Readjustment of the malaria vector control strategy in the Rusizi Valley, Burundi

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Abstract

Based on a longitudinal survey performed in 1982-1983, a vector control strategy was implemented from 1985 onwards in a malarial-dense area of Burundi. One annual round of indoor spraying with malathion greatly reduced both the parasite load and the parasite rate in the population until 1989. However, from 1990 to 1993, a progressive resurgence of malaria was observed in most villages. For the present study, two villages were selected on the basis of their differential response to house spraying. In the village of Mulira surrounded by rice fields, the excellent results observed in the past have been followed by recent increases in parasite rates. In the village of Murengeza, also located in the rice growing area but near a river, the spraying had less impact. The inoculation rate was found to be similar in both villages, but the transmission peak occurred at the end of April in Mulira, and two months earlier in Murengeza. Indoor spraying with lambda-cyhalothrin was carried out on 26 April 1993, one month too late according to the strategy intended. As no sporozoite mosquitoes were observed during the six months following spraying, this strategy should be maintained but, in villages near rivers, the application should commence much earlier, in mid-January. *Anopheles gambiae* Giles *sensu stricto* (Diptera: Culicidae) and *A. funestus* Giles were found to be very endophilic species, whereas the dominant *A. arabiensis* Patton was highly exophilic. Therefore it is recommended that treatments should not only be applied to human dwellings but also to other structures such as animal sheds, kitchens, etc, shown by earlier studies to be resting sites of *A. arabiensis*. This study underlines the need for regular reassessment in vector control programmes.

Introduction

One of the limitations of disease control programmes, and particularly those involving vector control, is that once the process of activities is streamlined, there may be some slackening in supervision and evaluation and, in particular spraying may not be completed on time. The vector control strategy, as a component of malaria control, should not be a static process. Based on the local situation, once a control strategy has been outlined, the output of such activities should be monitored regularly to improve upon or to adapt the stategy according to the conditions and situation present at the time.

In Burundi, a vector control strategy was developed for the Rusizi Valley, an area where malaria is endemic in the lowlands of the country (Coosemans & Barutwanayo, 1989). From a longitudinal survey undertaken in 1982-1983 (Coosemans, 1985), it was expected that one annual round of indoor-spraying with a residual insecticide at the beginning of April, just before the peak of transmission at the end

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of the rainy season, would reduce the parasite incidence and heavy parasitaemia. Indeed, a major decrease of the parasite rate and the parasite load, an indicator of malaria mobidity (Delacollette & Van der Stuyft, 1993; Coosemans *et al.*, 1994), was observed in the whole area from 1985 until 1989 (Coosemans & Barutwanayo, 1989; Barutwanayo *et al.*, 1991). However, this initial advantage levelled off and a progressive increase in malaria cases was observed in most villages during 1990-1993. Only in a few villages was there a slight reduction in malaria since the beginning of the programme.

In the present study we have investigated the transmission dynamics in two selected villages, before and after the annual treatment, in order to find an explanation for the recent increase in malaria and why some villages had never responded adequately to indoor sprays. Readjustment of the developed strategy will then be proposed in order to remedy these situations.

Materials and methods

Study area and vector control activities

The Rusizi Valley is a lowland area belonging to the northern part of the Tanganyika Graben situated between Lake Tanganyika (altitude 750 m) and the border with Rwanda (altitude 900 m). The valley is divided into two parts, Central Imbo and North Imbo. The annual rainy season starts in September and extends until May, with an average rainfall of about 700 mm/year. Before intervention began systematically in 1985, much variation in the prevalence rates of malaria was observed throughout the Valley. The highest level of endemicity was encountered in the rice-growing area, where the irrigation system supported high densities of vectors (Coosemans, 1985). Village-scale trials with one round of indoor spraying started in April 1985. For this purpose malathion (2 g a.i./m^2 wettable powder) was selected as the residual insecticide of choice because it has a relatively short residual activity of 3 months, which was considered sufficient to interrupt the peak transmission and, a shorter residual effect was preferred over a longer one in order to delay the appearance of resistance, by exerting minimal insecticide selection pressure on the anopheline populations. Encouraging results made it worthwhile to expand the sprayed area. Total coverage was achieved in 1989 for the Central Imbo (80,000 inhabitants) and in 1993 for all the Rusizi valley (160,000 inhabitants). In 1993, the staff of the malaria control programme decided to use an insecticide with a high residual activity, namely lambda cyhalothrin, in order to try to contain the malaria increase in the study area which had been noted in 1990-1992.

A previous study has shown that the months of January-February was the period with the highest vector density of the year, but at the same time the period with the lowest transmission, as was suggested by a low parasite incidence rate and a very low seroconversion rate (Coosemans, 1985). For this reason we decided to start the present survey in February 1994.

