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FINAL AND ANNUAL PROGRESS REPORT TO THE INTERNATIONAL DEVELOPMENT RESEARCH CENTRE, (IDRC) OTTAWA, CANADA

FINAL REPORT Technical and Financial Reports for the Programme: COMMUNITY PREVENTION OF MALARIA, (TANZANIA) File No. 3-P-89-0216

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Final and Annual Progress Report for Year Three Period 22 June 1993 to 20 August 1995

A. TECHNICAL REPORT

General Objective

The overall objective of the research programme is to determine and compare in village community trials, the performance of permethrin treated bednets versus similarly treated polypropylene bedcurtains on the reduction of clinical parasitological and entomological malaria indices.

Specific Objectives

- (a) To measure and compare the efffect of community use of permethrin treated polypropylene bedcurtains as opposed to bednets on clinical episodes of malaria, malaria parasitaemia and malaria vector dynamics.
- (b) To compare the duration of the insecticidal residual effect of permethrin on the two materials.
- (c) To evaluate, and compare the community acceptability, durability and costs of the two treatments.

Study Site

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The study is being carried out in six villages of Korogwe district, Tanzania. The main malaria vector, Anopheles funestus and members of the Anopheles gambiae s.l. which includes An. gambiae s.s., An. arabiensis, An. merus and possibly An. quadriannulatus are found at all seasons but increases after the heavy rains of April and May. All the villages are labour camps of Sisal Estates, four around Mombo and two around Korogwe townships. Houses are made of concrete blocks with tin or tile roofs. There is a dispensary not further than two kilometres away from each camp.

Study Design and Operations

All work in the villages was carried out in close consultation with the camp and estate administration. The camps were mapped and houses numbered. The reporting period covers ten months of the post intervention monitoring period. Baseline pre-intervention data collection was initiated in 1991. Intervention was first introduced into the villages in April 1993, being eight months behind schedule. Reimpregnation was done in the villages in October 1993; six months after the initial treatment. A second reimpregnation was done in May 1994.

Part One: Malariological and Entomological Studies

Malariological methods: Malariological observations were made only on children aged between 1 and 10 years, as in a holoendemic area they are more seriously by malaria than are adults. Children presenting voluntarily with or without either parents or guardians were met by arrangement in each village once every fortnight. A questionnaire was completed including questions about name, age, use of bednets, indication of fever during the previous two days, e.t.c. The axillary temperature was measured with an electronic thermometer. A blood slide was taken from each child, stained with Giemsa and immediately examined for malaria parasites. The number of malaria parasites per 200 white blood corpuscies were counted. The next day, results of the diagnosis were reported and chloroquine treatment given to positive cases. A mass blood survey was carried out on as many as possible of the children in each village after every six months.

In June 1993, cohorts of children aged between one and ten years in each village around Mombo were treated with Fansidar (pyrimethamine sulfadoxine) to clear any existing parasitaemia. A similar exercise was carried out in two villages around Korogwe in October 1993. The incidence of new malaria infections was actively followed up weekly for the first eight weeks and fortnightly for the subsequent weeks up to 20 weeks post treatment; when most of the children had converted to malaria positivity. However, the children were dropped from the study cohort if they gave a malaria positive slide or if they reported one night or infection risks. A similar trial had already been completed in 1992 during the preintervention period. The second trial was done to allow for comparison of malaria incidences before and after intervention.

Entomological methods: Mosquito collections were made in each village once every fortnight. Four main methods were employed in collecting mosquitoes, these included CDC light traps, pyrethrum spary catch, window exit traps and pit traps. Three houses were selected in each village for regular use (once in a month) of CDC miniature light traps, these were hung close to bednets which were occupied and untreated. The householder was responsible for connecting the trap to a rechargeable battery at dusk, and disconnecting it at dawn after tying the trap bag. Pyrethrum spray catch technique was used to collect indoor resting mosquitoes in ten designated houses in each village. The exercise was carried out once a month in intervening fortnights between light trap catches.

