednet was left to the inhabitants and, as a result, coverage rates between 6 and 65% were observed at sub-district level. Three intervention regions were specified based on the intervention start date. From November 1992 until March 1995, bi-monthly parasitological and entomological surveys were carried out in two areas each of Region 1 and Region 2. After introduction of impregnated bednets in Region 1 the proportions of children under 5 with high parasitaemia were reduced by 42 and 53% in the 2 parasitological survey areas, where the average bednet coverages were 55 and 44% respectively. In the survey areas of Region 2 (control) no significant change occurred during the same period. During the second part of the intervention from September 1994, when intervention was also operational in Region 2, significant decreases in the proportion of high parasitaemia (63 and 42%) among children under 5 years were obtained in both parasitological survey areas of Region 2 (average coverages of 51 and 29%). The positive output of the intervention was maintained and even reinforced in the survey areas of Region 1. Bednets as a tool for malaria control entail specific problems such as coverage, daily use, reimpregnation, and renewal of old and torn nets. Further evaluation has to point out the possible shift of the clinical spectrum and the age-specific admission of malaria cases to assess the long-term benefit of this control method.

keywords bednets, *A. gambiae*, *A. funestus*, *P. falciparum*, Burundi

correspondence W. Van Bortel, Laboratory of Medical Entomology, Prince Leopold Institute of Tropical Medicine, Nationalestraat 155, B-2000 Antwerpen

Introduction

The choice of vector control method depends on local malaria epidemiology and vector behaviour. In the central lowland of Burundi, indoor spray campaigns, begun in 1985, were very effective in reducing malaria prevalence and prevalence of high parasitaemia (Smits *et al.* 1995). A control measure must not only achieve a rapid initial reduction of the transmission, its sustainability is even more important (Coosemans & Carnevale 1995). Insecticide impregnated bednets have been shown to be effective against malaria disease (Curtis *et al.* 1991; Snow *et al.* 1987) and are considered a cost-effective tool for obtaining sustained control efforts (Brinkmann & Brinkmann 1995).

When people decide to buy and use bednets, both purchase and use will depend upon such factors as the nuisance level, perceptions of the causes of malaria and economic aspects. These factors are not directly related to the level of the malaria problem in the community, so the use of bednets is more inclined to reflect motivation of the inhabitants for buying and using bednets (Carnevale & Coosemans 1995; Van Bortel *et al.* 1996). In the Gambia the transition from small-scale control trials to the national impregnated bednet programme resulted in a reduction in the impregnation coverage (D'Alessandro *et al.* 1995). Likewise, the bednet coverage can be very incomplete even within a small area as observed in southern Burundi, district Nyanza Lac (Van Bortel *et al.* 1996). Any evaluation of the effectiveness of bednets as malaria control tool has to consider these operational constraints which can interfere with the positive impact.

To control malaria morbidity and mortality in a hyper-endemic, stable malaria region in Nyanza Lac district, the staff of the National Malaria Control Programme Burundi (NMCP-Burundi) decided to introduce deltamethrin-impregnated bednets. This method was not known to the inhabitants and the problems encountered during introduction of the bednets were described in a previous paper (Van Bortel *et al.* 1996). Briefly, the motivation for buying and using impregnated bednets appeared to depend essentially on the nuisance level caused by mosquitoes (mainly *Anopheles*). Very unequal bednet coverage was observed at subdistrict level. The question arises whether impregnated bednets can reduce morbidity at community level when the bednet coverage is related to the mosquito nuisance level rather than to the malaria problem. This paper reports the assessment of the impact of impregnated bednets on the malaria morbidity and on the malaria vector population in 4 study areas in the district Nyanza Lac, where a bednet programme started in January 1994.

Material and methods

Study area

The study was conducted in the district Nyanza Lac, a stable malaria region in southern Burundi (Coosemans 1989; Delacollette & Barutwanayo 1993) flanked by the Tanganyika Lake and the Zaire-Nile crest. Malaria is the first cause of morbidity with a yearly incidence rate of 1.17 admissions per person among children under 5 years. This burden is responsible for more than 30% of mortality in under-5-year-olds. The second and third major causes of death are diarrhoea and respiratory tract infections (Delacollette & Barutwanayo 1993).

The district is divided into 23 administrative areas called 'hills' which are themselves subdivided in 'sub-hills'. Most of the houses are scattered throughout the sub-hills, but in some of them the properties are lined up along roads. Most (90%) of the people farm food crops. Two other important economic activities in the region are rice cultivation and fishing. A census of the district carried out in May 1994 counted a population of 60 541 with 21.3% children under 5 years old and 18.5% 5–9-year-old children (Table 1).

The climate is of the tropical humid type with an average temperature of around 25°C, a dry season from June to September and an annual rainfall of 1100–1300 mm. The total rainfall from January until July 1993 and 1994 was 697 and 762 mm respectively.

Control programme

The bednet control programme in the district Nyanza Lac was designed in 3 steps to optimize human and material resources, to obtain better collaboration with the inhabitants and to assess the effectiveness of the programme. Three Intervention Regions were specified: in Intervention Region 1 (11 hills) the sale of the nets started in January 1994, in Region 2 (8 hills) in September 1994 and in Region 3 (4 hills) in September 1995 (Table 1, Figure 1). Two areas within Region 1 and Region 2 were selected for the entomological and parasitological evaluation of the control programme.

