BI *BioImpacts*, **2012**, 2(3), 159-166
doi: 10.5681/bi.2012.024
http://bi.tbzmed.ac.ir/

Assessment of Long-Lasting Insecticidal Nets (LLINs) on Vectors and Malaria Transmission in the Commune of Aguegues, Benin

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ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 18 June 2012
Revised: 20 August 2012
Accepted: 26 August 2012
ePublished: 31 August 2012

Keywords:
Olyset® Net
Anopheles
Plasmodium falciparum
Hemoglobin

ABSTRACT

Introduction: To overcome the problems of periodic re-impregnation of mosquito nets and low rates of treatment, the commune of Aguegues was chosen to evaluate the effects of Olyset® nets on malaria transmission and against *An. gambiae*. ***Methods:*** 87 old Olyset® nets installed five years ago were identified in the village 'Akpadon'. 10 untreated nets were installed in 10 structures of type "a bedroom and parlour" in the village 'Akodji'. Four rooms without nets were identified in the village 'Donoukpa'. Entomological and epidemiological evaluations were conducted during the May to October 2011. 24 sessions of capture or 2088 men-nights resulted in the capture of 30,608 mosquitoes. ***Results:*** The entrance of anopheles was significantly reduced in the village with Olyset® nets. 45% of mosquitoes captured inside rooms with Olyset® nets were found dead after 24 hrs of observation. Overall, parasitemia was very low in the treated village (4.52%). 18 (4.64%) cases of malaria fever were from Akpadon with 7.5% positive blade smear, 29 (10.98%) were from Akodji with 8.37% positive blade smear, and 80 (95.23%) come from Donoukpa with 38.09% positive blade smear. The Olyset® nets and untreated net were adjusted hemoglobin levels. ***Conclusion***: Olyset® net had a very high knock down effect and is an alternative in malaria control.

Introduction

The use of insecticide treated bed nets (ITNs) to protect vulnerable population from malaria parasite transmission is one of the main strategies recommended by the Roll Back Malaria (RBM) partnership (WHO 2002). Mosquito nets treated with pyrethroid insecticides have been shown to cause a decline in malaria morbidity and mortality in several trials carried out in different countries (Malima *et al* 2008, Lengeler 2004, Nevill *et al* 1996). However, there have been some problems which have influenced insecticide-treated nets' application for long periods of time. These problems comprise periodic re-impregnation, low treatment rates and effects of repeated washing (Sood *et al* 2011, Dev *et al* 2010). To overcome these problems, long-lasting insecticidal nets (LLIN) have been developed. These nets in which the insecticide treatment is intended to last for the lifetime of the nets are advocated by the World Health Organization for protection against malaria (WHO 2007). LLINs have covered the institutional net-buying market in recent years (Wise 2004, Teklehaimanot *et al* 2007). Olyset® is approved to be the longest of LLIN registered (Teklehaimanot *et al* 2007, N'Guessan 2001, Gonzales *et al* 2002, Graham *et al* 2006). Olyset® nets evaluated in other countries showed encouraging results (Faye *et al* 1998, WHO 2001). Cone bioassays showed that Olyset® nets in use in some Tanzanian villages for at least seven years retain their insecticidal activity (Tami *et al* 2004). However, biological tests performed on Olyset® nets of two years of use showed mortality rates below the threshold set (Lindblade *et al* 2005).

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Also, Lindblade *et al* (2005) and Gimnig *et al* (2005) recently reported that Olyset® nets poorly performed in laboratory and in field conditions against *Anopheles gambiae*. Results of a phase II trial carried out in India showed that Olyset® nets are wash resistant and effective for several weeks in providing the desired level of mortality in *Anopheles culicifacies* Giles and *An. fluviatilis* (Diptera: Culicidae) (Sharma *et al* 2006, Sharma *et al* 2009, Ansari *et al* 2006) and against *An. stephensi* and *Aedes aegypti* (Jeyalaksmi *et al* 2006).