Mosquitoes attempting to leave the houses through the windows were collected by trapping them with window exit traps. These were set late in the afternoon on windows of five houses in each village. The five houses comprised half of the houses where spray catches were carried out on the next morning. Three pit traps were dug in each village and collections made from horizontal pockets dug on the sides (walls) of each pit once every fortnight.

Bioassays to measure the residual effect of the insecticide on the fabrics in regular

use were carried out. The fabrics were brought to the laboratory for the tests.

Laboratory reared An. gambiae s.s. of the R70 strain held in W.H.O. bioassay cones were exposed for 3 minutes on various treated fabric surfaces once in two months. Controls were concurrently exposed to untreated fabric surfaces in regular use from the same villages.

Mosquito catches were sorted by species, sexed and counted. Female anophelines were dissected and scored for parity and for presence of sporozoites visible in salivary glands. A sample of female mosquitoes were dried and later on assayed for presence of *P. falciparum* malaria sporozoites using the ELISA technique of Wirtz et. al., (1987). Some samples from both sites were identified to sibling species using Polymerase Chain Reaction (PCR) technique.

Preliminary results on protective efficacy of the interventions necessitated further studies on biting patterns of the vectors. Mosquito biting patterns were studied by carrying out human landing catches overnight from 18:00Hrs. to 07:00Hrs. the next morning. Each hours' collection was kept separate in a paper cup. The mosquitoes were sorted by species and sex. After sorting, all female *An. gambiae* samples were preserved in Carnoys' solution for later identification of sibling species.

Results and Discussion

Table 1 shows for each village; the number of slides examined, proportion found positive for malaria, proportion with *Plasmodium falciparum* infection and proportion with high parasitaemia (i.e. with > 100 parasites/200 leucocytes) and or having body temperature > 37.4° C.

Table 1: To show the slide reading results for each village from June 1993 to April 1994; number of slides examined, proportion found positive for malaria, proportion with *P. falciparum* infection and proportion with fever and / or high parasitaemia (i.e. with 100 parasites/200 leucocytes).

Village	Sides exemined	% Pos.	% P. feldpærum	% High parealt i or °t>37.4°c
Mwatya	1133	42	08	3
Contract	732	44	81	3
Kikwajuni	429	35	78	2
Masuwint	216	26	89	18
Section 1	1197	47	98	6
Section 1	1050	47	94	11

Table 2: To show the relative mosquito densities (from July 1993 to March 1994 for female An. gambiae s.l. only), estimated using various methods (numbers of rooms or traps sampled are shown in brackets). Results of dissections for microscopic examination as parity and sporozoite rates are shown in the last two columns, (in brackets following each value are numbers of mosquitoes examined). The results have been divided into quarters of the year for each village.

Querter of		Mazgutta Densities	Dissection Persuits		
Year	Per room	Per noom Per L. Trip Per E. Trip			Sporo, Rates

MWELYA

Jul/Sept 93	0.3(20)	3.8(6)	7.7(10)	44(18)	0.0(15)
Oct/Dec 93	0.3(20)	0.8(9)	3.0(10)	-(0)	-{0}
Jon/Mar 94	, 0.8(28)	54.8(10)	11.9(12)	63(11)	-(0)

CONTRACT

Jul/Sept 93	0.0(10)	0.0(6)	0.1(10)	-(0)	-(0)
Oct/Dec \$3	0.2(20)	0.3(9)	0.1(10)	+(0)	-(0)
Jan/Mar 94	0.5(20)	3.8(12)	0.8(9)	-(0)	-(0)

			T		
Jul/Sept 93	0.8(12	12.9(6)	6.5(9)	48.2(29)	0.0(32)
Oct/Dec 93	0.1(14)	14.9(\$)	2.0(10)	35.3(34)	0.0(34)
Jan/Mar 94	0.6(20)	116(12)	2.4(15)	-101	~(0)