Bednet introduction, impregnation process and coverage

The bednets sold in Intervention Region 1 were imported from Thailand and those sold in Intervention Region 2 were sewn locally under the

Table 1 Data of the census of May 1994 and the bednet coverage data for the three Intervention Regions of Nyanza Lac district

	Intervention Region 1	Intervention Region 2	Intervention Region 3
Start of intervention	January 1994	September 1994	foreseen 1995
Number of families	6527	5861	1564
Number of inhabitants	28 796	25 183	6562
Percentage 0–4 years old children	20.9	21.9	21.1
Percentage 5–9 years old children	18.8	18.0	18.5
Number of bednets purchased	5061 (in April 1994)	5160 (in December 1994)	–
Percentage of families that bought at least one bednet (range)[1]	52.2 (10.0–64.8)	43.6 (5.5–61.8)	–
Percentage of families with at least one installed bednet (average coverage)	43.1	36.2[3]	–
Average percentage coverage in the different parasitological survey areas (range)[2]	A1 54.5 (46.2–70.2) A2 43.7 (14.3–77.1)	B1 51.0 (29.7–59.4)[3] B2 28.5 (1.7–44.6)[3]	–
Percentage coverage in the collection sites (sub-hill level)	CS-A1 70.2 CS-A2 77.1	CS-B1 59.4[3] CS-B2 44.6[3]	–

[1] Range: minimum and maximum observed value (hill level) in the intervention region.

[2] Range: minimum and maximum coverage (sub-hill level) observed in the parasitological survey areas.

[3] Assuming the same utilization rate as in Intervention Region 1 (Van Bortel *et al.* 1996).

supervision of the NMCP-Burundi. The bednets from Thailand were made of nylon, whereas the nets sewn in Burundi were made of a locally produced tissue composed of 2/3 nylon and 1/3 cotton. Only white nets of 1.2 m × 1.5 m × 1.8 m (width × height × length) were sold. The cost of the locally produced unimpregnated bednets was twice that of the imported ones. The NMCP-Burundi intended to ensure the availability of bednets on a long-term basis through promotion of the local nets. The impregnated bednets were sold at a promotion price of US$2 or 1% of the annual income of a family in this district. The selling price covered 41% of the cost of an imported net and 21% of the cost of a local net.

Health care facilities, schools and local administration were called on to promote the bednets as a tool for malaria control and to convince families to buy at least one. Staff of the health care facilities were responsible for health education to improve perceptions of the causes of malaria. The NMCP-Burundi provided the essential educational tools. Meetings were held in schools to explain to parents and children the usefulness of bednets in preventing malaria and to show how to install the nets. These meetings started 2 months before the distribution and were held daily in different parts of the district. The final decision for buying a net was up to householders. Special daily meetings were held to sell the bednets. All sub-hills were visited at least 3 times to give all the families the opportunity to buy impregnated nets.

The bednets were impregnated with deltamethrin EC 2.5%, 25 mg a.i./m^2 by the staff of the NMCP-Burundi using the method described in Coosemans and Carnevale (1995). The nets which were to be sold in the first intervention zone were treated in August 1993. Due to political problems distribution did not start until January 1994. As light, mainly ultraviolet, may affect the longevity of synthetic pyrethroids (Barlow *et al.* 1977), bednets were packed in plastic bags and stored in the dark until distribution. The staff of the NMCP-Burundi impregnated the nets for the second intervention (Region 2)

