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Ranking the Indicators: Another Approach for a Quick and Easy Evaluation of Malaria Vector Control Methods in Angola

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Abstract

This study proposes a new method, called “ranking”, to quickly assess four vector control methods: Long-Lasting Insecticide-Treated Nets (LLINs) alone, LLINs combined with insecticide-treated plastic sheeting (ITPS) (ZeroFly® model), ITPS (ZeroVector® model) alone, and indoor residual spraying (IRS) followed by ITPS installation. These methods were implemented in eight villages (two villages per vector control methods) near Balombo town, Benguela Province, Angola. Entomological and parasitological indicators were systematically analyzed, and a ranking system was applied to compare the efficacy of each method. Three entomological parameters-density, infectivity, and entomological inoculation rate (EIR)-and three parasitological indicators-plasmodial prevalence, parasite load, and gametocyte index-were evaluated. The evaluation with ranking of the efficacy of the four methods was different with entomological, or parasitological indicators. Gathering the entomological and parasitological ranks, showed that IRS followed by ITPS had the greatest efficacy, significantly reducing vector densities, EIR, plasmodial prevalence, and gametocyte indices. LLINs combined with ZeroFly® performed second, followed by ZeroVector® alone. The three methods including ITPS had almost the same overall efficacy, while LLINs alone ranked the lowest. Ranking these indicators provides a quick, and effective, approach for vector control evaluation. It clearly shows that, in the surveyed situation, the ITPS alone, or in combination with IRS, or LLIN, was of great help for malaria control, and could be recommended as a complement tool for the National Malaria Contro Programme.

Keywords Ranking; Entomological indicators; Parasitological indicators; Four methods of vector control; Long-lasting insecticide treated nets; Insecticide-treated plastic sheeting; Indoor residual spraying; Malaria

1 Introduction

In Angola, malaria is still a main public health problem, even if “the accuracy of malaria prevalence estimates has traditionally been somewhat compromised by incomplete reporting, inability to test due to stock-outs of the necessary materials, and other logistic challenges.” (Plucinski *et al.,* 2017).

Due to the presence of drug-resistant *P. falciparum* strains vector control is needed, but a recent house-spraying campaign was a failure (Somandjinga *et al.,* 2009) and the National Malaria Control Programme focused on increasing the scale of distribution of long-lasting insecticide-treated nets (LLINs).

In villages near Balombo town, it was observed that withdraw of used LLINs was common, with more than 50% of nets previously distributed being already torn, and discarded within two years (Carnevale *et al.,* 2021), emphasizing the need for a constant, and costly, redistribution of nets, and a new vector control method. Such behavior of no-use or misuse of nets was reported elsewhere (Koenker *et al.,* 2023).

Therefore, special attention was devoted to the recently developed tool called “insecticide-treated plastic sheeting” (ITPS) (or “durable lining” DL or “wall lining” WL) (Burns, 2005). It appeared interesting to be tested in Angola, where some studies on its acceptability had already been done (Messenger *et al.,* 2012). But its epidemiological impact, in the region, was still missing.

At the request of the National Malaria Control Programme, a comprehensive, long-term, village-scale, malaria vector control programme, was implemented around Balombo town. The objective was to compare the efficacy of ITPS (two models), used either alone, or in combination with LLINs model PermaNet® 2.0, or following two rounds of indoor residual spraying (IRS) with lambdacyhalothrin (Carnevale *et al.,* 2024).

The evaluation of malaria vector control program is often complicated with mathematical models sophisticated, and not always affordable (Molineaux and Gramiccia, 1980; Eckhoff, 2013). Therefore, we developed, and used, a simple method for a "quick and easy" evaluation of the Balombo vector control project by "ranking" the main entomological and parasitological indicators, and use them to compare the four methods of vector control.

The first phase of the project lasted five years: two years before implementation of vector control and three years after; with both entomological and parasitological field surveys.

2 Materials and Methods

2.1 Study sites

The National Malaria Control Programme (NMCP) selected eight villages in the Balombo (Benguela Province, Angola (12°21'S; 14°46'E). They were far enough from each other to avoid any contamination by mosquitoes, actively flying from one village to another, which would bias entomological evaluation. The site was already presented in several publications (Brosseau *et al.,* 2012; Carnevale *et al.,* 2021).