Study villages

Two villages were selected for the present study in the Central Imbo (fig. 1); these were chosen on the basis of their differential response to house-spraying with residual insecticide. In Mulira village (480 lots), after earlier success, an increase in the parasite rate and load had been observed since 1989. In Murengeza village (392 lots), the parasite rate had remained relatively high despite the annual malathion treatment applied since 1989. Both villages were treated with lambda-cyhalothrin (0.030 g a.i./m^2-100 g/kg WP Zeneca) on 26 April 1993.

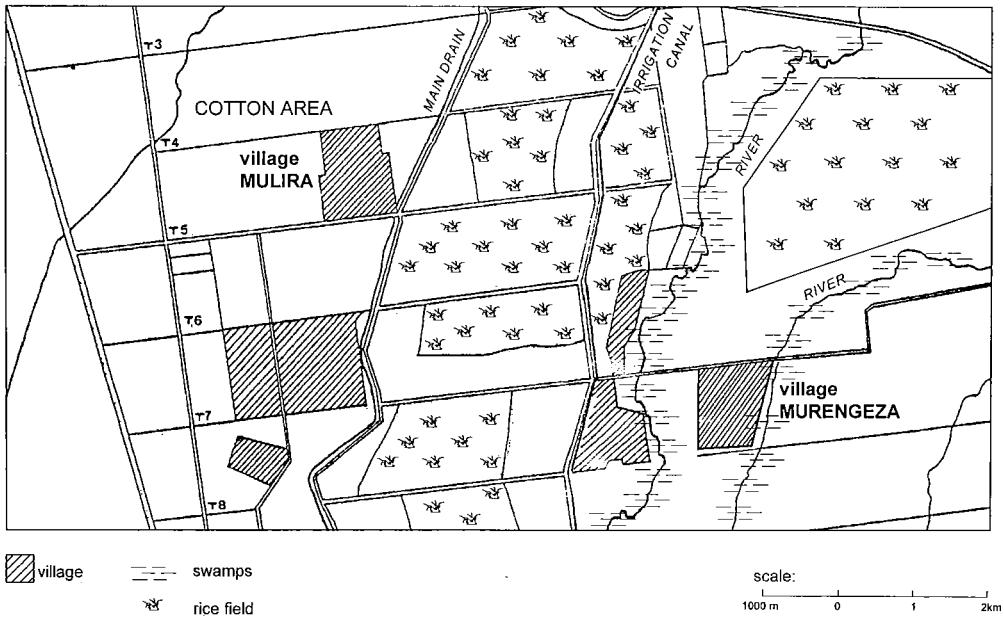

Fig. 1. Map of the area and the environment surrounding the two villages that were selected for the present study.

Human bait collections

Mosquitoes were collected fortnightly in the two villages from February until October 1993 (except that no catches could be organized in July and August). Five fixed stations were chosen in each village for night-biting collections (NBC), with 5 man-nights indoors (NBC IN) and 5 man-nights outdoors (NBC OUT). Two collectors made each man-night collection: one from 18.00 h to midnight and the second from midnight until 06.00 h. Collectors were rotated between different times and collection sites. The use of the night biting method was considered ethically acceptable because the collectors lived in these villages and remained under medical supervision.

Indoor-resting collections

Every night-catch was followed the next morning by pyrethrum spray collections (PSC) in five houses in the neighbourhood of the fixed stations.

Examination of the collected material

Night-biting mosquitoes were transported to the laboratory alive, in a refrigerated box, for morphological identification. Only *Anopheles gambiae* Giles *sensu lato* and *A. funestus* Giles were retained for this study. *Anopheles gambiae sensu lato* from one of the five houses were deep frozen in liquid nitrogen for isoenzyme analysis. The head and thorax of the remaining anophelines were kept on silica gel for later circumsporozoite antigen (CS) detection by an enzyme linked immunosorbent assay kit (supplied by Dr Wirtz, Walter Reed Army Institute of Research, USA) (Burkot *et al.*, 1984). Abdomens of unfed females were dissected the same day for parity determination (4479 specimens of *A. gambiae sensu lato* and 531 of *A. funestus*).

Abdomens of freshly fed females from the PSC were squashed on to filter paper for blood meal identification by ELISA (Beier *et al.*, 1988). Heads and thoraces of indoor-resting *A. gambiae sensu lato* were placed in liquid nitrogen for later isoenzyme analysis and CS antigen detection.

