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Co-Evolution of the Presence of Long Lasting Insecticide Treated Nets and Plasmodium falciparum Welch, 1897 Prevalence in Cahata Village (Benguela Province, Angola) during a Village Scale Long-Term of Malaria Vector Control Program

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Authors' contributions

This work was carried out in collaboration among all authors. Author PC elaborated the protocol, participated in field surveys, did analyzing of data and drafted the report. Authors JCT and VF participated in field surveys, author VF did microscopical examination of blood slides and verification, SC participated in the interpretation of human behaviour. Author FG finalized statistical analysis and writing of the document. All authors read and approved the final manuscript.

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ABSTRACT

A long term village scale vector control programme was implemented since 2007 in 8 villages around Balombo town (Benguela Province) to compare the efficacy of 4 methods of vector control (1,2). One of them are deltamethrin treated Long Lasting Nets ("LLIN") "Perma©Net 2.0" with a complete coverage of every sleeping units in 2008 in 2 villages, Caala and Cahata. Cahata was

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surveyed for 10 years with regular parasitological cross sectional parasitological surveys. During the 3 years following LLIN implementation, a check of their actual usage in every house, or on sleeping units of each patient examined during every parasitological surveys showed that 3 years after the full LLIN distribution less than 50% were still in use, and this percentage regularly decreased to reach 10% and less in 2015 (with 0% during the survey done in October 2005). But *Plasmodium falciparum* prevalence still decreased with low level until 2014 then it started (slightly) increasing in 2015, and the following years, which correspond to both the National malaria outbreak and the least percentage of LLIN used! But even at that time the plasmodial load was significantly lower in "LLIN users" than in non users showing some maintained personal protection.
Even 10 years after the full coverage in LLIN no rebound effect was actually noticed in such natural conditions of life and plasmodial prevalence was even 2 times lower than before the implementation of vector control.
Information gained were of great importance for the National Malaria Control program in term of nets replacement with 50% missing in 3 years underlining the need of "stronger" nets and community sensitization for the sustainability of positive results gained with LLIN.

Keywords: Vector control; plasmodia index; deltamethrin insecticide; impregnated nets; parasite load.

1. INTRODUCTION

Since their first trial in the 80' [1-5] and the demonstration of their striking efficacy in reducing malaria transmission and morbidity and overall infant mortality [6-8] pyrethroid treated nets (Insecticide Treated Nets "ITN" becoming "Long Lasting Insecticide Treated Nets, or "LLIN" with industrialized long lasting impregnation) became the main method for malaria vector control.

Recently it was estimated that interventions have averted 663 million clinical cases since 2000 (542-753 credible interval). Insecticide-treated nets and the most widespread intervention were the largest contributor (68% of cases averted) [9]. Therefore, scaling-up "Long lasting insecticide treated nets program - LLIN", could have averted some 450 million of malaria cases between 2000 and 2015. In spite of the spreading of pyrethroid resistance among the main malaria vectors [10-12], LLIN remain the basic tool for malaria prevention and involved in any National Malaria Control Program along with case management, quick and reliable diagnosis of malaria with rapid detection test, quick and efficient malaria treatment with artemisinin combined therapy etc. The ultimate goal being the elimination of malaria in a near future.

However, one of the key point to get actual malaria prevention with large-scale vector control programme is the sustainability of the operations with both long-term efficacy of insecticide treated materials [13-15] and their acceptability by the communities [16-23]. With the National Malaria Control Programme of Angola, a long-term village scale about malaria control project it was implemented, since 2007, in 8 villages around the town of Balombo (Benguela Province), to compare the efficacy of 4 methods of vector control including the classical Long Lasting deltamethrin Insecticide Treated nets (LLIN) model Permanet 2.0 (P 2.0), alone in 2 villages (Caala and Cahata) [24,25].

The multidisciplinary evaluation included entomology, parasitology and immunology [24,25,26,27] with studies on human behavior and perception by direct observation and focus group discussion.

We reported here observations done in Cahata (12°20'S ; 14°48'E) during each parasitological survey on the actual usage of LLIN, at house and individual level, of patients examined during the 3 years following the full coverage of every sleeping units to compare their respective evolution. We also wanted to verify the long-term evolution of Plasmodium falciparum prevalence and the eventuality of any "rebound effect".