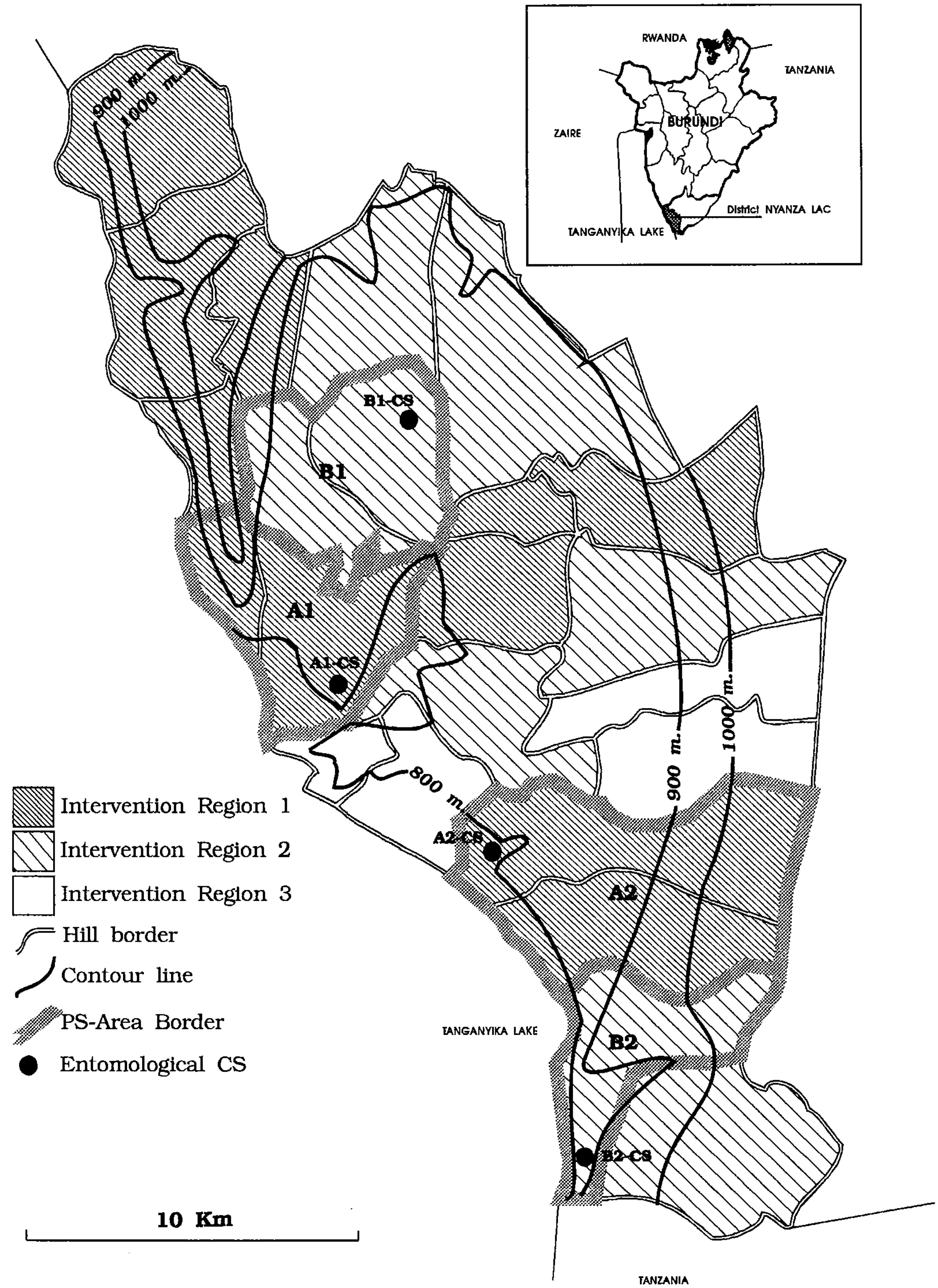

Figure 1 Map of Nyanza Lac district indicating the 3 intervention regions, the 4 parasitological survey areas (PSA) A1, A2, B1 and B2, and the 4 entomological capture sites (CS) A1-CS, A2-CS, B1-CS and B2-CS.

in August 1994 and distributed them from September 1994. In July and August 1994 70% of the bednets in Region 1 were reimpregnated free of charge in close collaboration with inhabitants and local authorities.

The overall estimate of the coverage rate, defined as the proportion of families with at least one installed bednet, was 43% in Region 1 and 36% in Region 2, but a significant difference in purchase and use of bednets, mainly related to the mosquito nuisance level, was noticed between sub-hills (Table 1) (Van Bortel *et al.* 1996).

Entomological survey

From November 1992 onwards a bimonthly human bait collection (HBC) was begun in 2 sub-hills each of Intervention Region 1 (A1-CS and A2-CS) and 2 (B1-CS and B2-CS) (Figure 1). The last HBC took place in September 1994. No collection was carried out in November 1993 and a total of 11 surveys were conducted.

A1-Capture Site (A1-CS; sub-hill Gasange; altitude 800 m) is located near the river Rwaba which forms a large depression. Rice is cultivated far away from this capture site. A2-Capture Site (A2-CS; sub-hill Mugere), which is surrounded by rice fields is situated in a depression near Lake Tanganyika at an altitude of 780 m. Mosquito collections in Intervention Region 2 were carried out in sub-hills Kazirabageni and Gisenga. Kazirabageni (B1-CS) is situated 500 m from the river Rwaba, at an altitude of 860 m. Banana trees grow between the river and the houses. Gisenga (B2-CS) is flanked by mountains and situated near the lake at an altitude of 820 m.

The HBC were organized inside and outside three fixed houses in each collection site. In November 1992, one HBC (one night; inside and outside the 3 houses) was performed in each collection site, but from January 1993 onward, two HBC with 3-day intervals were carried out every 2 months in all capture sites. On the night of the HBC one team of collectors from the villages worked from 1800 to 2400 h and another team from 2400–0600 h. Collectors remained under medical supervision and were rotated between surveys.

Collected mosquitoes were kept in a refrigerated box during the night. The following morning they were morphologically identified in the field laboratory. *Anopheles gambiae sensu lato* and *A. funestus* were dissected for parity and sporozoite determination. Salivary gland dissection was carried out on the mosquitoes from the HBC of November 1992, January and March 1993. From May 1993 an ELISA test for the detection of the *Plasmodium falciparum* circumsporozoite antigen was performed on the head and thorax which were kept on silica gel until testing (kit supplied by Dr R. Wirtz of the Walter Reed Army Institute of Research, USA) (Burkot *et al.* 1984).

Parasitological survey

The surveys were carried out at 2-monthly intervals from November 1992 until March 1995 in 4 parasitological survey areas (PSA). Two PSA are situated in Intervention Region 1 (PSA A1 and A2) and two in Region 2 (PSA B1 and B2) (Figure 1). The survey planned for November 1993 was not carried out and in total 14 surveys were conducted. Parasitological surveys were planned after entomological surveys.

Thick and thin blood films were analysed from repeated cross-sectional surveys among children 0–9 years old. Each child in the study area was invited and eligible for inclusion at every survey. An average of 198 ± 81 children under 5 years (an average of 15% of this age class in each survey area) and 148 ± 59 children between 5 and 9 years (an average of 13% of the age class in each survey area) participated in every survey. Blood films were stained with Giemsa and 200 microscopic fields of each thick film were examined under immersion oil (× 100 ocular lens 10). The parasitological index (PI) and the proportion of high parasitaemia (PHP) were calculated. Parasitaemia was considered high when the 200 examined microscopic fields were positive for at least one asexual *P. falciparum* form, corresponding to more than 2000 trophozoites/µl (Coosemans *et al.* 1995). Thin films were examined to specify the *Plasmodium* species.