Electrophoresis to study the isoenzymes of stored mosquitoes (heads and thoraces) was carried out for species identification, using allozyme keys of Miles (1978) and Bullini (1984). *Anopheles gambiae sensu stricto* and *A. arabiensis* Patton were separated by their Odh (octanol dehydrogenase) bands and later confirmed by Mpi (mannose phosphate isomerase). Two *A. gambiae sensu stricto* of the 16 cSS strain, homozygous for Odh 100 and Sod (Superoxide dismutase) 100 were run as standards on each cellulose acetate gel. The mosquito homogenates used for sibling species identification were further tested with the CS-ELISA to estimate the CS antigen positivity index in each sibling species. Preliminary tests on anophelines which were known to be carriers of *Plasmodium falciparum* sporozoites and controls (provided by J.P. Verhave, Nijmegen) showed that there is no inhibiting effect of the Enzyme Stabilization Liquid (dithiothreitol 2 mM; aminocaproic acid 2 mM and EDTA 2 mM) added to our mosquito homogenates on the CS-ELISA reactions.

Parasitological survey

Three parasite surveys for *Plasmodium* were carried out in March, June, and September 1993, in both villages. A minimum of 170 children under 5 years of age in Mulira (total inhabitants 2300) and of 160 in Murengeza (total inhabitants 1940) were examined at each survey. A list of children in each village is regularly updated in order to include the new born infants and to exclude the children more than 5 years old. At each survey about 10% of the registered children were missed. These surveys were part of the routine surveillance of the Burundi malaria control programme (Barutwanayo *et al.*, 1991). Samples of peripheral blood were taken from each child and thin and thick films prepared. Each thick film was examined for 200 microscopic fields (100× objective, 10× oculars). Identification of the species of *Plasmodium* was determined on a thin film. The prevalence of high parasitaemia was calculated as the proportion of thick films with all fields positive for at least one asexual form of *P. falciparum*, which corresponded to a parasite density of more than 2000 trophozoites/μl (Coosemans *et al.*, 1994).

Results

Man-biting rates

Man-biting rates are shown in figure 2, for night-biting collection (NBC) IN and OUTdoors. Man-biting rates were higher in Mulira than in Murengeza by a factor of about 2.0 for *A. gambiae sensu lato* and 3.5 for *A. funestus*. Prior to the application of insecticides, *A. funestus* inflicted 7.4% of the bites on humans in Mulira and only 2.2% in Murengeza. This proportion rose to about 25% after treatment in both villages. After spraying, the decrease in density of *A. gambiae sensu lato* and, to a lesser extent, that of *A. funestus*, was accelerated.

Degree of exophagy

The ratio of the man-biting rate outdoors/indoors was similar for *A. gambiae sensu lato* in both villages, but it increased from 2.1 (5847/2824) before treatment to 3.0 (769/259) after treatment. This increase of exophagy was more pronounced for the relatively endophagic species *A. funestus*: from 0.8 (200/259) to 3.9 (179/46) in Mulira and from 0.3 (15/50) to 1.4 (75/55) in Murengeza.

Parous rates and semi gravids

Heterogeneity χ^2 tests performed by species and survey showed no differences in the parous rate and the proportion of semi gravid females between samples caught biting in the houses and samples caught outside (results not shown). In Mulira, a parous rate of only 18% was observed for *A. gambiae sensu lato* in February rising to a peak at the end of April (69%). In Murengeza, the parous rate for *A. gambiae sensu lato* showed two peaks, one at the beginning of March (56%) and another in April (67%). After spraying with lambda-cyhalothrin, reductions of the parous rate were observed in both villages (table 1). The proportion feeding in the semi gravid state, indicating the proportion of females taking more than one bloodmeal per gonotrophic cycle, was highest during the early rainy season (September-October).

CS antigen positive index

Before the insecticide treatment, sporozoite rates in *A. gambiae sensu lato* were similar for samples collected