KIKWAJUNI

Jui/Sept 93	10.6(12)	62(5)	31(B)	56(39)	4.7(42)
Oct/Dec 93	6.7(15)	61(9)	3.8(8)	43(61)	0.0(84)
Jan/Mar 94	9.5(11)	32(9)	22.1(7)	33.3(42)	2.2(44)

SECTION I

Jul/Bept \$3	0,1(20)	2.0(6)	0.1(10)	-(0)	-(0)
Oct/Dec 93	0.0(10)	4.8(9)	0.1(10)	-(0)	-(0)
Jun/Mar 94	0.3(40)	1.8(16)	1.8(15)	-(0)	-101

SECTION R

Jul/Sept 93	0.1(20)	1.33(9)	0.1(10)	50(2)	0.0(2)
Oct/Dec 93	0.1(20)	1.3(9)	3.0(7)	62.5(5)	0.0(13)
Jan/Mar 94	0.9(39)	2.0(7)	40(20)	33.3(16)	0.0(17)

MASUWINI

Malaria prevalence was found to range from between 26% and 47%. As expected, *P. falciparum* infections were found to be the most predominant; with proportions ranging from 79% to 98% of the infections. High parasitaemias suspected to be associated with clinical malaria in children were found to range from 2% to 16% of the infections. Other malaria infections include *Plasmodium* malariae and *Plasmodium* ovale. A few mixed infections were also observed.

Table 2 above shows results on vector abundance and dissection for *An. gambiae* s.l. only. The results for each village are divided into quarters of the year. Peak mosquito densities were observed during the October - December and April - June periods of the year. Parity rates have been estimated to range from between 33% and 63%.

Estimates for sporozoite rates by dissection have indicated a considerable level of transmission (4%) in the villages without any intervention while no mosquito with sporozoites have been observed in the intervention villages. After intervention, very few mosquitoes could be collected for dissection and subsequent microscopic examination. The ELISA technique (see Table 3) could however detect some infected mosquitoes after intervention.

"sole 3: To show a comparison in Sporozoite Inoculation Rates (SIR - i.e. number of infective bites ar person per night), before and after intervention in four villages. Sporozoites have been detected by the ELISA technique, following each value the number in parentheses indicate total mosquitoes assayed. The biting rates (in terms of mosquito bites per person per night) have been estimated from light trap catches following the relationship developed by Lines et. al. 1991 (i.e. multiplying average light trap catch per night by 1.5). The last column gives an estimate for the percent reduction in sporozoite inoculation rates following intervention.

	8.	fore Intervention	<u> </u>		After Intervention			
Village	Bites /person /right	Sporo. Rate(%) (ELISA)	\$.I.R	Bites /Person /night	Spore. Rate(%) (El.ISAI	Ş.I.R	% Reduction	
Mweiya ^b	20	4,40 (159)	0.86	32	0.00 (107)	0.00	100	
Kikwajumi ^c	60	4.37 (183)	2.63	75	0.90 (110)	0.67	74	
Section I ^b	27	5.33 (206)	1.42	6	2.27 (89)	0.14	80	
Section N ^c	83	2.35	1.93	4	2.81 (71)	0;10	89	

* - bednet village; ⁶ - bedcurtein village

Table 3 above shows stimates for the reduction in malaria infection risks posed by An. gambiae s.l. only in intervention villages. The other vector but of lesser ignificance is An. funestus. The sporozoite detections were done by the ELISA

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technique. Estimates for the vector biting activity are based on light trap catches and their relationship to human landing catches. The data from Table 2 for vector density per light trap per night may be multiplied by 1.5 (Lines et al., 1991) to convert them to estimates of what the biting rates per person would have been in a room in the absence of their associated untreated bednet. Muliplying these inoculation rates (SIR). A reduction was achieved in all the trial villages ranging from 74% to 100%.