While the protective efficacy and effectiveness of ITNs have been well established (Choi *et al* 1995, Goodman *et al* 1999, Schellenberg *et al* 2001, ter Kuile *et al* 2003a, b, c, Meltzer *et al* 2003, Phillips-Howard *et al* 2003, Wiseman *et al* 2003, Lengener 2004), many challenges remain: access and availability of ITNs, cost of ITNs on the open market in resource poor setting, timely re-impregnation of bad nets and issues of proper ITN adherence, and deployment and use. Recently, local perceptions of the acceptability of bed nets and insecticide and the determinants of ITN possession and use have been explained in some studies (Winch *et al* 1994, 1997, Binka and Adongo 1997, Agyepong and Manderson 1999, Schellenberg *et al* 2001, Alaii *et al* 2003).

In view of this, the commune of Aguegues was chosen to evaluate the Olyset® nets on malaria transmission and against *An. gambiae*, the main vector of the disease.

Materials and methods

Study area

Aguegues community is made up of 21 lakeside villages and covers a surface area of 103 km^2 which only 52 km^2 out of the surface is habitable. Aguegues is situated in the south west of Ouémé and has the same latitude of the Porto-Novo municipality. At the lower part of the river Ouémé, Aguegues is composed of small islands of Alluviale and is annually submerged by floods for three to five months. Three villages from this commune were selected for study. 87 old Olyset® nets installed five years ago were identified in the village Akpadon. These Olyset® nets were intact containing no holes. Akpadon was taken as treated village. 15 structures (a bedroom and parlour) were identified in the treated village. The nets were installed in the bedrooms. 35 children under 5 years slept under these nets. 10 new nets were bought from the pharmacy and installed in the bedrooms of 10 structures in the village Akodji considered as control village #1. 22 children under 5 years slept under these nets. 4 bedrooms without nets were identified in 4 structures in the village Donoukpa considered to control village #2. 7 children under 5 years old slept in these rooms. The study was conducted from May to October 2011.

Olyset® net

Olyset® net is a new type of mosquito net developed by Sumitomo Chemical Co. Ltd. (Japan), in which the insecticide (permethrin used at a concentration of 2% w/w, about 860 mg/m2) is incorporated by fusion into a fiber composed of polyethylene resin. Compared to an impregnation by immersion where the product is simply placed on the fibers, the manufacturing process of Olyset® nets incorporates the insecticide in the support at the time of polymerization. As the incorporation of the insecticide in the substrate is performed at high temperatures, only permethrin - from all the compounds present pyrethroids - supports such a process without being altered in its molecule. The effectiveness of Olyset® is guaranteed for a minimum of 5 years, during which the nets does not need to be impregnated again.

Some characteristics of Olyset® nets

- WHO Specification No. 331/LN (July 2006)
- Insecticide and dosage: Permethrin 2% (w/w)
- Fiber: Polyethylene Monofilament
- Denier>150 denier
- Flammability: Class 1 (16-CFR 1610)
- Weave type: knitting
- Size: (160 x 180 x 150) (180 x 190 x 150), (160 x 180 x 210)
- Area: 13.08 m^2, 14.52 m^2, 17.16 m^2
- Color: blue

Entomological evaluation

Hand collections of indoor resting mosquitoes were done by suction tube and flashlight. It lasted 4 nights by month in the three study villages from 6 pm to 6 am. 29 houses were selected (15 for treated villages, 10 for control village #1, 4 for control village #2) and used for sampling indoor resting mosquitoes. Density per man per night of collection was calculated. Data were pooled for each month and then statistical analysis was carried out. Field collected mosquitoes were then brought to the laboratory to be processed further (entomological parameters).