2.2 Tools used for vector control

Four tools were used for vector control: Long-lasting insecticide-treated Net (LLINs) PermaNet® 2.0; Insecticide-treated plastic sheeting (ITPS), model ZeroVector®: presented in rolls to be cut at the dimension of the walls; Insecticide-treated plastic sheeting (ITPS), model ZeroFly® presented as tarpaulins; Indoor residual spraying (IRS) with lambdacyhalothrin, two rounds followed by installation of insecticide treated plastic sheeting (ITPS).

2.3 Entomological methods

2.3.1 Mosquito collections and biological analysis

Entomological surveys were regularly done, every two months, in the eight villages, and were based on the classical CDC Light Traps (CDC-LT). For each session, 10 traps were installed, inside initially randomly selected houses, which were kept the same for the whole entomological surveys. Mosquitoes were collected and sorted out at the genus level, *Anopheles* species were determined on the spot with classical keys, then isolated in plastic tubes, and kept in dry atmosphere until their identification at the species level (*An. gambiae* complex) and for circumsporozoite ELISA analysis to get the infectivity of specimens caught i.e. a "sporozoite index" (s).

2.3.2 Entomological analysis

Three indicators were analyzed: density, infectivity and inoculation rate.

Absolute vectors numbers caught by the traps were converted into "capture rates" i.e. number of specimens per trap. For each year, and each village, the total number of *An. gambiae* and *An. funestus* ("Main Vectors" or "MV") collected was divided by the total number of CDC-LT implemented to get the "number of *Anopheles* vectors/trap" ("ma") considered as a reliable indicator of the "density/house."

Combining these data with infectivity rates ("s") gave some proxy of the "indoor risk of inoculation" or "entomological inoculation rate" (EIR), "h" with the Ross 'formula: h=ma.s.

Density, infectivity and EIR were analyzed for each vector control method, before (two years) and after (three years) implementation of control with same "weight" for each indicator; some "correcting factor" should obviously biased the ranking method.

2.4 Parasitological methods

Three indicators were considered: *Plasmodium* prevalence (also called “plasmodial prevalence” or “plasmodic index”, “PI”), parasite load (also called “parasitaemia”, “PL”), and gametocyte index (“GI”). Their evolution was analyzed according to villages and vector control methods, considering the same two periods: before (two years) and after (three years) vector control implementation; as it was done for entomological evaluation.
Such as for entomological indicator we gave the same “weight” for each parasitological indicator; some “correcting factor” should obviously bias the ranking method.

The evaluation of parasitological impact of vector control was based upon cross-sectional surveys (CSS) performed every two months, two weeks after entomological surveys, following the protocol implemented in Côte d’Ivoire (Henry *et al.,* 2005).
Classical thick blood films (TBF) were done in the field, and microscopically examined with double check of 10% of slides. Surveys were done on random samples of ≤15-year-old symptomless children.
Erythrocytes with *Plasmodium* were counted versus 200 white blood cells (WBC) to estimate the parasite load /ml of blood, assuming 8,000 WBC/ml of blood.
As *Plasmodium falciparum* was largely predominant against other *Plasmodium* species (few *P. malariae*, often associated with *P. falciparum*) analysis were done gathering all *Plasmodium* species.
The parasitological surveys were done as a follow-up of immunological studies (Brosseau *et al.,* 2012).

3 Results

3.1 Entomological results

3.1.1 *Anopheles* species caught

During five years; 202 catching sessions with CDC Light Traps were conducted in the eight villages, representing a total of 1,880 trap-nights, which caught 1,153 *Anopheles* specimens.

An. gambiae and *An. funestus* only were considered as the main vectors (“MV”) for further analysis.

3.1.2 Evolution of density according to vector control method

The average decrease after vector control was slightly different according to the method of vector control, with an average of 70%, from 61% with LLIN alone, to 83% with durable lining alone (Table 1).