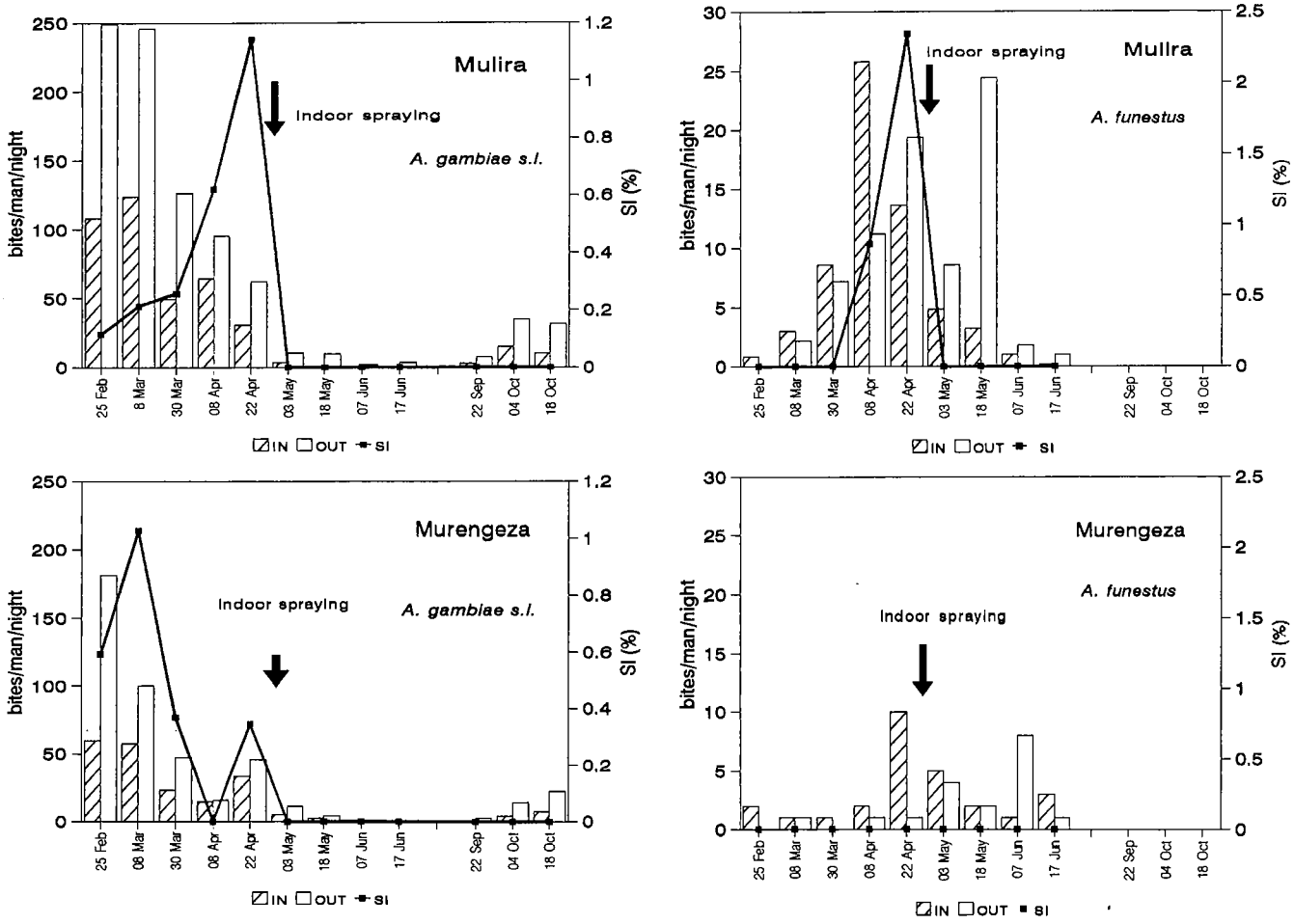

Fig. 2. Man-biting rates inside (IN) and outside (OUT) the houses and mean CS antigen positive index (SI) in *A. gambiae sensu lato* and *A. funestus* in two villages of the Rusizi Valley, Burundi, February-October 1993.

Table 1. Proportions of parous and semi-gravid females among anophelines collected during night bite catches (indoors + outdoors), before and after indoor spraying on 26 April 1993.

	Mulira		Murengeza	
	Parous rate % (Ne)	Semi-gravids % (N)	Parous rate % (Ne)	Semi-gravids % (N)
A. gambiae sensu lato				
25 Feb 1993	17.7 (543)	8.7 (595)	43.4 (530)	14.8 (622)
8 March	37.6 (612)	14.4 (715)	56.2 (203)	19.4 (252)
30 March	37.9 (383)	7.7 (415)	18.4 (207)	17.2 (250)
8 April	40.7 (567)	9.3 (625)	47.8 (115)	11.5 (130)
22 April	68.9 (424)	11.1 (477)	67.4 (224)	17.0 (270)
	lambda-chyhalothrin spraying			
May-June*	50.8 (122)	9.6 (135)	26.2 (84)	10.6 (94)
Sept-Oct*	41.7 (321)	18.7 (395)	42.2 (144)	29.1 (203)
A. funestus				
March 1993	32.7 (49)	0.0 (49)	1/2	1/4
April*	58.6 (210)	13.9 (244)	44.4 (36)	14.3 (42)
	lambda-cyhalothrin spraying			
May-June*	27.9 (172)	9.0 (189)	37.7 (61)	14.1 (71)

*Pooled catches for several nights; Ne: total number of unfed anophelines; N: total number of unfed and semi-gravid anophelines.

Table 2. Estimation of the sporozoite rate determined by the detection of CS antigen in *Anopheles gambiae sensu lato* and *A. funestus* collected during night bite catches (indoors + outdoors) before and after house spraying.