Due to several events in Caala it was not possible to do the same in-depth regular observations while parasitological surveys have nevertheless been done.

2. MATERIALS AND METHODS

In 2007, at the beginning of the trial, Cahata was composed of 138 houses; 484 inhabitants and 442 sleeping units (a sleeping unit is anything regularly used to sleep such as mattress, etc).

The free of charge distribution of LLIN PermaNet© 2.0 were done in 2 steps:

- February 2007: distribution of 310 LLIN with the objective of "at least one LLIN/house";
- February 2008: distribution of 195 LLIN with the objective of "one LLIN/sleeping unit" to get full coverage;
- December 2008: 25 supplementary LLIN to achieve the full coverage according to already noticed some "miss of LLIN".

Therefore, a total of 530 LLIN were distributed for 442 sleeping units, and it was considered that the full coverage was thus obtained.

In Cahata, as well as in other villages, each house received a number (on the door) and localized by GPS to report each Plasmodium positive case on a computer map to notice any eventual cluster [27].

Parasitological evaluation was based upon regularly done cross sectional surveys ("CSS") on randomized samples according to the number of the houses, during each survey a thick blood films was prepared for further microscopical observation and some other drops of blood were taken on a filter paper for immunological analysis [26].

The present study was devoted to the LLIN use during the years following their distribution and the concomitant evolution of the Plasmodium falciparum prevalence ("Plasmodic Index" or "PI").

From 2011, i.e. 3 years after full coverage in LLIN, and during the 5 following years, simultaneously of blood slides a check was systematically done on the presence, or not, of LLIN still used in house or on sleeping unit of each patient involved in the 10 cross sectional parasitological surveys done.

Blood slides were microscopically observed in the Medical Department of the Sonamet© company in Lobito (which supported these studies in the framework of their Malaria Control Programme) with double check on randomized 10% of slides in the Parasitology Laboratory of OCEAC (Yaoundé).

Parasitaemia it was estimated in counting the number of Plasmodium versus 200 white cells then estimated as the number of parasites/µl considering 8000 WC/µl of blood.

Parasitaemia was analyzed by the non-parametric Mann-Whitney test and graph pad software.

3. RESULTS

3.1 Parasitological Long Term Surveys

A total of 4530 blood thick films were made during the cross sectional surveys done from 2007 to 2018 and 926, i.e. 20.4%, were found positive with P.falciparum parasite (Table 1).

Several points must be noticed (Fig.1.):

- a strong and significant reduction of plasmodial prevalence occurred after the first and the full coverage in LLIN in 2008 then the Plasmodial Index regularly decreased for almost the 5 following years,
- Between 2011 and 2014: only 29 of the 1379 thick films examined were P. falciparum positive i.e. a Plasmodic Index (PI) of 2.10%; and for the period 2011-2015 the PI was 3.04% (n= 1643);
- The plasmodic prevalence was thus maintained at a low level till 2015 where it was observed an increase also noticed the following years and which could be related (in part) to the National Malaria outbreak which occurred in 2015;
- 11 years after the first distribution of LLIN the plasmodial prevalence was still lower that before any vector control implementation in the village (27.4% versus 55.5% i.e. 2 times less).

3.2 Observations on the Presence of LLIN

3.2.1 Check of the presence of "at least 1 LLIN /house"

A total of 438 houses were visited during the 10 field surveys done between 2011 and 2015 and the actual presence of "in use" LLIN nets were noticed in 111 i.e. 25.3% of houses (Table 2).

Thus 3 years after the full coverage in LLIN, around 40% of house have still "at least one net/house" and this percentage regularly decreased with time (Fig. 2) becoming around 20% after 5 years and around 10% the 6th year following LLIN distribution.

3.2.2 Observations at individual level ("sleeping units")

A total of 1643 sleeping units were examined along with the CSS done between 2011 and 2015 and the actual presence, or not, of LLIN was noticed for each patient (Table 3).