Table 1 Evolution of the average of densities of *An. gambiae* and *An. funestus* caught by CDC Light Traps in paired villages, according to the method of vector control (VC) implemented, before and after vector control implementation. (LLINs = long-lasting insecticide-treated nets; ZF = insecticide-treated plastic sheeting model ZeroFly®; ITPS = insecticide-treated plastic sheeting model ZeroVector®; IRS = indoor residual spraying).

Methods of VC	Years 2007-2008 (= before VC)	Years 2009-2010-2011 (= after VC)	Difference
LLINs	0.671	0.263	-60.8%
LLINs+ZF	0.732	0.230	-68.6%
ITPS ZV	0.421	0.070	-83.4%
IRS then ITPS	0.501	0.127	-74.7%
Total	0.583	0.173	-70.4%

3.1.3 Evolution of infectivity according to vector control method

A total of 1,130 *Anopheles* were analyzed with ELISA and 28 were positive, *i.e.* a general positivity index of 2.48%, with 7.27% (n=55) for *An. gambiae*; and 3.60% (n=500) for *An. funestus* (Table 2).

Table 2 Evolution of infectivity rate of main vectors before vs. after implementation of each vector control (VC) method. (LLINs = long-lasting insecticide-treated nets; ZF = insecticide-treated plastic sheeting model ZeroFly®; ITPS = insecticide-treated plastic sheeting model ZeroVector®; IRS = indoor residual spraying; E+ = specimens positive with ELISA test).

Method	Before VC			After VC		
	E+	n	%	E+	n	%
LLINs	3	106	2.83	2	65	3.08
LLINs+ZF	5	119	4.20	0	56	--
ITPS ZV	3	66	4.54	1	21	4.76
IRS then ITPS	6	84	7.14	2	38	5.26
Total	17	375	4.53	5	180	2.78

The overall infectivity was the same before (4.53%; n=375) and after (2.78%; n= 180) vector control (χ^2=0.98; p=0.32; OR=0.60 [0.22-1.66]).
Even not statistically different, we could consider that infectivity "increased" by 10% in LLINs sample; remained the same in LLINs+ZF sample; increased by 5% in ITPS ZV and decreased by 26% in IRS then ITPS sample.

3.1.4 Evolution of the entomological inoculation rate (EIR) according to the vector control method

By combining the evolution of the "average density/trap" (Table 1) and the infectivity (Table 2), it is possible to estimate some proxy of the evolution of the risk of receiving one infected bite in one day, or one month (Table 3), according to the method of vector control implemented.
The entomological inoculation rate (EIR) decreased by #60% with LLIN and, similarly, by 80% with other methods, with an average of 82% after implementation of vector control. A level similar to the one usually reported by other vector control trials with LLINs.

Table 3 Evolution of the monthly entomological inoculation rate, before and after vector control (VC). (ma= density/trap, assuming it means the landing rate; s= infectivity, usually called sporozoite index; h= inoculation rate).

Method	Before/after VC	ma	s	h	diff
LLIN alone	Before	0.671	0.0283	0.569	
	After	0.263	0.0308	0.243	- 57.3%
LLIN+ZF	Before	0.732	0.0420	0.922	
	After	0.230	0.0278*	0.192	-79.2%
ITPS ZV	Before	0.421	0.0455	0.574	
	After	0.070	0.0476	0.099	- 82.7%
IRS then ITPS	Before	0.501	0.0714	1.073	
	After	0.127	0.0526	0.200	- 81.3%
Total	Before	0.583	0.0453	0.792	
	After	0.173	0.0278	0.144	-81.8%

Note: * assuming an infectivity similar to the average.