Three minutes bioassays on dry freshly impregnated bednets and bedcurtains gave 100% mortality. Table 4 shows subsequent mortalities up to six months when reimpregnation was carried out. The bioassay results shows that the killing effect slowly declined to 80% and 74% towards the end of six months for bednets and bedcurtains respectively. However, the effect was restored back to 100% following reimpregnation.

Table 4: To show three minutes bioassay results as % mortalities at intervals of two months post treatment and post retreatment. Sample size 'n' in terms of number of mosquitoes bioassayed on each months' test is indicated in parentheses below each mortality value.

FARRIC		MOWTHS P	ONT THEATMENT	_					
	•	2							
		·		<u> </u>	f	<u> </u>	2	<u>+</u>	
RECHET	1007	90	9 7	80	100	1009	82		£=
A =	(100)	(1009	(107)	(941)	(160)	(196)	(200)	(708)	(967)
CLINTAIN	100	95		74	100	100	\$ 3	34	
n	(100)	(100)	(110)	(100)	11001	11001	(100)	(120)	(190)

No adverse reactions to pyrimethamine-sulfadoxine were encountered in the course of both incidence trials. During the first trial in 1992, a total of 336 children were recruited in the study in five villages. A parasite clearance failure was recorded in four children seven days after drug administration (1.1%; 4/336) one of which was at gametocyte stage. The second trial in 1993 did similarly exibit even a lower level of parasite clearance failure (0.6%; 2/328). This observation corroborates well with an earlier study done in Muheza in 1991. However, two separate studies around Muheza have recently shown some evidence for unexpectedly high levels of Fansidar resistance to the tune of 60% (J. Mhina, pers. comm.; J.Trigg, pers. comm.).

Figures 1a to 1e shows for each village, the proportions of children free from malaria before and after parasites were cleared using sulfadoxine-pyrimethamine (Fansidar) both before and after intervention. The graphs tend to show a similar pattern, with a slow reversion to positivity for the first six weeks. This was followed by a sharp slope which tends to tail off at week sixteen. Estimates for the force of infection 'f' for each village was thus worked out between the two

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for log proportion of children free from malaria over weeks six to sixteen.

Table 5 summarizes the values for the Force of infection before (f_1) and after (f_2) intervention. In Mombo area where Anopheles arabiensis are the only exclusive vectors; bednets were found to to provide a superior protection (58% to 68%) in comparison to bedcurtains (28%). On the contrary, in Korogwe area where both An gambiae and An arabiensis vectors exist in sympatry; neither bednets nor bedcurtains were found to provide detectable protection against malaria infection.

Table 5: To show for each village; the size of the cohort in number of children as well as the estimated forces of infection f_1 (1993; pre-) and f_2 (1994; post) intervention.

Village	Number	f ₁ (1992)	f ₂ (1993)	% Protection
Contract	50	0.091	0.037	58.2
Kikwajuni	21	0.055	0.039	28.0
Mwelya	80	0.171	0.055	67.7
Section I	71	0.045	0.079	0.0
Section II	52	0.124	0.156	0.0

These findings suggests that, perhaps the two populations experience different exposure patterns in the two areas; resulting either from varied human or vector behaviours. A human behaviour study was carried out by a Social Scientist and established that there was no difference in human behaviours between the two areas which could be having such exposure implications.

Further investigations on the vector biting pattern has revealed some significant difference in the peak biting time, and more important; a difference in the proportions of late biters between the two places. Figure 2 shows the biting activity of the vectors in the two areas. In Mombo area, the peak biting time was found to be immediately after midnight then dropping towards morning hours whereas in Korogwe the same was found to be after 2:00Hrs. stretching up to 4:00Hrs. In Muheza where *An. gamblae* s.l. comprise over 95% of the complex population, the biting peak was observed to be similar to the observation (Magesa et al., 1991) in Mombo where *An. arabiensis* abounds by over 96%. However it is interesting to note that when the two siblings exist in sympatry, (as the case in Korogwe) their biting behaviour becomes modified as shown above.