Table 1. Parasitological cross sectional surveys in Cahata from years 2007 to 2018 (Nb PF+ = number of P. falciparum positive thick films; Nb ex= number of thick films examined; % (PP = percentage of positive thick films or Plasmodial Prevalence)

Years	Nb PF+	Nb ex	% (PP)
Y 2007	413	744	55.5%
Y 2008	242	655	36.9%
Y 2009	124	616	20.1%
Y 2010	46	691	6.7%
Y 2011	8	157	5.1%
Y 2012	10	662	1.5%
Y 2013	7	441	1.6%
Y 2014	4	119	3.4%
Y 2015	25	264	9.5%
Y 2016	13	57	22.8%
Y 2018	34	124	27.4%
Total	**926**	**4530**	**20.4%**

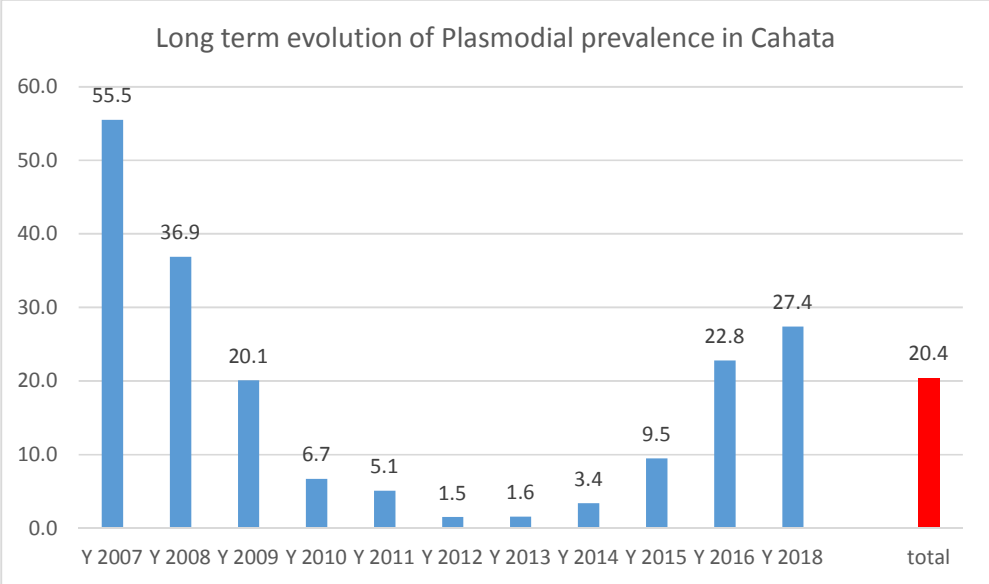

Fig. 1. Long-term evolution of plasmodial prevalence in Cahata

Table 2. Number of houses checked every year along with blood film surveys and percentage of houses with "at least one net/house"

Years	Nb houses visited	Nb house with "at least 1 net/house"	%
Y 2011	49	21	42.9%
Y 2012	163	54	33.1%
Y 2013	112	25	22.3%
Y 2014	33	4	12.1%
Y 2015	81	7	8.6%
Total	**438**	**111**	**25.3%**

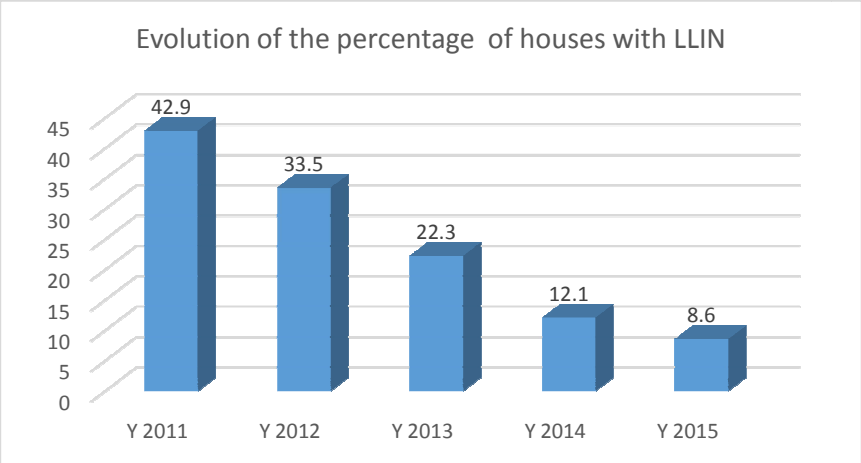

Fig. 2. Evolution of percentages of houses with "at least one net/house" from 3 years after full coverage