Evaluation

The baseline period from November 1992 until September 1993 covers 6 surveys. Three periods, each covering 7 months (4 surveys), were considered to assess the impact of impregnated nets in the four study areas:

(1) Period 1, from January 1993 to July 1993, covered the pre-intervention phase.
(2) Period 2, from January 1994 to July 1994, followed bednet intervention in Region 1.
(3) Period 3, from September 1994 to March 1995, followed bednet intervention in Region 2 and reimpregnation of bednets in Region 1.

To compare entomological (parity rates) and parasitological parameters (PI, PHP) of the pre-intervention period (Period 1) with those of the intervention periods (Periods 2 and 3), a summary risk ratio (intervention/pre-intervention) with 95% confidence interval (95% CI) was computed using stratified Mantel–Haenszel analysis.

Results

Entomological survey

Baseline period: November 1992–September 1993

Anophelines caught by the human bait collections inside (IN) and outside (OUT) the houses consisted mostly of *A. gambiae sensu lato* (68%) and *A. funestus* (23%). Of the *A. gambiae* complex, only *A. gambiae sensu stricto* was found in this part of the Imbo valley (Smits *et al.* 1996). The remaining 9% of collected *Anopheles* mosquitoes belonged to the species *A. ziemani*, *A. marshallii*, *A. squamosus*, *A. maculipalpis* and *A. pharoensis*.

Inside the houses 59.6% of *A. gambiae* (n=9379) and 67.2% of *A. funestus* (n=2899) were caught. No seasonal variation of the endophagic rate (indoor density/(indoor+outdoor density)) was observed for *A. gambiae* (Pearson's χ^2, 5 d.f., P=0.66). *Anopheles funestus* was slightly more endophagic (71.7%) during the dry season from June to September than in the rainy months (60.9%) (Pearson's χ^2, 5 d.f., P<0.001).

Important differences in mosquito night bite densities were observed between capture sites and seasons (Table 2). Man-biting densities were very low in the collection site situated at the highest altitude (B1-CS; 860 m), whereas they were very high in A2-CS, which is surrounded by rice fields. In this capture site a maximum of 295 *A. gambiae* bites/person/night (B/P/N) inside the houses was reached during the rainy months January–March.

The sporozoite rates were similar for Anophelines caught inside and outside the houses (*A. gambiae*, IN: 58/1495, OUT: 20/922; *A. funestus*, IN: 6/925, OUT: 12/394) but a stratified analysis by survey was not possible due to the low frequency of positive Anophelines. The sporozoite rate depends mainly on the survival rate of the vector population, the last parameter being estimated by parity rate. Parity rates for both vector species were not significantly different between the HBC inside and outside the houses (Mantel–Haenszel χ^2 stratified by collection; P>0.17 for each capture site). The Mantel–Haenszel χ^2 was not valid for comparison of *A. funestus* at B1-CS. We can thus reasonably estimate the sporozoite rate for the indoor biting population by combining the positive mosquitoes of the indoor and outdoor catches, in order to achieve a more reliable estimate of the sporozoite rate (Table 2).

Malaria transmission in this region is high and perennial. In A2-CS, the daily entomological inoculation rate of *P. falciparum* (B/N/P × sporozoite rate; *A. gambiae* and *A. funestus* combined) of an unprotected person spending the night inside the house was more than one infective bite per person per night throughout the year (Table 2). The transmissions in A1-CS and in B2-CS were lower than in A2-CS, but infective bites also occurred during the dry season (July–September). Only in B1-CS were no infected mosquitoes found during the rainy months from January until May.

Intervention of impregnated bednets

After introduction of bednets (comparing Period 1 with Period 2), declines of 50 and of 75% of the *A. gambiae* man-biting density were observed at CS-A1 and CS-A2 respectively. During the second period a reduction of 11% in the *A. gambiae* man-biting rate was observed in the control collection sites (B1-CS, B2-CS). The introduction of impregnated bednets had no consistent effect on the *A. funestus* man-biting density in the intervention collection sites, whereas an important reduction was noted at the B2-CS site (Table 2).

Parity rates of both vector species decreased significantly in A2-CS (Mantel–Haenszel χ^2 stratified by collection, P<0.01) after intervention, with

Table 2 Calculation of the average daily entomological inoculation rate of *Plasmodium falciparum* (He) by *Anopheles gambiae* and *A. funestus* in unprotected persons inside houses before and after the introduction of impregnated bednets