A Chi-square test for association was used to test for any significant difference in proportions biting late (biting between 5:00Hrs and 7:00Hrs.) between the two areas on three separate weeks. Significant differences were established for each week as shown on Table 6. A Mantel-Haenszel test on three weeks'pooled data was carried out to take care of weekly confounding by changes in time of moonlight. The test gave a very significant result (with a Chi value of 30.98; p

<<< 0.001) This perhaps suggests that, people in Korogwe get more bites especially during morning hours after they have woke up. From the available limited data, it has been suggested that such a difference may account for the observed difference in protection levels between the two areas.

Results from sibling species identification using polymerase chain reaction (PCR) technique has revealed that; around Mombo area, (n = 193), *An. arabiensis* is the most abundant vector species accounting for 96.3% of the complex population. There are also a few *An. gambiae* (3.1%) as well as *Anopheles merus* (0.6%). Around Korogwe, (n = 205) there is a comparatively reduced proportion of *An. arabiensis* (59%) with an increased proportion of *An. gambiae* (36%). About 2.9% of the samples were undoubtedly confirmed as belonging to *An. merus* group. However, in a few (2.1%) of the samples examined; there was observed a band usually expected in *Anopheles quadriannulatus* but which also occasionally occurs in *An. merus*. It could thus not be conclusively determined whether or not the said proportion belong to either of the sibling group.

	Early	Late	Chi-value	p value
Week 1				
Korogwe	51	12		
Mombo	49	21	4.4	< 0.05
Week 2				
Korogwe	107	12		
Mombo	972	19	23.2	< < 0.001
Week 3				
Korogwe	46	8		
Mombo	711	40	6.4	< 0.025

Table 6: To show a comparison between early (18:00 to 05:00Hrs.) and late (05:00 to 07:00Hrs.) biters for the two localities (Mombo and Korogwe) on three consecutive weeks.

The above finding makes the identification results doubtful on the one hand but very interesting on the other. This would thus warrant further investigation in order to confirm the presence or absence of *An. quadriannulatus*, this will be done by making more collections in habitats favoured by the two siblings in question; i.e. animal baits and outdoor resting sites. The collected samples will be accompanied with correlated ovaries for possible cytotaxonomic identifications.

More studies are envisaged to try and understand the biting pattern of sympatric populations of the An. gambiae comples. Such knowledge is considered essential

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in the planning of malaria control using pyrethroid treated fabrics in areas where *An. gambiae* s.l. siblings exist in sympatry.

Conclusions and Recommendations

- 1. The use of permethrin treated fabrics was found to reduce the malaria risk (in terms of Sporozoite Inoculation Rate) to people sleeping outside nets/curtains by over 74%.
- 2. Fewer mosquitoes were caught resting indoors in houses where permethrin treated fabrics have been put up.
- 3. The fabrics remained potent up to six months of regular use when potency got reduced. The potency was restored back to the original level by reimpregnation.
- 4. Results of the malaria incidence trials indicate that undetectable levels of protection were achieved in an area with sympatric populations of the members on the An, gambiae complex. This may be due to their modified biting habits when they exist in sympatry.
- 5. Sibling species identification using PCR technique has for the first time suggested the possible existence of *An. quadriannulatus* in the study area.
- 6 Previous reports on socioeconomic aspects had indicated that, though people in the villages do appreciate the protection provided by polypropylene bedcurtains against mosquito bites; if given a choice, they would prefer a conventional bednet instead, most probably for aesthetic reasons.
- 7. In places like Mombo where permethrin treated fabrics are more effective, polypropylene bedcurtains were found to reduce malaria incidences by 28%. Given the burden of the disease, such protection is considered meaningful. This makes the tool "better than nothing" in situations of the currently existing desperation.
- Where treated fabrics are effective, bednets have been found to be superior to bedcurtains. This has been observed right from experimental hut to village scale trials.
- 9. The response of sympatric populations of the members of the An. gambiae complex to pyrethroid treated fabrics need further attention since it is likely to be having some negative implications to this promising malaria control tool.