Table 1. Mosquitoes captured by structures

Villages	Structures	Catchers per structure	Catchers	Sessions	Men-nights	Captured mosquitoes per village	Captured mosquitoes per structure
Akpadon	15	03	45	24	1080	10800	0720
Akodji	10	03	30	24	0720	15120	1512
Donoukpa	04	03	12	24	0288	14688	3672
Total	29	09	77	72	2088	30608	5904

Mosquitoes landing on bed nets were collected four nights per month in the treated villages and on baits in without net village. Informed consent was obtained from the human volunteers participating in the study as baits. Human volunteers were allowed to sleep inside the bed nets and mosquito collections were done from dusk-to-dawn (6 pm to 6 am). Exit traps were fixed on the windows. Three mosquito catchers were deployed in each structure. In structures with nets, one catcher was assigned to the collection of mosquitoes landing on nets. A second catcher was installed in a fixed position of the room and was responsible for the capture of mosquitoes landing on the legs. The third catcher was installed in a fixed position of the parlour and was responsible for the capture of mosquitoes landing on the legs. In structures without mosquito net, three catchers were deployed. One catcher was assigned to the room to capture mosquitoes resting on walls, baits and exit traps. A second catcher was immobile responsible for the capture of mosquitoes landing on the legs. A third catcher was installed in the lounge and was responsible for the collection of mosquitoes landing on the legs. Total of the mosquitoes collected from the walls, exit traps, off the bed nets and on the legs of catchers were considered to represent the total number entering the house. The number of mosquitoes that landed on the bed nets was specially noted. Insect collectors were rotated every six hours. Collected by all the methods, *An. gambiae* mosquitoes were dissected and their parity or nulliparity were evaluated from the tracheolar skeins, and gland infections with Plasmodium were registered.

Epidemiological evaluation

Mass blood surveys were conducted in the selected villages at the end of each month of the study (May, June, July, August, September, and October, 2011). All of the 29 houses were surveyed and blood smears of all of children less than 5 years old were collected regardless of occurrence of fever. In addition, active surveillance for malaria fever was also carried out fortnightly in all the study villages. All fever cases were identified and blood smears were collected, stained and examined under the microscope. A radical treatment was given to all the malaria fever cases according to the National programme of malaria control of Benin in both control and experimental villages.

Statistical analysis

The data generated from the study were subjected to Tukey test at 5% probability using SPSS 16.

Results

Impacts on entomological parameters

24 sessions of capture or 2088 man-nights resulted in the capture of 30,608 mosquitoes. The presence of Olyset® nets significantly reduced the number of mosquitoes captured in the treated village compared to other villages. 720 mosquitoes were caught in a structure with Olyset® nets by 3 catchers during 24 sessions against 1512 mosquitoes in a structure with untreated nets and 3672 mosquitoes in a structure without net.

The results in Table 1 show that untreated nets also reduced the number of mosquitoes resting in bedrooms. Of a total of 720 mosquitoes caught in a structure with Olyset® nets throughout the study, 87 mosquitoes were captured in the bedroom with the nets against 633 mosquitoes captured at the parlour. In this structure 324 mosquitoes caught were found dead. 15 mosquitoes caught were gorged. Of a total of 1512 mosquitoes captured in a structure with untreated net, 939 were captured in bedroom against 573 in parlour. 254 mosquitoes collected in this structure were gorged. Of a total of 3672 mosquitoes captured in a structure without net, 2798 mosquitoes were captured in bedroom against 874 mosquitoes captured in parlour. 3048 mosquitoes captured in this structure were gorged. The results of these observations are illustrated via percentage in Fig. 1.

In one of the structures with untreated mosquito nets, 113 mosquitoes were caught on the nets while no mosquito was captured on the Olyset® net. Of 2798 mosquitoes collected in one of the structures without mosquito nets more than half of mosquitoes were captured on exit traps and walls. Table 2 shows the average number of mosquitoes caught per night resting on the catchers' legs and landing on nets / bait.

Table 2. Mosquitoes caught per night resting on the catchers' legs and landing on nets/bait

Villages	An. entering the room	An. landing on net/bait	Dead (%)
Akpadon	09.70±05.20	00.00±00.00	45
Akodji	20.80±08.90	12.00± 06.20	00
Donoukpa	50.60±20.70	30.20 ± 10.30	00