			Anopheles gambiae		*Anopheles funestus*		
Capture site	Period	*n*	B/P/N	SI (*n*)	B/P/N	SI (*n*)	He
A1-CS	11 1992	1	11.0	0.147 (34)*	5.0	0/3*	1.62
	01–03 1993	4	28.3	0.017 (181)*	15.0	0.023 (130)*	0.82
	05 1993	2	15.2	0.030 (100)	17.0	0.030 (67)	0.96
	07–09 1993	4	1.4	0.067 (30)	1.3	0.000 (33)	0.09
	11 1993	0	not done				
	01–04 1994	*4*	*11.6*	*0.019 (321)*	*8.6*	*0.006 (179)*	*0.26*
	05 1994	*2*	*12.0*	*0.051 (118)*	*10.7*	*0.022 (90)*	*0.85*
	07–09 1994	*4*	*0.7*	*0.000 (14)*	*0.1*	*0/2*	*0.00*
A2-CS	11 1992	1	57.7	0.055 (91)*	15.7	0/11*	3.17
	01–03 1993	4	294.6	0.005 (400)*	26.2	0/5*	1.47
	05 1993	2	91.7	0.006 (474)	91.2	0.009 (337)	1.39
	07–09 1993	4	18.9	0.049 (225)	19.8	0.013 (156)	1.18
	11 1993	0	not done				
	01–04 1994	*4*	*76.7*	*0.002 (1635)*	*39.3*	*0.005 (609)*	*0.38*
	05 1994	*2*	*24.8*	*0.000 (237)*	*66.2*	*0.002 (525)*	*0.13*
	07–09 1994	*4*	*1.4*	*0.000 (39)*	*0.4*	*0/9*	*0.00*
B1-CS	11 1992	1	1.7	1/8*	0.7	0/3*	0.21
	01–03 1993	4	6.0	0.000 (56)*	0.3	0/5*	0.00
	05 1993	2	4.2	0.000 (48)	0.0	0/4	0.00
	07–09 1993	4	0.0	–	0.5	1/12	0.04
	11 1993	0	not done				
	01–04 1994	4	8.0	0.024 (208)	0.8	0.032 (31)	0.22
	05 1994	2	2.0	0.065 (31)	1.8	0.000 (21)	0.13
	07–09 1994	4	0.1	0/1	0.3	0/5	0.00
B2-CS	11 1992	1	9.3	0.177 (17)*	0.0	–	1.65
	01–03 1993	4	5.8	0.019 (104)*	10.8	0.032 (155)*	0.46
	05 1993	2	26.2	0.012 (166)	50.3	0.024 (335)	1.52
	07–09 1993	4	0.1	0/1	0.3	1/4	0.08
	11 1993	0	not done				
	01–04 1994	4	17.1	0.028 (215)	1.8	0.042 (24)	0.47
	05 1994	2	1.8	0.067 (15)	0.2	0/1	0.12
	07–09 1994	4	0.1	0/1	0.0	–	0.00

n, number of night collections in 3 fixed stations; B/P/N, number of bites/person/night; SI, circumsporozoite antigen index and the number (*n*) of mosquitoes tested. Inside and outside catches combined; He, number of infected bites/person/day.
*Detection of sporozoites by dissection of salivary glands.
Italic, bednet intervention started in January 1994; bold, bednet intervention started in September 1994.

summary parity rates ratios (Period 2/Period 1) of 0.56 (95% CI 0.50–0.63) for *A. gambiae* and of 0.67 (95% CI 0.64–0.79) for *A. funestus*. In A1-CS, the parity rates ratio was not significantly different from one for either vector species (Mantel–Haenszel χ^2, $P>0.25$). In both non-intervention sites (B1-CS, B2-CS) a significant increase in the parity rate of *A. gambiae* was observed during Period 2 (Mantel–Haenszel χ^2, $P<0.01$). The ratio was 2.48 (95% CI 1.98–3.12) in B1-CS and 1.64 (95% CI 1.27–2.12) in B2-CS. The sample size of *A. funestus* was too small for this analysis.

A comparable trend was observed for sporozoite indexes in different collection sites (Table 2). After the introduction of impregnated bednets a decrease of 86% in the inoculation rate occurred in A2-CS with an estimated number of 370 positive bites from January to September 1993 (before intervention). A reduction of 50% was observed in A1-CS where the estimated number of positive bites before the intervention reached 165. In B1-CS and B2-CS, no positive inoculation was detected after the intervention; however, observations took place only during the months with the lowest vector densities.

A slight but significant decrease in the endophagic rate of *A. gambiae* was observed after introduction of bednets, from 59.6 to 53.2% (odds ratio 1.30 (95% CI 1.19–1.42), $P<0.001$), whereas *A. funestus* showed no change in behaviour.

Parasitological survey

Baseline period: November 1992–September 1993

Plasmodium falciparum is the most frequent malaria parasite in this region (98% of the positive slides, 81% in non-mixed infections, and 17% in combination with *P. malariae* or *P. ovale* or both). Figure 2a and b shows the evolution of the PI and the PHP by survey observed in the 4 survey areas. Almost no seasonal variation occurred during this baseline period.

No significant difference was found between B2 and A2 or between B2 and A1 for the 2 parasitological parameters (Mantel–Haenszel χ^2 stratified by survey, $P>0.09$ for both age groups; except for the comparison B2/A2 of the PHP among children under 5, $P=0.047$). Both PI and PHP were significantly lower (Mantel–Haenszel χ^2 stratified by survey, $P<0.01$ for both age groups) in PSA B1 than in PSAs A1 and A2 (except for comparison B1/A1 of the PHP in the age group 5–9 years, where no significant difference was observed).

Intervention with impregnated bednets

In PSAs A1 and A2, a reduction of the PI was observed between the reference Period 1 (January–July 1993) and the post-intervention Period 2 (January–July 1994). This decline of at least 23% is significant for both age groups (Table 3, Figure 3). In PSA B1, without intervention after Period 1, no significant change was noted in the PI between Periods 1 and 2 in the 2 age groups. In PSA B2, without bednet intervention in Period 2, a low but significant reduction of 8% was observed in children below 5 years.