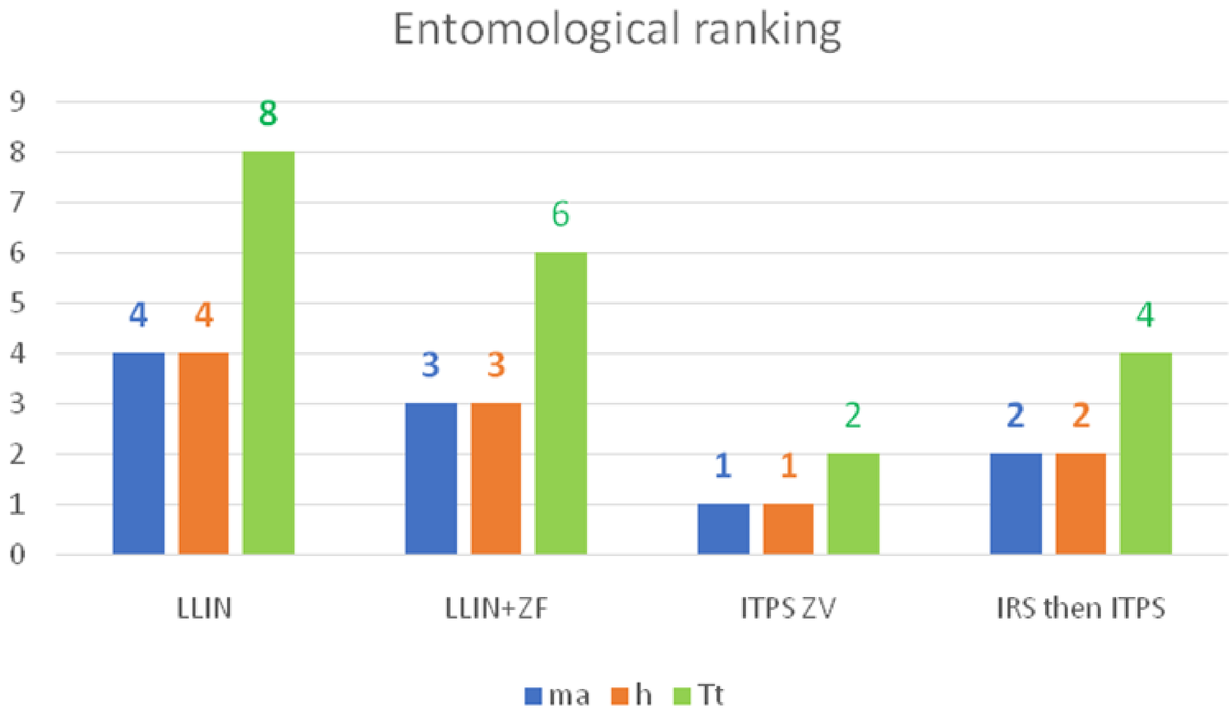
3.1.5 Entomological ranking

At entomological level; we considered two parameters: density ("ma") and inoculation rate ("h") (Table 4), as the infectivity (s) remained similar. We did a ranking, for each method of vector control with, arbitrarily, number 1 for the main impact and 4 for the lowest.
For density (ma), and EIR (h), the ranking was 1 for insecticide treated plastic alone, then 2 for the association in time of ITPS following house spraying; 3 for the association LLINs + durable lining and 4 for LLINs alone. Considering the two indicators the ranking was ITPS≥IRS+ITPS≥LINs+ZF>LLINs (Table 4).

Table 4 Entomological ranking (ma= density/trap, assuming its means the landing rate; h= inoculation rate).

Method VC	ma	h	Total
LLINs	4	4	8
LLINs + ZF	3	3	6
ITPS ZV	1	1	2
IRS then ITPS	2	2	4

The best entomological efficacy of ITPS ZV alone clearly appeared (Graph 1).



Graph 1 Entomological ranking (LLIN= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

3.2 Parasitological results

3.2.1 Evolution of the overall plasmodial prevalence

A total of 190 surveys were regularly performed. *P. falciparum* was largely predominant, and analysis of data considered all plasmodial infections together.

In the sample of symptomless ≤15-year-old children, trophozoites of *Plasmodium* were microscopically noticed in 5,330 of the 20,188 thick films done, *i.e.* an overall plasmodic index of 26.4%, and gametocytes were noticed in 553 films, i.e. an overall gametocyte index of 2.73%.

Each one of the four methods vector control induced a significant reduction of the plasmodic index (Table 5).