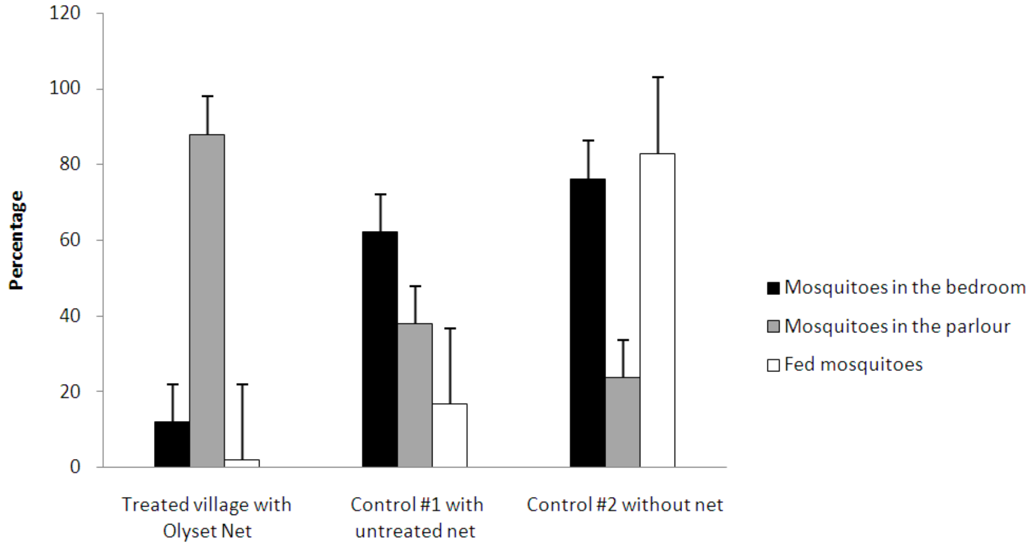

Fig. 1. Mosquitoes caught in bedroom, in parlour and fed mosquitoes in structure of each village.

This table also shows the results of night catches on human volunteers positioned in rooms with or without protective nets and the entrance rate of mosquitoes in rooms. The entrance of anopheles was reduced in the village with Olyset® nets as compared to the village with untreated nets and the village without nets. No mosquito was caught on the Olyset® net. This suggests that Olyset® nets have a strong irritant effect on the mosquitoes as they remain in contact with the nets for a very short time. 45% of mosquitoes captured with Olyset® nets inside rooms were found dead after 24 hrs of observation. With the total mortality being 45%, it was noted that an immediate mortality (IM) was 27.6% against a delayed mortality that was 17.4%. The knock down effect of Olyset® nets was very high. The results obtained with Olyset® nets showed a significant difference with those obtained with the untreated net, which in turn shows a significant difference with the structure without a mosquito net.

Dissection of mosquitoes captured inside rooms with Olyset® nets showed an entomological inoculation rate of 0.46. In the village with untreated nets the entomological inoculation rate was 2.07. The entomological inoculation rate was 22.83 in the village without mosquito net. These results can be observed in Table 3.

Table 3. Entomological inoculation rate in the village with Olyset® net, untreated nets and no mosquito net

Villages	Dissections	Positive	AR*	AD*	EIR*
Akpadon	312	015	0.048	09.7	00.46
Akodji	422	042	0.099	20.8	02.07
Donoukpa	616	278	00.45	50.6	22.83

*AR: Anthropophilic rate; AD: Aggressive density; EIR: entomological inoculation rate

There was a significantly lower rate on parity. The results in the village treated with Olyset® nets showed that the average parity was 16%. In the village with untreated nets the average parity was 38.7% and in the village without nets the average parity was 60.2% (Fig. 2). There was a statistically significant difference in the parity rate of all villages compared ($p<0.0001$).

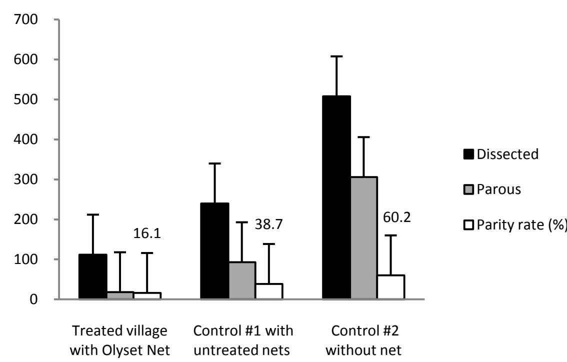

Fig. 2. Parity rate of mosquitoes in villages with Olyset® net, untreated nets and without net.