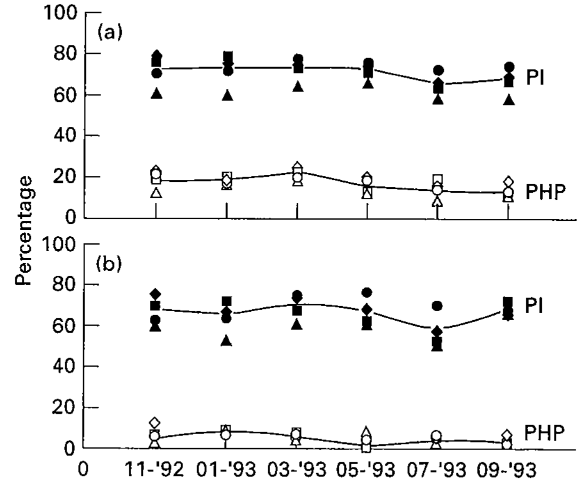

Figure 2 Evolution of the parasitological index (PI, grey symbols) and the proportion of high parasitaemia (PHP, white symbols) by survey observed in the 4 parasitological survey areas (PSAs). The solid lines give the evolution of the weighted means of the PI or the PHP of the 4 PSAs (□, A1; ◇, A2; △, B1; ○, B2). The weights are inversely proportional to the variance of the observed PI or PHP value in the PSA. a, Children under 5 (mean number of children per survey is 253 ± 73); b, children between 5 and 9 years (mean number of children per survey is 179 ± 67).

The prevalence of high parasitaemia in children under 5 years was significantly reduced by 42% in A1 and by 53% in A2 (Table 3). A similar decrease was observed in the older age group (5–9 years) but this was significant only in A2. No significant change in risk ratio (RR >0.89) of PHP was observed during the control period (Period 2) in areas B1 and B2.

The impact of the bednets in the B areas was measured by comparing Periods 1 and 3. These 2 periods do not cover the same period of the year but since no seasonal variation was observed during the period November 1992 until September 1993 (test of homogeneity of PI and PHP, $P>0.05$ for both age classes) the comparison was viable. After intervention, the parasitological index was reduced by almost 33% in both age classes (Table 3). The risk ratio of the PHP was significantly different from 1 for the children under 5 years (reduction of more

Table 3 Seven-months comparison between intervention and pre-intervention periods (Period 2/1 and 3/1) of the parasitological index (PI) and the proportion of high parasitaemia (PHP). Summary risk ratio (RR) for the different parasitological survey areas (PSA) with 95% confidence interval (95% CI)

			PI			PHP		
Period[1]	PSA	Age (years)	mean[2] % Period 1	RR[3]	(95% CI)	mean[2] % Period 1	RR[3]	(95% CI)
2/1	A1	0–4	74.3	0.77	(0.71–0.84)**	18.0	0.58	(0.44–0.77)**
		5–9	67.0	0.76	(0.68–0.85)**	1.9	0.55	(0.29–1.05)
	A2	0–4	72.7	0.75	(0.70–0.81)**	19.1	0.47	(0.37–0.59)**
		5–9	66.5	0.77	(0.71–0.84)**	6.2	0.58	(0.37–0.91)*
	B1	0–4	61.9	0.95	(0.86–1.05)	12.7	1.16	(0.86–1.55)
		5–9	55.7	0.99	(0.88–1.11)	4.4	0.89	(0.49–1.60)
	B2	0–4	74.4	0.92	(0.87–0.98)**	17.1	0.90	(0.72–1.12)
		5–9	71.9	0.97	(0.89–1.05)	5.7	0.92	(0.54–1.56)
3/1	B1	0–4	61.9	0.67	(0.59–0.75)**	12.7	0.37	(0.24–0.56)**
		5–9	55.7	0.68	(0.60–0.78)**	4.4	Sample size too small	
	B2	0–4	74.4	0.69	(0.63–0.75)**	17.1	0.58	(0.45–0.76)**
		5–9	71.9	0.67	(0.59–0.75)**	5.7	0.54	(0.29–1.01)

[1] Comparison of periods: 2/1 Period 2 over Period 1; 3/1 Period 3 over Period 1. Period 1 (January–July 1993) pre-intervention period; Period 2 (January–July 1994) start of bednet distribution in Intervention Region 1; Period 3 (September 1994–March 1995) start of bednet distribution in Intervention Region 2. The mean number of children per survey in the age class 0–4 years is 185 ± 78 and in the age class 5–9 years 137 ± 52.
[2] Weighted mean: the weights are inversely proportional to the variance of the observed PI or PHP value of each survey.
[3] Significance test of summary risk ratio: Mantel–Haenszel summary χ^2 stratified by survey.
*$P<0.05$; **$P<0.01$.

than 40%). In the older age group (5–9 years) the 40% reduction was not significant, mainly due to the low frequency of high parasitaemia during the intervention period in this age group. During Period 3 the lower levels of the PI and the PHP in PSAs A1 and A2 were maintained and even reinforced (Figure 3).

Discussion

A high coverage of impregnated and installed bednets is most desirable to achieve mass killing of the vector population. This would result in a reduction in the inoculation rate of malaria, whereby unprotected persons will benefit from the wide use of individual protection. In experimental trials the coverage often exceeded 90%. In these circumstances, transmission was often reduced by 90% or more and malaria-related morbidity was reduced by 60% (Carnevale & Coosemans 1995).