Table 5 Evolution of plasmodic index, before and after, implementation of each vector control (VC) method (OR= Odds Ratio) (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

Method of VC	Before VC	After VC	Difference	OR
LLINs	44.2% (n=3,104)	15.3% (n=2,737)	-65.4%	0.23 [0.20-0.26]
LLINs + ZF	45.4% (n=2,113)	11.5% (n=2,354)	-74.7%	0.11 [0.09-0.12]
ITPS ZV	35.7% (n=2,053)	10.1% (n=2,535)	-71.7%	0.20 [0.17-0.24]
IRS then ITPS	40.3% (n=2,521)	11.0% (n=2,771)	-72,7%	0.18 [0.16-0.21]
Total	41.7% (n=9,791)	11.9% (n=10,397)	-71,5%	0.19 [0.18-0.21]

With plasmodic index as indicator the efficacy of the four methods of vector control were LLINs+ZF>IRS then ITPS>ITPS ZV>LLINs.

3.2.2 Evolution of parasite load ≥1,000 par./ml

The impact of vector control was analysed with a threshold of parasite load of ≥1,000 par./ml of blood. Each method induced a significant reduction of the parasite load (Table 6).

Table 6 Evolution of parasite load ≥1,000 par./ml of blood before, and after, each implementation of each vector control method (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

Methods of VC	Before vector control	After vector control	difference
LLINs	26.6% (n=1,372)	18.6% (n=4,200	-30%
LLINs+ZF	29.3% (n=960)	15,6% (n=270)	-47%
ITPS ZV	20.4% (n=732)	13.3% (n=256)	-35%
IRS then ITPS	24.1% (n=1,015)	11.1% (n=305)	-41%

With parasite load as indicator the efficacy of vector control could be considered as LLINs+ZF>IRS then ITPS and ITPS>LLINs.

3.2.3 Evolution of gametocyte index

After vector control the gametocyte index was significantly reduced by 66%; from 4.16% (n=9,791) to 1.40% (n=10,397) and the drop of gametocyte was obtained by each one of the four methods (Table 7).

Table 7 Evolution of gametocyte index of symptomless ≤15-year-old children before, and after; implementation of each vector control method (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

	Before vector control	After vector control	Difference
LLINs	4.28% (n=3,104)	2.01% (n=2,737)	-53%
LLINs+ZF	4.97% (n=2,113)	1,61% (n=2,354)	-68%
ITPS ZV	3.11% (n=2,053)	1,10% (n=2,535)	-67%
IRS then ITPS	4.0% (n=2,521)	0.90% (n=2,771)	-77%

With gametocyte index as indicator the efficacy of each method of method control appeared as IRS then ITPS>LLINs+ZF>ITPS ZV>LLINs.

3.2.4 Parasitological ranking

Considering the Table 5, 6 and 7 the parasitological ranking could be estimated as indicated Table 8.

Table 8 Ranking of the parasitological efficacy of the four methods of vector control according the three parameters analyzed (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

	PI	PL	GI	Total
LLINs	4	4	4	12
LLINs+ZF	1	1	2	4
ITPS ZV	3	3	3	9
IRS then ITPS	2	2	1	5