Impacts on parasitological and clinical parameters

Blood smears obtained from children less than 5 years old classified into the age groups have yielded the percentage parasitemia as in Fig. 3. Whatever the village, the prevalence is low in the age group (6-11). It rises in the age group (12-23) and descends in the age group (24-35) and stabilizes. The Olyset® nets have a significant impact on parasitemia because whatever the age group, parasitemia is very low in the Olyset® nets village as compared to other villages. Parasitemia is low regardless of the age group in the village with untreated nets compared to the village without net. Fig. 3 shows the percentage of children under 5 years old whose parasitemia test is positive. Parasitemia is very low in the treated village (4.52%). It is 73.12% in the untreated nets village. These two villages show a significant difference compared to the village without a mosquito nets with a prevalence of 33.22% (Fig. 4).

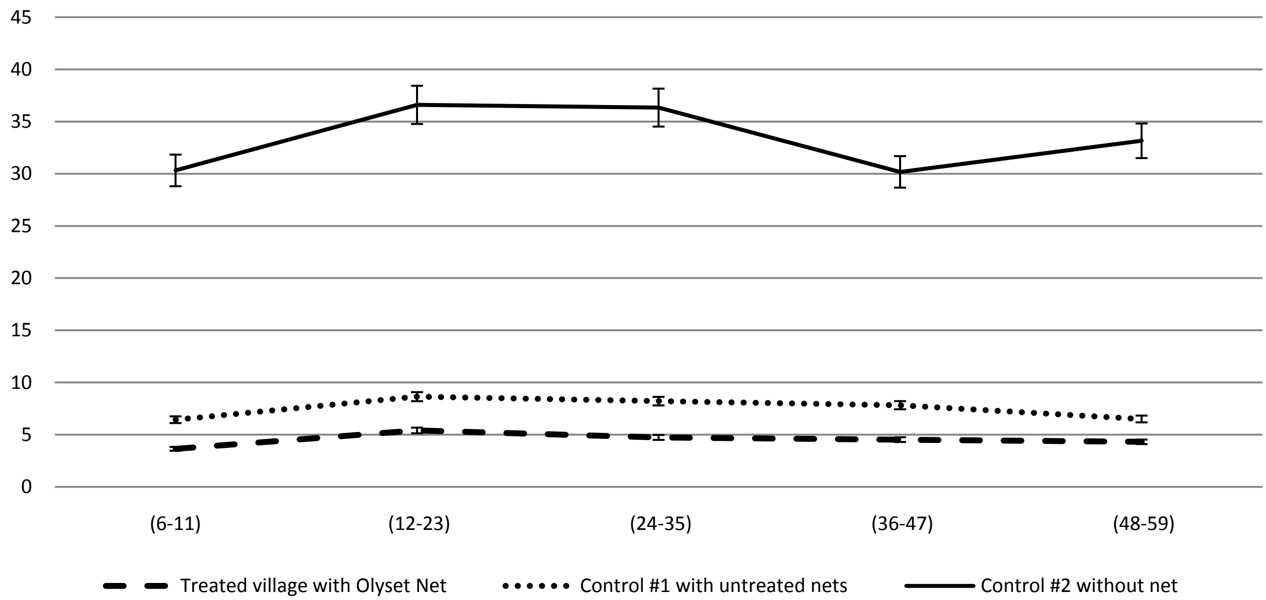

Fig. 3. Percentage of children from 6 to 59 months whose parasitemia test is positive by age and village.

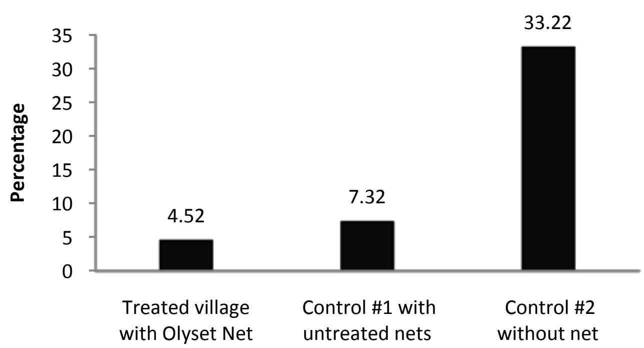

Fig. 4. Prevalence of malaria parasitemia in children according to village.