In our study we used a coverage rate expressed by the percentage of families having purchased and properly set up at least one impregnated bednet (Van Bortel *et al.* 1996). A coverage of 70% was obtained in Gasange (A1-CS) and of 77% in Mugere (A2-CS). No substantial reduction of vector density was observed after the introduction of impregnated bednets. However, the survival rate decreased significantly in one collection site. The entomological inoculation rate was reduced by 86% in A2-CS where the highest transmission was noticed before intervention (an estimation of 370 positive bites

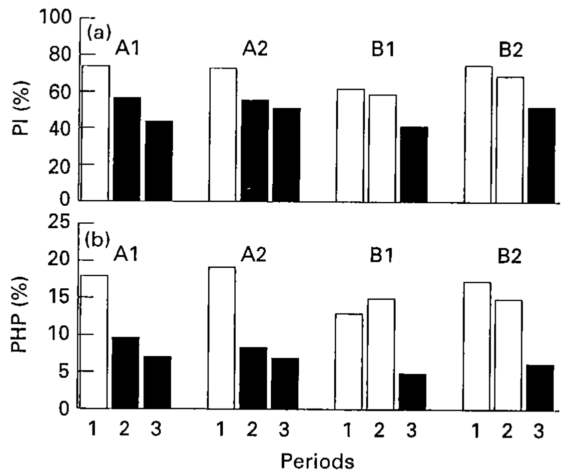

Figure 3 Evolution of a, the parasitological index (PI) and b, the proportion of high parasitaemia (PHP) for children under 5 years of age in the 4 parasitological survey areas A1, A2, B1 and B2. □, Pre-intervention; ■, intervention. Period 1: 01–07 1993; Period 2: 01–07 1994; Period 3: 09 1994–03 1995. The mean number of children per survey is 185 ± 78.

from January to September 1993). In A1-CS with a slightly lower coverage and an initial lower transmission (165 positive bites from January to September 1993) a reduction of only 50% of the entomological inoculation rate was estimated during intervention.

In Tanzania and Burkina Faso (Curtis *et al.* 1991) the survival and sporozoite index of the vector population decreased greatly, attributed to the killing of large numbers of mosquitoes, but there was little to suggest that the use of impregnated bednets reduced the survival of the mosquito population in the Gambian trial (Lindsay *et al.* 1993). This difference, observed between the trials, is probably due not only to the difference in study design (Rozendaal 1989) but also to the initial composition of the vector population and its behaviour and to the use of bednets by the population. The different impacts observed in CS-A1 and CS-A2 can be explained by the motivation to use the bednets correctly, which is related to the importance of the nuisance level in A2-CS (Van Bortel *et al.* 1996).

Thomson *et al.* (1995) found no effect on the sporozoite rate in a village where only 47% of the beds had treated nets. The lowest coverage observed in one of the sub-hills of Nyanza Lac district was below 2%; in this sub-hill no effect of bednets on malaria vector population was assumed and so any impact of the impregnated bednets on malaria morbidity at community level would be unlikely. Indeed, the entomological data can provide information on the impact of bednets at community level (mass-effect or impact on unprotected persons) whereas the parasitological data give information of the impact at community and individual level. However, the entomological follow-up gives only an idea of the mass-effect in one particular site, whereas the parasitological follow-up provides information of a larger area.

Despite the important differences of inoculation observed between collection sites and surveys, the variation of PI and the PHP was small, which justifies the comparison of different periods in the same PSA. After introduction of bednets reductions of 42% (PSA A1) and 53% (PSA A2) in the prevalence of high parasitaemia among children below 5 years was observed. Bednet coverage in the former was 55% and in the latter 44%. During the same period no reduction was noted in PSA B1 and B2. In the same way, treated bednets in Region 2 led to a significant reduction of the PHP (63% in PSA B1, coverage 51%; 42% in PSA B2, coverage 29%). These reductions indicate a significant decline in malaria morbidity due to the introduction of bednets. While asymptomatic carriers of malaria parasites are frequent in endemic areas, a correlation exists between clinical manifestation of malaria and parasite densities in the blood. Delacollette and Van der Stuyft (1993) found a remarkable association between the malaria morbidity incidence rate and the high parasitaemia incidence rate (HPIR) in the same region: 96% of the variability of the malaria morbidity was explained by the HPIR. The HPIR was obtained from the daily parasitological incidence rate and the proportion of high parasitaemia (PHP). Likewise, Coosemans *et al.* (1995) proposed the PHP as a simple and reliable indicator of relative changes in malaria morbidity that can be used for the evaluation of malaria control programmes.

A significant reduction of the PHP in children between 5 and 9 years old was seen only in PSA A2. This decrease of the PHP (RR=0.58) was similar to that observed in younger children (RR=0.47). The reductions in the other PSA were not significant due to the rather low frequency of high parasitaemia in this age group through acquired immunity. Malaria cases are also almost absent from this age group. The clinical spectrum of the disease and age-specific malaria admission differed between regions with different levels of transmission (Snow *et al.* 1994). The question arises whether sustained implementation of this vector control method in a high malaria transmission region might lead to a shift of the problems from younger to older children (Lines & Armstrong 1992). After one year of intervention we observed a similar reduction of the PHP in older and in younger children. This seems to contradict the formulation of the problem, but these children grow up in the absence of vector control and the impact of intervention is likely to be marked (Lines & Armstrong 1992). The evaluation of sustained vector control programmes has to point out the possible shifting of the clinical spectrum and of the age-specific malaria admission to assess its long-term benefit. However, this evaluation has mainly to be directed towards children grown up under a vector control programme.

Despite the unequal coverage (Table 1; Van Bortel *et al.* 1996), intervention with treated bednets in the Nyanza Lac district resulted in a significant reduction in the estimated malaria morbidity at community level and, to a lesser extent, in the prevalence of infection. Apart from the coverage, other operational aspects have to be considered (e.g. reimpregnation, replacement of torn bednets, daily use) which can interfere with the positive impact on malaria morbidity. Careful follow-up of these aspects is required to obtain a tool that can be applied for sustained vector control. To reinforce the effectiveness of vector control measures, additional efforts have to be implemented, especially easy access to antimalarials.