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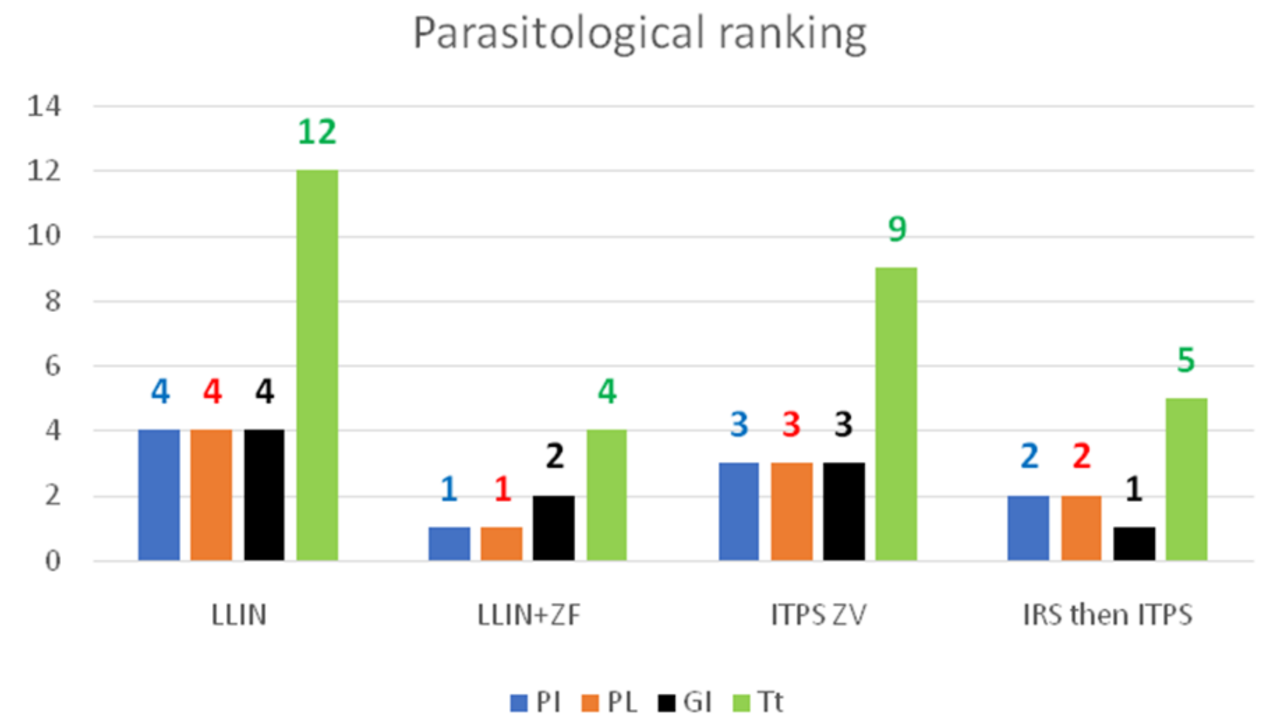
The best parasitological efficacy of the combination LLIN + ITPS ZF and IRS then ITPS clearly appeared (Graph 2).



Graph 2 Parasitological ranking (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

3.3 Overall ranking

Ranking entomological and parasitological efficacy procure an interesting proxy of the efficacy of each one of the four methods of vector control implemented (Table 9).

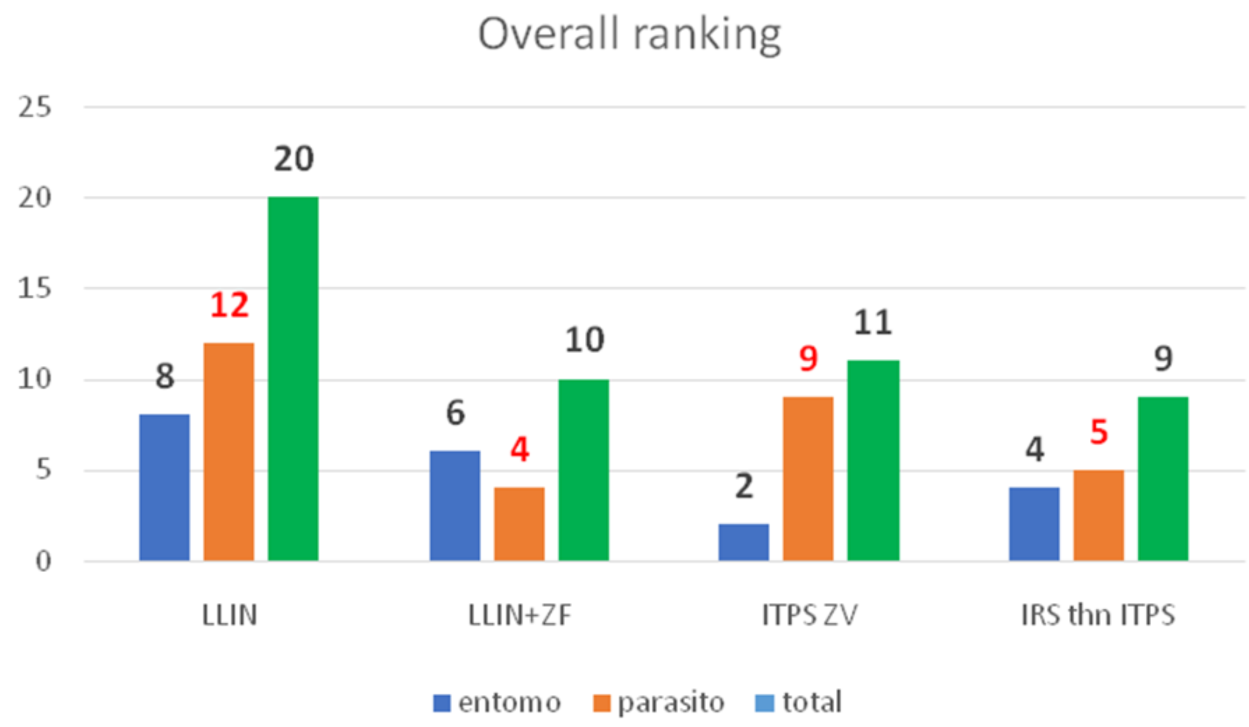
Table 9 Ranking of entomological and parasitological efficacy of each one of the four method of vector control implemented (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying; ma= density per trap; h= inoculation rate; Tt = total; PI= plasmodic index; GI= gametocyte index; Tt= Total).