On all cases of fever of malaria observed, 18 are from the village treated with Olyset® net, 29 are from the village with untreated nets and 80 are from the village without net. As well, we obtained 4.64% of fever cases in the village with Olyset® net, 10.98% in the village with untreated nets and 95.23% in the village without net. Of 384 smears performed in cases of fever in the village with Olyset® net, 29 are positive i.e., 7.5%. Of 264 smears performed in cases of fever in the village with untreated net, 22 are positive i.e., 8.37%. In these villages there is no significant difference in parasitemia of plasmodium in case of fever. 84 blades made in the village without net, 32 or 38.09% were positive. There is a significant difference between this village and two other villages concerning the parasitemia associated with fever (Table 4).

Table 4. Collected slides and positive cases of plasmodium and fever cases

Parameters	Treated village	Control village #1	Control village #2
Fever cases	18	29	80
Thick film/blood smears made	384	264	84
Positive slides with plasmodium	29 (07.50%)	22 (08.33%)	32 (38.09%)

The Olyset® nets and untreated nets have corrected the hemoglobin versus the village without net. The results are reported in Table 5.

Table 5. Hemoglobin level of children (%)

Hemoglobin level	Treated village	Control village #1	Control village #2
Mild (10.0-10.9 g/dl)	69.00	65.00	24.23
Moderate (8.0-9.9 g/dl)	25.00	27.00	48.43
Severe (<8.0 g/dl)	06.00	08.00	27.34

Discussion

In this study, the entomological method revealed the power of individual and collective nets protection. The untreated nets significantly reduced the entrance rate of mosquitoes in the rooms thereby reducing biting rates of mosquitoes. This observation is consistent with other studies in Africa and in India (Sreehari *et al* 2007). Modern methods of vector control recommend the use of insecticide-treated bednets. However, untreated nets in good condition are by itself an insurmountable physical barrier normally. This simple observation leads to the question: what is the behavior of *An. gambiae* inside houses in absence of mosquito nets or existence of untreated, intact or deteriorated nets? The mere use of intact mosquito nets decrease from 75% to 80% on blood feeding rate of *An. gambiae* from the two other experimental situations, which induces a stronger exophily females (32%) still searching for their blood meal. It is well known that untreated nets may offer some protection to their users, but the degree of protection regarded is around 30-100 percents (D'Alessandro *et al* 1995, Maxwell *et al* 2006). However, an intact mosquito nets does not confer complete protection, since 16.79% of females are found gorged, without any of them been captured under the net.

During sleep, the sleeper may have a body part in contact with the net, allowing females *An. gambiae* from biting through the tulle. An intact net, therefore represents an effective physical barrier, but not total against mosquito bites. In addition to the physical barrier, the Olyset® nets have an irritant power against mosquitoes. This gives an excitorepellent, repellent and killing effect against mosquitoes. This is explained by the fact that no mosquito was captured on the Olyset® nets throughout the study. This also justifies the fact that 45% of mosquitoes caught in the structures with Olyset® nets are found dead. In sensitivity area of Anopheles to insecticides, ITNs have a lethal effect not on the anopheline population, reducing the density but also on the longevity of vectors and thus the infectivity of Anopheles mosquitoes and malaria transmission in the village. The protection obtained is communal and causes a reduction of about 60 percent. 100 of parasite loads and the fever attributed to malaria (Snow *et al* 1988, Carnevale *et al* 1991). In area of anopheline resistance to pyrethroids, recent studies conducted in experimental huts showed that ITNs reduce the rate of entrance of anopheles in houses, increasing the exit rate, limiting the rate of human blood and inducing some mortality (Darriet *et al* 1998). Thus, even in areas of resistance, ITNs limit the host-vector contact and, therefore, confirm personal protection to its user. This confirms our results that given in the catches, more mosquitoes are in the parlours than in the bedrooms in structures with Olyset® net. Of a total of 720 mosquitoes caught in one structure with Olyset® nets throughout the study, 87 mosquitoes were captured in the bedroom with the Olyset® nets against 633 mosquitoes captured at the parlour.