Acknowledgements

We are grateful to the authorities of the Ministry of Public Health of Burundi and the authorities of the Nyanza Lac district for their collaboration. The ELISA kits were provided by Dr R. Wirtz of the Walter Reed Institute, Washington. We acknowledge SKB as source of the positive control/capture antigens; the laboratory of New York University produced the Mab cell line. This study used reagents produced with support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Disease. This work was supported by the Belgian Development Co-operation (AGCD), the Vlaamse Vereeniging voor Ontwikkelingssamenwerking en Technische Bijstand (VVOTB), and the Special Programme of Research and Training in Tropical Diseases (TDR, World Bank, UNDP, WHO, ID 910447). Data analysis was supported by a grant of Compagnie Maritime Belge, Société Générale de Banque, Union Minière, Cimenteries CBR and Tractebel.

References

Barlow F, Hadaway AB, Flower LS, Grose J & Turner C (1977) Some laboratory investigations relevant to the possible use of new pyrethroids in control of mosquitoes and tsetse flies. *Pesticide Science* 8, 291–300.

Brinkmann U & Brinkmann A (1995) Economic aspects of the use of impregnated mosquito nets for malaria control. *Bulletin of the World Health Organization* 73, 651–658.

Burkot TR, Williams JL & Schnieder I (1984) Identification of *Plasmodium falciparum* infected mosquitoes by a double anti-body enzyme-linked immunosorbent assay. *American Journal of Tropical Medicine and Hygiene* 33, 783–788.

Carnevale P & Coosemans M (1995) Some operational aspects of the use of personal protection methods against malaria at individual and community level. *Annales de la Société belge de Médecine tropicale* 75, 81–103.

Coosemans M (1989) Recherche épidémiologique dans un foyer de paludisme peu stable en Afrique Centrale. Prospection géographique dans la région naturelle de l'Imbo (République du Burundi). *Mémoires, Académie Royale des Sciences d'Outre-Mer Classe des Sciences Naturelles et Médicales* 22 (3), 1–62.

Coosemans M & Carnevale P (1995) Malaria vector control: a critical review on chemical methods and insecticides. *Annales de la Société belge de Médecine tropicale* 75, 13–31.

Coosemans M, Van der Stuyft P & Delacollette C (1995) A hundred per cent of fields positive in a thick film: a useful indicator of relative changes in morbidity in area with seasonal malaria. *Annals of Tropical Medicine and Parasitology* 89, 581–586.

Curtis CF, Lines JD, Carnevale P, Robert V, Boudin C, Halna JM *et al.* (1991) Impregnated bednets and curtains against malaria mosquitoes. In *Control of disease vectors* *in the community* (ed CF Curtis), Wolfe/CRC Press, London, pp 5–46.

D'Alessandro U, Olaleye BO, McGuire W, Langerock P, Bennett S, Aikins K *et al.* (1995) Mortality and morbidity from malaria in Gambian children after introduction of an impregnated bednet programme. *Lancet* 345, 479–483.

Delacollette C & Barutwanayo M (1993) Mortalité et morbidité aux jeunes âges dans une région à paludisme hyperendémique stable, commune de Nyanza-Lac, Imbo Sud, Burundi. *Bulletin de la Société de Pathologie Exotique* 86, 373–379.

Delacollette C & Van der Stuyft P (1993) High parasitaemia incidence rates can be used to estimate malaria morbidity rates. *Annals of Tropical Medicine and Parasitology* 87, 537–539.

Lindsay SW, Alonso PL, Armstrong Schellenberg JRM, Hemingway J, Adiamah JH, Shenton FC *et al.* (1993) A malaria control trial using insecticide-treated bednets and targeted chemoprophylaxis in a rural area of The Gambia, West Africa. 7. Impact of permethrin-impregnated bednets on malaria vectors. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 87, Suppl. 2, 45–51.

Lines J & Armstrong JRM (1992) For a few parasites more: inoculum size, vector control and strain-specific immunity to malaria. *Parasitology Today* 8, 381–383.

Rozendaal JA (1989) Impregnated mosquito nets and curtains for self-protection and vector control. *Tropical Diseases Bulletin* 86, R1–R41.

Smits A, Coosemans M, Van Bortel W, Barutwanayo M & Delacollette C (1995) Readjustment of the malaria vector control strategy in the Rusizi Valley, Burundi. *Bulletin of Entomological Research* 85, 541–548.

Smits A, Roelants P, Van Bortel W & Coosemans M (1996) Enzyme polymorphism in the *Anopheles gambiae* Complex (Diptera: Culicidae) related to feeding and resting behaviour in the Imbo Valley, Burundi. *Journal of Medical Entomology*, 33, 545–553.

Snow RW, Bastos de Azevedo I, Lowe BS, Kabiru EW, Nevill CG, Mwankusye S *et al.* (1994) Severe childhood malaria in two areas of markedly different falciparum transmission in East Africa. *Acta Tropica* 57, 289–300.

Snow RW, Rowan KM & Greenwood BM (1987) A trial of permethrin-treated bednets in the prevention of malaria in Gambian children. *Transactions of the Royal Society of Tropical Medicine and Hygiene* 81, 563–567.

Thomson MC, Adiamah JH, Connor SJ, Jawara M, Bennett S, D'Alessandro U *et al.* (1995) Entomological evaluation of The Gambia's National Impregnated Bednet Programme. *Annals of Tropical Medicine and Parasitology* 89, 229–241.

Van Bortel W, Barutwanayo M, Delacollette C & Coosemans M (1996) Motivation à l'acquisition et à l'utilisation des moustiquaires imprégnées dans une zone à paludisme stable au Burundi. *Tropical Medicine and International Health* 1, 71–80.