	Entomology			Parasitology			Total	Total
Indicator	ma	h	Tt	PI	PL	GI	Tt	Tt
LLIN	4	4	8	4	4	4	12	20
LLIN+ZF	3	3	6	1	1	2	4	10
ITPS ZV	1	1	2	3	3	3	9	11
IRS then ITPS	2	2	4	2	2	1	5	9

Considering entomological and parasitological data together, it appeared that inside house spraying followed by installation of ITPS, on the walls, inside the house, procured the best efficacy in malaria control. Then were classified ITPS ZeroVector® alone, quite similarly the combination of treated mosquito nets+ insecticide treated plastic sheeting ZeroFly®; then LLINs alone.

Adding treated nets to treated plastic sheeting greatly increased the amount of insecticide in the house, with a small better entomological impact but not observed at parasitological level, and combining these indicators the impact was comparable between ITPS alone or combination ITPS+LLINs.

The overall ranking gathering entomological and parasitological one shows an almost similar level with ITPS, alone or combined with LLINs or following IRS, despite a clear difference in entomological and parasitological impact, mainly with ITPS alone (better entomological impact) or in combination with LLIN (better parasitological impact) (Graph 3).



Graph 3 Overall ranking of each indicator for each vector control method (LLINs= long-lasting insecticide treated nets; ZF= insecticide-treated plastic sheeting model ZeroVector; ITPS= insecticide-treated plastic sheeting; IRS= indoor residual spraying).

4 Discussion

Evaluation of vector control is crucial for improving malaria containment and, according to World Health Organization, new complementary indicators would be very valuable.

The Balombo project was designed to assist the National Malaria Control Programme in choosing appropriate, adapted tools for vector control, as addition to their other services, including case management with ACT and intermittent presumptive treatment for pregnant women.

The project was the first long-term village-scale malaria vector control trial planned, and implemented, in the region to compare, both, the entomological and the epidemiological efficacy of insecticide-treated plastic sheeting, alone or in combination with insecticide treated nets, or following house spraying, while considering long-lasting insecticide-treated nets as the “gold standard.” The project was carried out in completely natural conditions. The protocol followed the one implemented in Côte d’Ivoire to evaluate the efficacy of lambdacyhalothrin-treated-nets, with regular entomological, then parasitological surveys done every two months in areas with a high level of kdr-based pyrethroid-resistant *An. gambiae* population (Henry *et al.,* 2005).

Ranking entomological indicators and parasitological indicators according to their effectiveness gave different information, and this showed how important it is to implement comprehensive surveys. Combining entomological and parasitological indicators it appeared that, in the condition of the trial, at village-scale level, and on a long-term basis, the best method was the combination of indoor spraying followed by insecticide-treated plastic sheeting installation, and, almost equally, the combination of long-lasting insecticide-treated mosquito nets and insecticide-treated plastic sheeting or insecticide-treated plastic sheeting alone. Then, long-lasting insecticide plastic sheeting alone. While it has also to be taken into consideration that association of two methods largely increased the amount of insecticide and therefore could be involved in insecticide resistance pressure.