Table 3. Number of sleeping units with/without LLIN checked with the 10 parasitological cross sectional surveys in Cahata (LLIN+ = with LLIN; LLIN-= without LLIN)

Years	LLIN+		LLIN-		total
	Nb	%	Nb	%	
Y 2011	68	43.3	89	56.7	157
Y 2012	155	23.4	507	76.6	662
Y 2013	96	21.8	345	78.2	441
Y 2014	19	15.9	100	84.0	119
Y 2015	24	9.1	240	90.9	264
Total	**362**	**22.0**	**1281**	**78**	**1643**

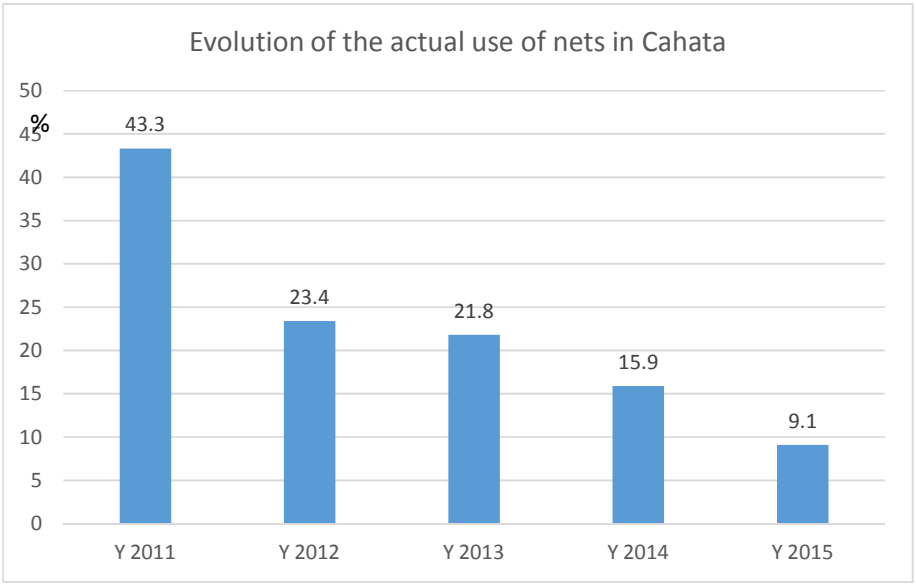

Fig. 3. Evolution of percentages of people having still mosquito nets installed over their beds. Such survey on LLIN use it was stopped in 2015 as in October not a single nets was found while checking 124 "beds"

The use of nets decreased rapidly as 3 years after the full coverage in LLIN (in December 2008) 40% of people have still a net on their "bed" and 4 years after some 80% of people have no net (Fig 3), less than 10% of people are still correctly protected after 7 years.

3.2.3 Use of net at house and individual level

Comparing the data dealing with the use of net at house or individual level showed clearly the same quite linear trends (Fig 4) which confirms how relevant it could be (and easier to do) to work at house level as it was already demonstrated in another village (Capango) of the Balombo village scale vector control project [27].

3.3 Plasmodial Prevalence and Use of LLIN

From Table 1, 2 and 3 it is possible to compare the concomitant evolution of plasmodial prevalence and the percentage of LLIN still present at house level (Fig 5a) or individual (sleeping unit) level (Fig 5b).

It thus appeared that the plasmodial prevalence started to increased when the coverage of houses or sleeping units reached 20% and it was boosted by the outbreak of malaria in 2015 showing the absolute need of vector control in such worsening situation.

3.4 Plasmodial Prevalence and Individual Use of LLIN

54 of the 1643 blood films made with checking the actual presence, or not, of LLIN on sleeping units of every patients (Table 3) were found positive with Plasmodium falciparum (i.e. an overall 3.29% plasmodial prevalence) with a striking difference between LLIN users or not (Table 4). Actually 8 plasmodial infection were diagnosed among LLIN users (# 15%) while 46 were observed among non-users (# 85% of infection).