	A. gambiae sensu lato				*A. funestus*			
	Mulira	%	Murengeza	%	Mulira	%	Murengeza	%
25 Feb 93	1/865	0.1	4/674	0.6	0/6	–	0/1	–
8 March 93	3/1406	0.2	7/682	1.0	0/21	–	0/4	–
30 March 93	1/389	0.3	1/271	0.4	0/42	0.0	0/3	–
8 Apr 93	3/482	0.6	0/122	0.0	1/116	0.9	0/9	–
22 Apr 93	7/613	1.1	1/289	0.4	3/128	2.3	0/61	0.0
26 Apr 93	lambda-cyhalothrin spraying							
May-June*	0/129	0.0	0/105	0.0	0/199	0.0	0/75	0.0
Sept-Oct*	0/422	0.0	0/199	0.0	–		–	

*Pooled catches for several nights.

indoors and outdoors (Mantel-Haenszel χ^2 stratified by month $P=0.97$ in Mulira, $P=0.77$ in Murengeza). The low χ^2 value obtained by the Mantel-Hanszel test for *A. funestus* could not be validated, however, cumulative results suggested that similar sporozoite rates were also found in Mulira (IN: 2/165; OUT 2/148).

In Mulira, the CS antigen positive rate of *A. gambiae sensu lato* oscillated around 0.2% in February-March but increased significantly at the end of April to 1.1% (χ^2 for trend $P < 0.005$) (table 2, fig. 2). During the same period a similar increase of the CS antigen positive rate was observed in *A. funestus* from 0%(0/69) to 2.3%(3/128) (fig. 2). In Murengeza few *A. funestus* were caught, and only *A. gambiae sensu lato* (13/2029) was found to be positive. In contrast in Mulira, no significant trend of the CS antigen positive rate was observed (χ^2 for trend $P=0.36$), although a peak of 1% was recorded at the beginning of March (ordinary χ^2 with 4 df; $P < 0.001$) (fig. 2).

After treatment none of the 855 *A. gambiae sensu lato* and the 284 *A. funestus* collected in both villages was found to be antigen positive. Based on the binomial distribution, the upper 95% confidence interval of the sporozoite rate for the two vector species combined was 0.32%.

Inoculation rate

The total inoculation rate (number of infective bites/man/night, of both vector species) during the pre-treatment period was approximately the same in both villages, with an average number of infective bites /man/night of 0.56 in Mulira and 0.49 in Murengeza outside the houses and of 0.34 and 0.23 inside the houses, respectively. This inoculation rate followed a bimodal curve in both villages (fig. 3), with one peak at the beginning of March and a second one at the end of April. However an important difference was observed between the two villages: in Mulira the main peak was observed at the end of April (IN: 0.68, OUT 1.16 infective bites/man/night), whereas the main peak in Murengeza was observed two months earlier (IN: 0.59; OUT: 1.03). Greater weight should be attached to the inoculation rates inside the houses since almost all the children and the majority of adults stay in the houses after nightfall.

The gambiae *complex*

Anopheles arabiensis was the dominant species in the biting vector population. From the NBC prior to treatment, only 2.5% (9/367) of the *A. gambiae sensu lato* were identified as *A. gambiae sensu stricto*. There was no significant difference in relative abundance of *A. gambiae sensu stricto* between indoor and outdoor biting catches. (5/171 IN and 4/196 OUT; χ^2 test $P=0.59$), nor between the villages (Mulira: 5/225; Murengeza: 4/142; χ^2 test $P=0.72$).

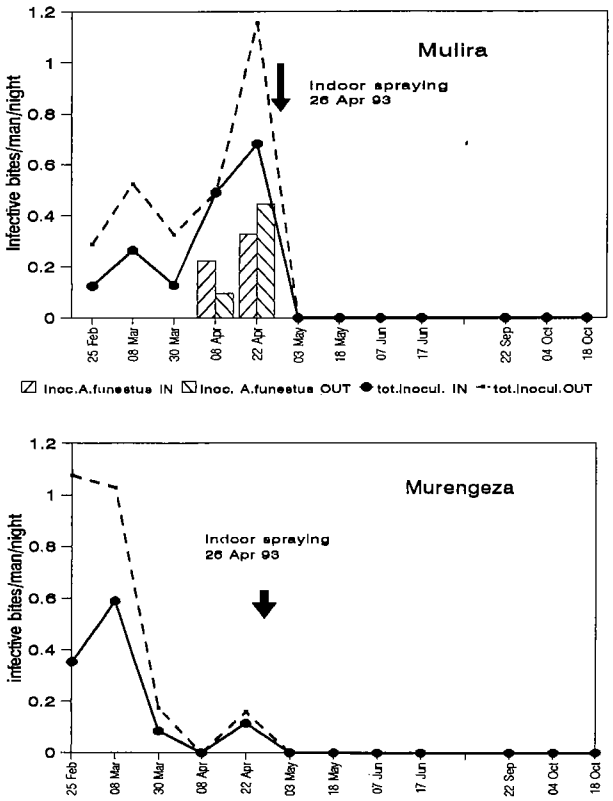

Fig. 3. Total entomological inoculation rate (*A. gambiae sensu lato* plus *A. funestus*) in the two study villages (number of infective bites/man/night indoors and outdoors), with histograms showing the contribution of *A. funestus* to the inoculation rate.