Biomarkers were recently developed (Drame *et al*., 2010; Brosseau *et al.,* 2012; Marie *et al.,* 2015) which procured interesting immunological information on the contact between anopheles and human being, and its evolution with implementation of long-lasting insecticide treated-nets.

With a not so easy to do immunological technic Brosseau *et al* (*loc.cit*.) analysed the human antibody (Ab) response to *Anopheles* whole saliva, used as biomarker of *Anopheles* exposure, investigated over a period of two years (2008-2009), in children between 2 to 9 years old, before and after the introduction of three different malaria vector control methods; deltamethrin treated long lasting impregnated nets (LLIN) and insecticide treated plastic sheeting (ITPS-Zero Fly®) (ITPS-ZF), deltamethrin impregnated Durable (Wall) Lining (ITPS-DL-ZeroVector®) alone, and indoor residual spraying (IRS) with lambdacyhalothrin alone in these villages around Balombo.

They considered that these three vector control methods significantly reduced malaria infections among the children studied and IRS significantly reduced the human-*Anopheles* contact. The number of *Anopheles*, positive blood smears, and the levels of anti-saliva IgG Ab were most reduced when LLIN and ITPS-ZF were used in combination, compared to the use of one vector control method alone, either ITPS-DL or IRS. Therefore, as a combination of two vector control methods is significantly more effective than one control method only, this control strategy should be further developed at a more global scale.

This immunological conclusion was in the line of our easy to do ranking combining classical entomological and parasitological indicators and it underlined the need of comprehensive studies before drawing any conclusion on the efficacy of vector control.

For Burkhot and Graves (1995) “Estimating malaria transmission in the human is fraught with problems of reconciling clinical illness with parasitological status. It follows that there is a role for entomological assessments as an independent outcome variable and as a process indicator.”

In the framework of comparative studies entomological and parasitological parameters were recently studied in Dakar (Sénégal) (Diallo *et al.,* 1998a; Diallo *et al.,* 1998b; Diallo *et al.,* 2000).

A malaria survey was also carried out in the small village about 45 km North-West of Antananarivo (Madagascar) to evaluate the level of transmission of *P. falciparum* by entomological, parasitological and serological indices (Ralisoa *et al.,* 1991).

The heterogenity of entomological and parasitological indices, according to the landscape, was recently studied in Kenya (Otambo *et al.,* 2022), and it was concluded that “the heterogeneity and seasonality on vector densities, parasite transmission, and *Plasmodium* infections in humans, emphasizes the importance of tailoring specific adaptive environmental management interventions.”

The choice of vector control methods must be based on comparative, comprehensive, evaluations but, quite often, the comparative evaluation of several methods, and several indicators, need sophisticated, mathematical, analysis (Dietz *et al*., 1974; Molineaux *et al*., 1978; Molineaux and Gramiccia, 1980; Dietz, 1988; Molineaux and Dietz, 1999; Smith *et al.,* 2006) not always available.

This is why we preconise, as first approach of the evaluation, a simple and easy method, the ranking, which procures a quick proxy of the best adapted method, combining entomological and parasitological indicators, to which could easily be added other indicators such as biomarkers, or other such as clinical impact, overall infant mortality (Lengeler *et al.,* 1996) etc.

Ethical Approval

Ethics Statement This Balombo study was conducted in accordance with the Edinburgh revision of the Helsinki Declaration and was approved by the National Malaria Control Program of the Ministry of Health of Angola, the Ethical authority in charge of approving studies on malaria research in Angola. Written consent (signed by the head of each household) was obtained for all individuals enrolled in the study by the SONAMET Company in its Malaria Control Program (MCP) which is responsible for malaria surveillance and control amongst company employees and their families (Brosseau *et al.,* 2012).

These entomological analyses are a part of a comprehensive evaluation of a vector control programme done with the Angola National Malaria Control Programme and Benguela Provincial Public Health Authorities.

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Competing Interests

Authors have declared that they have no competing financial interests, non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

No a.i. used for this article.

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