3.5 Parasitaemia According to use/no use of nets and Period

The analysis of parasitaemia according to the presence or not of LLIN (Fig. 6) shows that parasite load:

- were similar in users and not users of net (LLIN+ and LLIN -) during the period 2001-2014 (P value= 0.55) and the year 2015 (P value= 0.29);
- were significantly higher in 2015 than in 2011-2014 (P value < 0.0001) showing the impact of the malaria outbreak, noticed also at plasmodic index level;
- were similar in 2015 and 2016 (P value= 0.23);
- were significantly different in February 2018 compared to February 2007 (P value= 0.00010), compared to 2011-2014 (P value =< 0.0001) and to 2016 (P value = 0.0176).

It thus appeared a significant increase of parasite load in 2015, along with the withdrawal of net and the national and provincial malaria outbreak, and this increase of parasitaemia was statistically significant in non-users on LLIN but not among LLIN users who get still a personal protection with their nets.

3. 6 Parasitological situations in February 2007 (before the 1rst distribution of nets) and in February 2018

Comparing the P. falciparum prevalence in children ≤ 15 years old in February 2007 (i.e. before the first distribution of nets) and 11 years later, in February 2018, showed that no "rebound effect" occurred in these natural situations and even 11 years later the plasmodic index of children was significantly lower than before any vector control: respectively 71.7% (n= 113) and 31.6% (n= 79) (X2= 30.4; OR= 0.18 [0.09-0.34]).

It is also worth underlining the great decrease of gametocytic index from 10.6% to 2.53%.

4. DISCUSSION AND CONCLUSION

The First Conference on Malaria in Equatorial Africa held in Kampala (1950) discussed the possibilities of malaria eradication thanks to newly available DDT to be used for inside residual spraying (IRS) of every houses in malarious areas. Two schools of thinking appeared: those who could be called "go ahead" and advocated immediate action to reduce as much as possible the burden of malaria and to get in a relatively short time its eradication. In addition, those who could be called "wait and see" waiting for more scientific information and seeing what could be the impact (negative impact?) of reducing the transmission or parasite prevalence (or load) on "acquired immunity" of people of endemic malarious areas and possible rebound effect or epidemic outbreak.

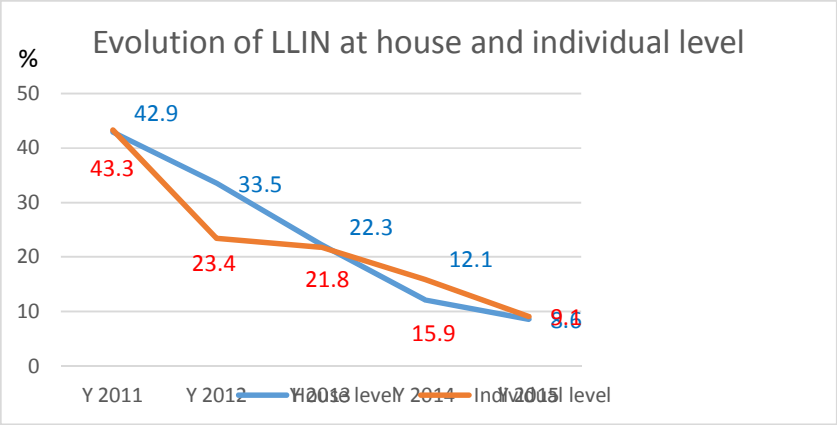

Fig. 4. Yearly evolution of the percentage of houses with at least one LLIn and sleeping unit still covered with LLIN

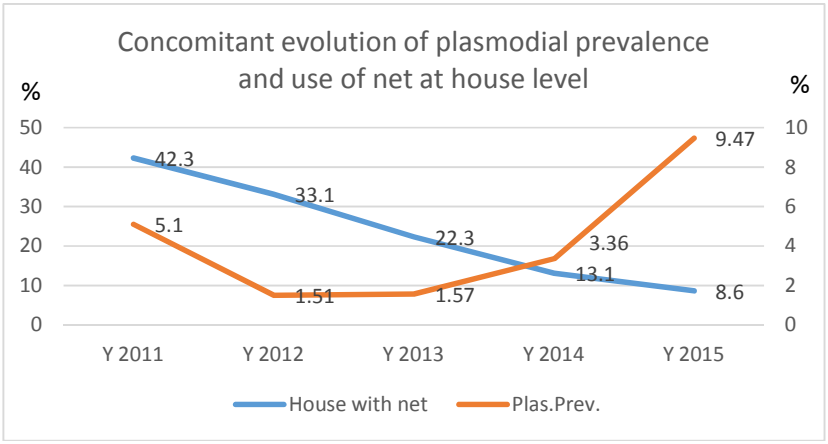

Fig. 5a. Concomitant evolution of plasmodial prevalence and nets use at house level