Table 3. The pre-spraying ratio of the indoor man-biting rate to the man-fed indoor resting density by village (February-April 1993).

NBC (IN)/man-night (PSC/house) (HBI)		(*) NBC (IN)/man-night × (frequency of species in NBC) (PSC/house) × (frequency of species in PSC)	
Mulira			
A. funestus 305/25 (150/20) (69/75) $\frac{12.2}{6.9} = 1.8$	*A. gambiae s. l.* 1880/25 (118/20) (43/49) $\frac{75.2}{5.2} = 14.5$	(*) *A. gambiae s.s.* 75.2 (5/225) 5.9 (4/8) $\frac{1.7}{3.0} = 0.6$	(*) *A. arabiensis* 75.2 (220/225) 5.9 (4/8) $\frac{73.5}{3.0} = 24.9$
Murengeza			
A. funestus 111/25 (101/25) (37/37) $\frac{4.4}{4.0} = 1.1$	*A. gambiae s. l.* 979/25 (85/25) (39/40) $\frac{39.2}{3.3} = 11.8$	(*) *A. gambiae s.s.* 39.2 (4/142) 3.4 (16/45) $\frac{1.1}{1.2} = 0.9$	(*) *A. arabiensis* 39.2 (138/142) 3.4 (29/45) $\frac{38.1}{2.2} = 17.4$

NBC (IN): night bite catches indoors; PSC: pyrethrum spray collection; HBI: Human blood index (No. positive for man/No. examined in the PSC); (*): HBI by species is not known, and has not been taken into consideration.

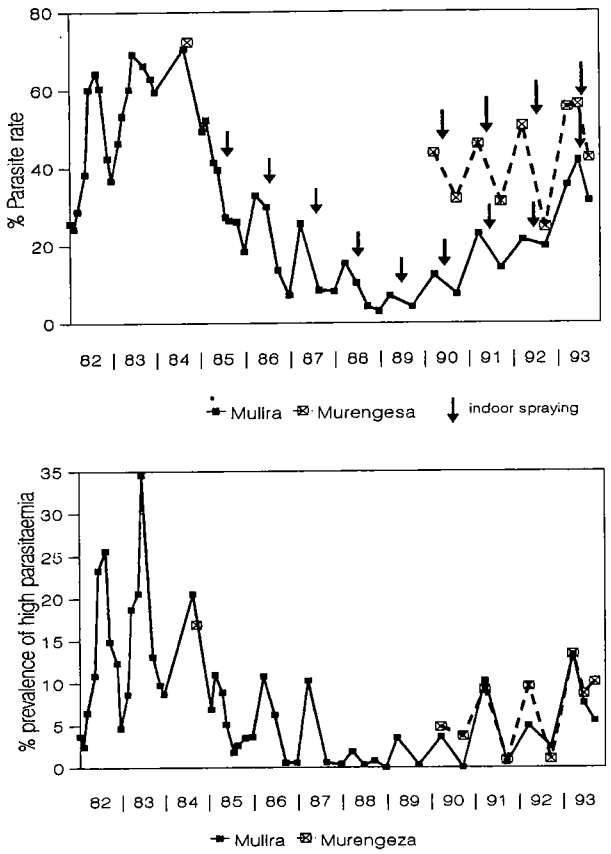

Fig. 4. Impact of indoor spraying with malathion from 1985 to 1992 and with lambda-cyhalothrin 30 mg a.i./m^2 on 26 April 1993 on the parasite rate and prevalence of high parasitaemias (> 2,000 trophozoites/μl) in two villages of the Rusizi Valley (Burundi). (Pre-intervention period 1982-1984).

However, a much higher proportion of *A. gambiae sensu stricto* occurred among indoor-resting females compared to biting females (20/53 and 9/367; χ^2 test $P < 0.0001$). After house-spraying, among the 140 *A. gambiae sensu lato* collected biting from both villages during May-October, only one specimen was identified as *A. gambiae sensu stricto* and the others were all *A. arabiensis*. The proportion of *A. gambiae sensu stricto* (0.7%) appeared to be less than that observed before spraying but the difference was not significant (Fisher exact test: $P = 0.29$).

Mosquitoes collected before the annual spray and identified by electrophoresis revealed the presence of 1/222 (0.45%) positive *A. arabiensis* and 1/22 (4.5%) positive *A. gambiae sensu stricto*. This apparent difference in infectivity rate is not statistically significant (Fisher exact test: $P = 0.11$).