Administrative Aspects: During the reporting period, the Project leader travelled to San Diego, California to attend the First International Congress of vector Ecology; from 3 to 8 October, 1993.

References

Lines J.D. Curtis C.F. Wilkes T.J. and Njunwa K.J. (1991) Monitoring human biting mosquitoes in Tanzania with light traps hung beside mosquito bednets. Bull. ent. Res. <u>81</u>, 77-84.

Magesa S.M. Wilkes T.J. Mnzava A.E.P. et al., (1991) Trial of pyrethroid impregnated bednets in an area of Tanzania holoendemic for malaria Part 2. Effects on the malaria vector population. Acta Tropica <u>49</u>, 97-108.

Wirtz et. al. (1987) Comparative testing of *Plasmodium falciparum* sporozoite monoclonal antibodies for ELISA development. Bull. WHO. <u>65</u>, 39-45.

B. FINANCIAL REPORT

A Financial report summary for yesr three period and beyond, from 22 June 1993 to 20 August 1995 for the Recepient (Institute) Administered Funds (RAP) in Tanzanian Shillings is shown on Table A below. A Final Financial Report Summary for the Project duration is shown on Table B attached.

Table A.FINANCIAL REPORT SUMMARY FOR YEAR THREE PERIOD22 June 1993 to 20 August 1995

PROJECT TITLE: COMMUNITY PREVENTION OF MALARIA PROJECT NUMBER: CENTRE FILE 89 0216

Institute Administered Funds (RAP) in T.Shs.

Item	Original Budget	Actual Expenses	Variance	Estimates for Next Period
1. Salaries	221200	57500	163700	-
2. Field allowances i. Expt. Huts ii. Vill. Studies iii. Socio Studies	- 9232000 1000000	- 10107986 1954935	- 875986 954935	-
3. Travel i. Fuel ii. Maintenance iii. Insurance	2520000 700000 100000	2451421 1275165 132025	68579 575165 32025	-
4. Data Processing	1000000	100000	900000	2500000
5. Equipment	350000	751577	401577	-
6. Stationery	300000	313865	13865	
7. Local Consultancy	638000	60000	578000	500000
8. Internat. Comm.	350000	63000	287000	100000
	16411200	17267474		3100000

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Table B.FINAL PROJECT FINANCIAL REPORT SUMMARY22 June 1991 to 20 August 1995

PROJECT TITLE: COMMUNITY PREVENTION OF MALARIA

PROJECT NUMBER: CENTRE FILE 89 0216

Institute Administered Funds (RAP) in T.Shs.

ltem	Original Budget	Actual Expenses	Variance	Estimates for Next Period
1. Salaries	364400	208700	155700	
2. Field allowances i. Expt. Huts ii. Vill. Studies iii. Socio Studies	1040400 17195200 2115000	692300 15876486 3114587	348100 1318714 999587	- - -
3. Travel i. Fuel ii. Maintenance iii. Insurance	4650000 1250000 300000	A439548 2084413 289 20 0	210452 834413 10800	- -
4. Data Processing	1500000	331000	1169000	2500000
5. Equipment	854500	1361684	507184	-
6. Stationery	700000	485865	214135	-
7. Local Consultancy	1138000	120000	1018000	500000
8. Internat. Comm.	350000	323000	63000	100000
	31457500	29326783	;	3100000

Summary of IDRC Funds Remittance

Date

Amount

	CAD	T.Shs.
17 April 1991	19500.00	3265396.45
23 January 1992	20240.00	4138763.25
23 February 1993	15925.00	4415551.00
05 May 1994	39000 00	14602169.00
TOTAL	94665.00	26421879.70

Submitted by ----

Stephen M. Magesa PROJECT LEADER

INSTITUTION FINANCE OFFICER

Analysis of variance

The estimated costing for most items shows little change from the original budget. However, item (5) do reveal an apparent variance. This resulted from the urgent need for purchasing locally additional bednets to cover a shortfall of 245 pieces.