Parasitological and clinical data show that ITNs offer an individual protection to children that result in significant reduction of high parasitemia and frequency of malaria attacks. This prevention afforded by ITNs is now considered an established fact in Sahara, south of Africa (Snow *et al* 1988; Carnevale *et al* 1991). The multidisciplinary study conducted in Kafiné, under natural conditions, demonstrates that this protection against mosquitoes results in an effective reduction of about 2 times, heavy *Plasmodium falciparum* infection and morbidity. It is feared that the collective effect leading to one limitation of the transmission and, therefore, the prevention of malaria at the village level, be reduced in the case of a pyrethroid resistant anopheline population. The effectiveness of ITNs has been measured primarily through epidemiological criteria. The reduction in mortality of about 20% from all causes when the nets are installed correctly and on a large scale as well as the decline in malaria morbidity - by half, including the severe clinical forms were observed in the most studies conducted throughout the world, particularly in sub-Saharan Africa. These results should, however, be qualified. Moreover, opponents of ITNs point out that these results were obtained in studies of short duration, and that is singularly lacking hindsight to assess their long-term effectiveness. In this regard, the decrease in transmission, as it is durable, represents a very interesting objective.

The number of infected bites, that is to say, those that transmit the parasite, is lowered from 90% to 75% depending on location, or more. At the very least, the elimination of seasonal peaks of transmission is regularly confirmed by studies in Africa. However, opponents argue that ITN is no proportional relationship between the level of entomological transmission and morbidity, and that, beyond a certain threshold though very low, reducing the transmission can have neither a significant impact on the number of malaria attacks, or on mortality. But they do not consider the distribution of access based on age and season, and consequently the cumulative effects of a close repetition of infectious episodes, let alone other intervention options that are available when the pressure of morbid alleviates, with less episodes of fever, care seeking, appears more systematic and eases access to drugs. In practice, it has been found that a well-controlled use of ITNs reduced the transmission itself and was accompanied by a significant reduction in the number of malaria attacks in subjects at risk and most likely with the slightest severity of each. The perverse effects caused by the use of impregnated mosquito nets are not always properly assessed. The irritant, repellent pyrethroids used for nets treatment can lead to changes in behavior of the mosquito, which will sting more readily outside of homes and possibly at other times.

Several authors have attended the possibilities of actually satisfactory results in reducing the human-vector contact; however they are subject to discussion because they are subject to confounding factors. Thus, the sudden fall in density of aggressive anopheles, is interpreted primarily as a reduction in the size of the vector population, and may be related to a huge diversion of anthropophilic species usually endophilic and that would go outside the houses and / or to other hosts. In each case, the transmission may be displaced in space or time, making less appropriate use of ITNs. In addition, lower infection rate of mosquitoes may be not due to a shorter lifespan, which constitutes the intent, but for blood meals taken outside the homes and / or non-human hosts, therefore not infected, which has no impact on longevity of Anopheles which might come back. In worse condition, these mosquitoes can carry genes for resistance to insecticides to be hidden so quickly. However, it was found that the reduction of transmission does not appear to affect the parasites in the evolution of drug resistance, eventually selected by the mosquitoes. This result was not evident in the sense that the flow of parasites between infected and/or sick modified during any work, can result in genotypic reconstructions.Since we found the efficiency of impregnated mosquito nets in the short term, we must make it permanent. The protection afforded by ITNs is largely dependent on their maintenance and lack of resistance to insecticides.

Ethical issues

No ethical issues to be declared.

Conflict of interests

No conflict of interest to be declared.

Acknowledgements

I am grateful to the medical laboratory's team of the hospital of Aguegues. I thank the heads of households for their participation in the study. I thank the people of Aguegues for their participation in the study. I also thank the children of the commune of Aguegues. Special thanks go to the team of Entomological Research Centre of Cotonou (CREC).

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