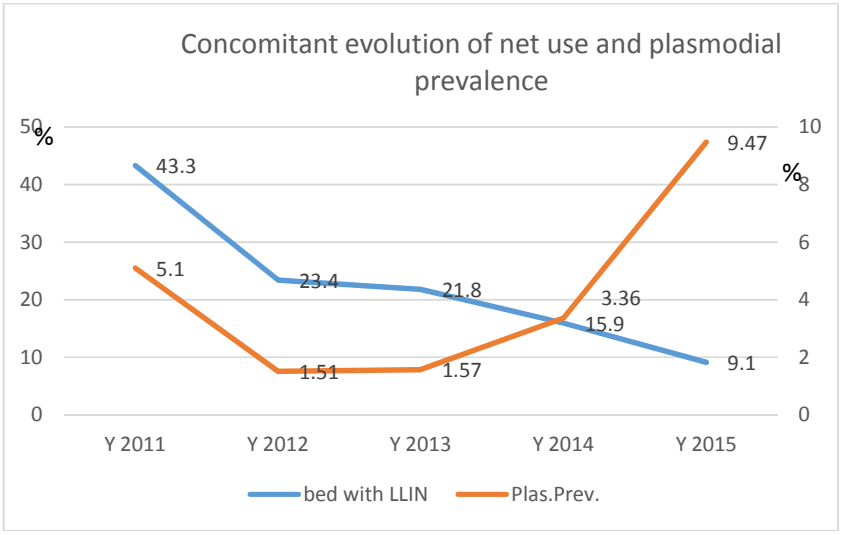

Fig. 5b. Concomitant evolution of plasmodial prevalence and nets use at individual level

Table 4. Evolution of Plasmodial prevalence and LLIN used at individual level in Cahata (Nb ex. = number of thick blood films; Nb PF+ = number thick film with Plasmodium falciparum; Nb PF+ in LLIN + = number of positive thick film among LLIN user; PF+ in LLIN- = number of positive thick films among non-users of LLIN)

Years	Nb ex.	Nb (%) PF+	Nb PF+ in LLIN+	Nb PF+ in LLIN-
Y 2011	157	8 (5.10)	1	7
Y 2012	662	10 (1.51	0	10
Y 2013	441	7 (1.59)	2	5
Y 2014	119	4 (3.36)	1	3
Y 2015	264	25 (9.47)	4	21
Total	**1643**	**54 (3.29)**	**8**	**46**

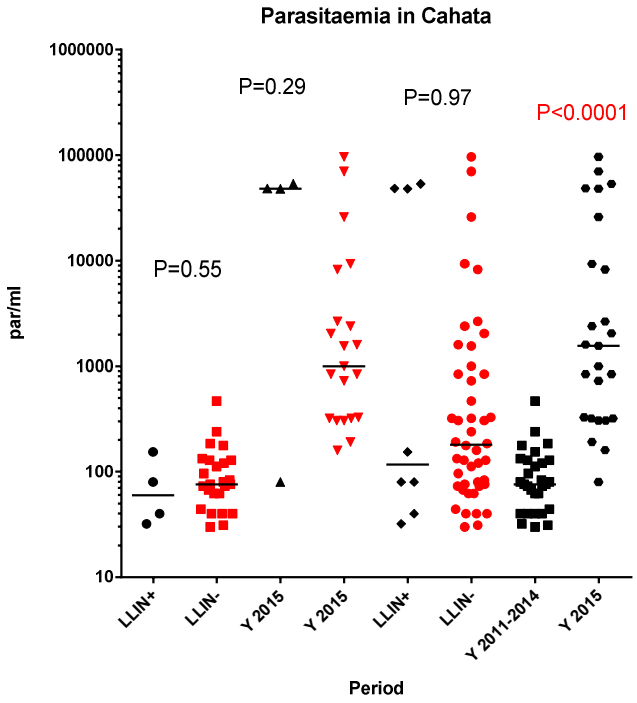

Fig. 6. Evolution of P. falciparum load according to the situation (with vs without LLIN) and years

The Conference recommended the implementation as soon as possible of malaria control with “modern tools” in one hand and to develop some “pilot zone” to experiment other several tools or protocols without waiting the results of other trials (28-30).