For item (2) Field allowances, an apparent variance was realised under subitems (i) & (ii) due to the fact that the Government allowance rates were officially changed in response to cost of living and devaluation of the Tanzanian Shilling. For items (4) and (7) Data processing and Local consultancy; much remain to be done in order to get the work published in peer review journals. This has resulted into the observed variance and need for extended budget.

1. Data processing

An additional modest budget will be required this year for data processing (this is indicated in the original proposal). Having collected all the important data, final data analysis will have to be undertaken in order to produce several good quality publications to appear in international scientific journals. A considerable amount of time is expected to be spent on data cleaning; requirement for hiring additional hands as well as overtime payments will be necessary. The Academic Medical Centre in Amsterdam had earlier indicated willingness to assist in data analysis as soon as cleaning is completed.

2. Local consultancy

During the next period, one local consultant will be required on regular basis to assist and advise on data cleaning and preliminary analysis. The costs only include his subsistence allowance of US \$25 a day. He will not require any consultancy fees.

Following the reported findings in the Technical Report above, interest has developed in trying to further understand the biting behaviours of sympatric populations of members of the *An. gambiae* complex. Efforts will be made to try and complete the studies on the above in the year 1995.

Malaria Incidence in Kikwajuni Village Proportions Free from Malaria Infection

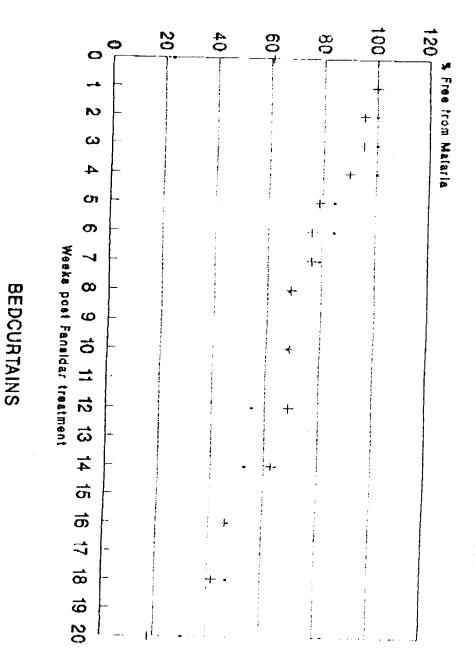
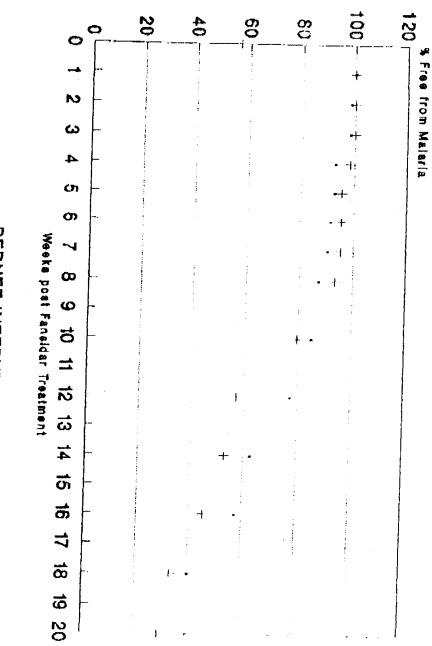


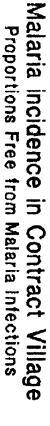
Fig. ta: To show the malaria incidence before and after intervention with permethrin treated curtains in Kikwajuni village (Mombo) Before intervention ۰ł۰ After intervention