Degree of endophily of man-biting vectors

Before spraying, the ratio of the indoor man-biting rate to the man-fed indoor-resting density provides a good estimation of the resting behaviour and can be used as a predictor of residual spray efficacy (Molineaux *et al.*, 1976). Assuming an average of three people per house and assuming that the mosquito feeding frequency averaged once per 2.5 days, we could expect, in case of complete endophily, 7.5 females resting indoors if there is one bite per man per night, or a ratio of 0.13. Table 3 shows the calculated ratios for *A. gambiae sensu lato* and *A. funestus*. Endophily was found to be strong for *A. gambiae sensu lato* slightly less in *A. funestus*, but almost absent for *A. arabiensis*. Since *A. arabiensis* greatly outnumbered *A. gambiae sensu stricto* the overall populations of *A. gambiae sensu lato* appeared to be strongly exophilic.

Parasitological surveys

Figure 4 shows the trend in prevalence of the infection and of high parasitaemia since 1982. No significant changes in the *P. falciparum* parasite rate and prevalence of high parasitaemias occurred between March and June 1993 in both villages (fig. 3). In September, a significant decrease of high parasitaemias (χ^2 for trend: $P < 0.025$) was observed in Mulira (from 13.4% in March to 5.5% in September), whereas the proportion remained almost unchanged in Murengeza (10%) (χ^2 for trend: $P = 0.41$).

Discussion

For the initial strategy in the Rusizi Valley, annual indoor-spraying with a residual insecticide was planned for the beginning of April. From 1985 to 1989, a major decrease of malaria was observed both in the parasite rate (from 75-60% to <10%) and the prevalence of high parasitaemia (>2,000 trophozoites/μl: from 35% to <5%) (fig. 4), the latter being a good indicator of relative changes in morbidity in areas with seasonal malaria (Delacollette & Van der Stuyft, 1993; Coosemans *et al.*, 1994). Thereafter, from 1990 to 1993, a progressive increase of malaria rates was observed in most villages, including Mulira. Moreover, in one village (Murengeza), no consistent reduction of the parasite rate was observed after treatments started in 1989.

The peaks of sporozoite inoculation in Mulira and Murengeza in 1993 were at the beginning of April and February respectively (fig. 3). This explains why the annual spray rounds performed in April were more effective in Mulira than in Murengeza. This difference in seasonality is probably related to anopheline breeding sites. Mulira is surrounded by rice fields, whereas Murengeza has a permanent river with several swamps (fig. 1) influencing the anopheline populations dynamics and the malaria transmission. This emphasizes that, even in the same general area, different patterns of transmission may occur.

The decrease in density of *A. gambiae sensu lato* and, to a lesser extent, of *A. funestus*, after treatment was considered to be mainly due to a natural decrease of vector populations at the beginning of the dry season, but this was probably accelerated by the treatment as demonstrated in a previous study where a control area was included (Barutwanayo *et al.*, 1991).

The present study raises the question as to why the previous treatments from 1985-1989 were so successful, given the highly exophilic behaviour of the major vector *A. gambiae sensu lato* with a NBC/PSC ratio of more than 10, compared to a maximum value of 2.8 in Nigeria (Molineaux *et al.*, 1976). We put forward two hypotheses. Firstly, of the two members of the *gambiae* complex present in the area, *A. gambiae sensu stricto* is a much better vector than *A. arabiensis*. For example, White *et al.* (1972) found that the sporozoite rate in *A. gambiae* was nearly 14-fold greater than that in *A. arabiensis* at Segera in Tanzania. Our limited data on the two sibling species suggested a similar difference, but it was not statistically significant. *A. gambiae sensu stricto* is highly endophilic and thus much exposed to indoor treatments (NBC/PSC ratio <1) (table 3), whereas *A. arabiensis* avoids contact with the insecticide because of its exophilic behaviour (NBC.PSC ratio > 17) (table 3). Considering the low proportion of *A. gambiae sensu stricto* (2.5%), complete elimination of this species could result in a decrease of transmission of only 20%.

Secondly, engorged *A. arabiensis* escape from human dwellings and rest in other treated shelters. Before the vector control programme started, Coosemans *et al.* (1989) observed a high human blood index for *A. gambiae sensu lato* collected by PSC from non-residential structures, such as animal sheds (37%) or separate cooking huts (57%) of which 96% (605/631) were *A. arabiensis*. This figure is very near to the proportion of *A. arabiensis* found in the present study by night-biting collections (97.5%), but is in contrast to a lower relative abundance of *A. arabiensis* (33/53 i.e. 62%) sampled by PSC from human dwellings only. This suggests that most of the *A. arabiensis* biting inside human dwellings then shelter in other types of structures. Thus provided that all these are sprayed, the probability of *A. arabiensis* contacting a lethal dose of insecticide should be high.