It is interesting to note that this subject on reduction of immunity following vector control was put back on the agenda with the arrival of ITNs as new tool for malaria vector control and their scaling up (31). Some “con” considering that their undoubtfully efficacy at short and middle term would in fact be followed by a “rebound” in long-term due to the drop in immunity induced by the reduction in inoculations and suggest that any intervention aiming to reduce transmission will not, on a long-term basis, reduce the burden of malaria in the majority of epidemiological contexts observed in tropical Africa” (32) while “pro” did not agree on that (33) and consider that it is worth to go ahead for malaria control with all available tools combined in a comprehensive programme. And the several hundred millions of malaria cases averted in 2000-2015 how right they are in promoting control involving both

vector control and case management [11] while developing new tools, insecticide and drugs.

The Balombo project (Benguela Province, Angola) was elaborated with the National Malaria Control Programme to compare the efficacy of classical tools such as PermaNet© 2.0 deltamethrin long lasting net or lambdacyhalothrin inside residual spraying used either alone or in association with the recently developped Insecticide Treated Plastic Sheeting (ITPS) [34-39] as the association of several methods was advocated [40]. The project was elaborated, implemented and analyzed in the long term basis, 10 years, to check, moreover the short term efficacy but also a long term parasitological impact and human behavior in keeping or withdrawing the net, especially in the village of Cahata, where large distribution of Permanet was done in two steps to actually cover any sleeping units since 2008.

The trial was done in complete natural situation and it reflects the reality and not any experimental conditions. After the full coverage in PermaNet it was soon observed a striking and significant reduction of Plasmodium falciparum prevalence and parasitaemia as expected but also an interesting and worth noticing general trends of decrease of Plasmodial prevalence during 6 years; from 55.5% at the beginning of the trial to 3.4% some 7 years after i.e. a 94% reduction.

Still on a long term basis 3 points must be underlined:-from 2015 the plasmodial prevalence increased and this could be related to the National and Provincial Malaria outbreak but also to the general withdrawal of nets, only 10% of people had still some net on their bed and they were greatly torn (which could explain why people removed them). - 3 years after full coverage some 40% of sleeping unit were still covered, some 40% of houses had still “at least one net/house”. Thus in 3 years more than half of nets “disappeared” and in 5 years some 80% of houses had no more nets. This has to be kept in mind when thinking at the replacement of nets to get the targeted 80% coverage need for spatial impact (people protected even if no net). - but even when outbreak occurred the parasite load of “LLIN users” did not significantly increased compared to the period 2001-2014 (showing that some personal protection was still maintained) while it actually increased in non-users of nets showing the increase of malaria risks linked to the usual human behavior.

Nevertheless it has to be mentioned that even if it increased since 2015 the plasmodial prevalence remained lower than those noticed in others villages of the areas where no same organized vector control measures were undertaken [25] and no rebound was ever noticed in such natural conditions.

These observations must be considered with the increasing risk of exposure of population to vectors bite and malaria parasite transmission according to their usual behavior and the needs of stronger nets with the needs of regular social sensitization campaigns.

As it was well observed in a study done in a three years study in Soumousso village (Burkina Faso), the motivation for the use of bednets decreased after less than a year and having a net is not using a net. In order to bridge the gap between possession and use of bednets, concerted efforts are required to change behaviour by providing accurate information [41], starting by knowing the perception of the disease by the targeted communities. The problems for net usage in the long term were raised [42]. For Paré-Toé (2009), if the individuals are not sufficiently motivated to use ITNs in their daily life, their widespread use on a long-term basis will not be successful [41]. And the Cahata (Angola) observations demonstrated the withdraw of nets (#50% in 3 years) with some collective protection for some years but when the coverage is too low plasmodial prevalence increased again and further vector control operations are clearly needed with other measures (clinical and social) to sustain malaria control for its schedule elimination.

CONSENT

As per international standard or university standard, respondents’ written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s). This analysis is a part of a comprehensive evaluation of a vector control programme done at the request and with the Angola National Malaria Control Programme and Provincial Public Health Authorities.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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