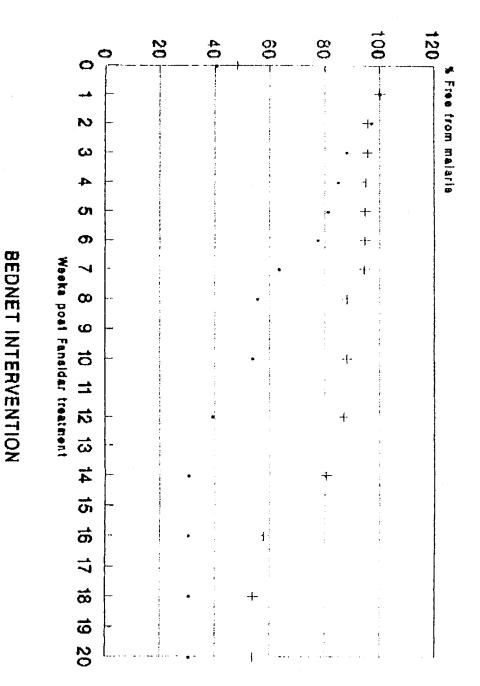


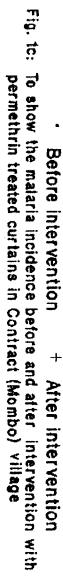
BEDNET INTERVENTION



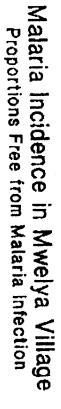
Malaria incidence in Section I Village Proportions Free from Malaria







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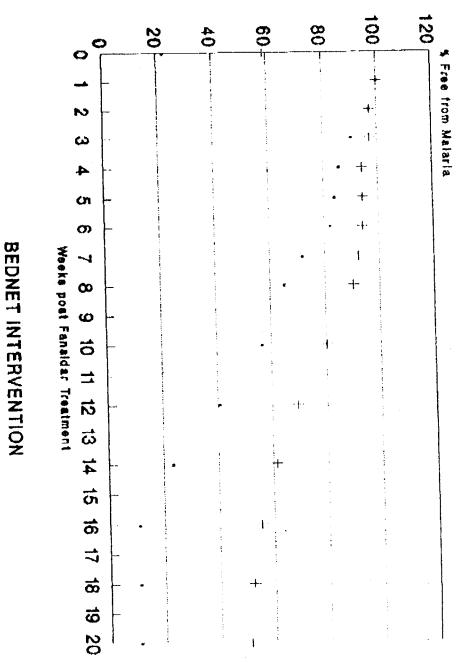
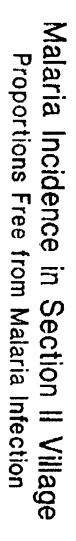
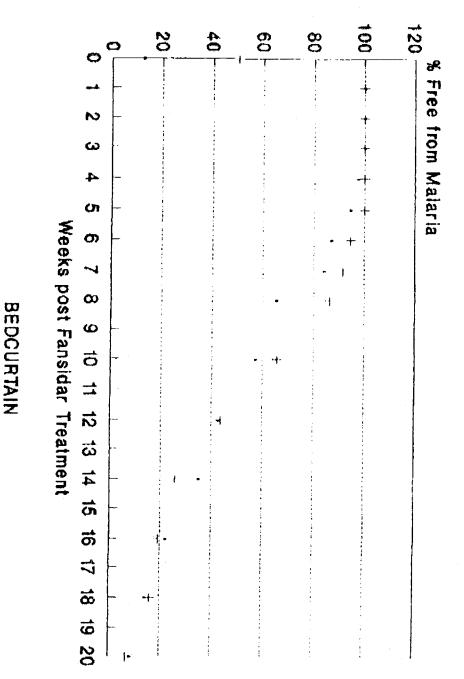


Fig. ត្ត. To show the malaria incidence before and after intervention with permethrin treated bednets in Mwelya village (Mombo) **Before intervention** 4. After intervention







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