Indoor spraying with lambda-cyhalothrin was applied on the 26 April 1993, which is now considered to have been one month too late in Mulira and three months too late in Murengeza. Unfortunately, the date of treatment had to be delayed by four weeks, for lack of funds which were not released in time. Similar delays occurred in 1990, 1991 and 1992, and this is considered to have led to the progressive increase of malaria from 1990 to 1993 in most of the area. Despite the delay, no infective anopheline (0/1139) was found following lambda-cyhalothrin treatment for the six months from May onwards. It should be noted that with the expansion of the sprayed area, supervision of the spray squads has deteriorated, and sensitization of the local authorities and the general population has become more difficult.

Conclusions

This investigation emphasizes that the timing of residual insecticide spraying is crucial in obtaining maximum benefit. Local authorities (and donor agencies) should therefore stock-pile the insecticide and release the allocated funds sufficiently in advance of the transmission season. House-spraying with an insecticide having an effective residual action (ca 3 months) should be applied to the major inhabited parts of the Rusizi Valley during late March and the beginning of April in order to curtail the peak of malaria transmission which occurs in April-May. Since no malathion resistance has been detected so far in the *A. gambiae* complex (M. Barutwanayo, unpublished data), this relatively short-acting insecticide can still be used in the major part of the Rusizi Valley, where irrigated rice is cultivated. In villages near rivers and natural swamps, application of an insecticide with a longer residual effect, such as a synthetic pyrethroid, is recommended, and should be implemented in mid January at the latest, before the upsurge of vector densities and infective biting. This treatment would then be effective for at least 6 months. Since few *A. arabiensis* remain in houses after a blood meal, and a substantial proportion of this predominant vector rest in other shelters, it is important for the spraying to cover all types of structures, including toilets, kitchens, animal sheds and store houses.

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References

Barutwanayo, M., Coosemans, M., Delacolette, Ch., Bisore, S., Mpitabakana, P. & Seruzingo, D. (1991) La lutte contre les vecteurs du paludisme dans le cadre d'un projet de développement rural au Burundi. *Annales de la Société Belge de Médecine Tropicale* **71** Suppl. 1, 113–125.

Beier, J., Perkins, P., Wirtz, R., Koros, J., Diggs, D., Gargan, T. & Koech, D. (1988) Bloodmeal identification by direct enzyme-linked immunosorbent assay (ELISA), tested on *Anopheles* (Diptera: Culicidae) in Kenya. *Journal of Medical Entomology* **25**, 9–16.

Bullini, L. (1984) Enzyme variants in the identification of parasites and vectors: methodological aspects of the electrophoretic approach. pp. 53–69 *in* Newton B.N. & Michal F. (Eds) *New approach to the identification of parasites and their vectors.* Tropical Diseases Research World Health Organization ser 5.

Burkot, T.R., Williams, J.L. & Schneider, 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.

Coosemans, M. (1985) Comparaison de l'endémie malarienne dans une zone de riziculture et dans une zone de culture de coton dans la Plaine de la Rusizi (Burundi). *Annales de la Société Belge de Médecine Tropicale* **65**, Suppl. 2, 187–200.

Coosemans, M. & Barutwanayo, M. (1989) Malaria control by antivectorial measures in a chloroquino-resistant area: a successful experience in a rice-growing area of the Rusizi Valley (Burundi). *Transactions of the Royal Society of Tropical Medicine and Hygiene* **83**, Suppl., 97–98.

Coosemans, M., Petrarca, V., Barutwanayo, M. & Coluzzi, M. (1989) Species of the *Anopheles gambiae* complex and chromosomal polymorphism in a rice growing area of the Rusizi Valley (Republic of Burundi). *Parassitologia* **31**, 113–122.

Coosemans, M., Van der Stuyft, P. & Delacollette, Ch. (1994) Hundred percent of fields positive in a thick film, a useful indicator of relative changes in morbidity in areas with seasonal malaria. *Annals of Tropical Medicine and Parasitology* **88**, 581–586.

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

Miles, S.J. (1978) A biochemical key to adult member of the group of species (Diptera: Culicidae). *Journal of Medical Entomology* **15**, 297–299.

Molineaux, L., Shidrawi, G.R., Clarke, J.L., Boulzaguet, R., Ashkar, T. & Dietz, K. (1976) The impact of propoxur on *Anopheles gambiae s.l.* and some other anopheline populations, and its relationship with some pre-spraying variables. *Bulletin of the World Health Organization* **54**, 379–389.

White, G.B., Magayuka, S.A. & Boreham, P.F.L. (1972) Comparative studies on sibling species of the *Anopheles gambiae* Giles complex (Dipt., Culicidae): bionomics and vectorial activity of species A and species B at Segera, Tanzania. *Bulletin of Entomological Research* **62**, 295–317.

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