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Research Article

Evaluation of the level of Sensitivity of Anophees to the Usual Insecticides in Public Health in the

Kailo Health Zone

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Abstract

Vector control is one of the national malaria control strategies in the DRC, which is mainly based on the use of insecticides using the mass distribution of long-lasting insecticide-treated nets and intra-home spraying of insecticide. The general objective of this study is to evaluate the level of sensitivity of anopheles to insecticides used in public health

We carried out a descriptive study that consisted in describing the bio-ecological parameters of anopheles gites and determining their behaviour towards conventional insecticides. After carrying out quality control on the consistency of the data collected, they were entered into Excel 2010 and the analyses were carried out on the Statistical Package for the Social Sciences 23 (SPSS 23) software.

The gambiaesl A nopheles tested showed different behaviours depending on the site and type of insecticides, and the Deltamethrin test showed a mortality rate of 97% in Lokando and 52% in Lonioma. After pre-exposure to the PBO, mortality was 98% at RVA. The permethrin test recorded a mortality rate of 77% at Lokando and 31% at Lonioma.

An increase in the sensitivity of Anophelesgambiae sl was observed after pre-exposure to 5% PBO. This resistance is largely of metabolic origin linked to mono oxygenase P450. In view of these results, it is imperative to consider new control strategies to control the emergence of this resistance.

Keywords: Anopheles, Sensitivity, Insecticide-usual

Introduction

Malaria, a parasitic disease caused by Plasmodiums and transmitted via a mosquito of the genus Anopheles.

The Anopheles is the most dangerous arthropod in the world, vector of malaria which continues to be the main cause of morbidity and mortality in sub-Saharan Africa. As a result, malaria is a real public health problem. According to WHO estimates, 216 million cases of malaria were recorded in 2016, an increase of 5 million compared to the previous year [1], The number of associated deaths reached 445,000, almost the same as in 2015,

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and there is now stagnation in the fight against malaria.

Worldwide, more than 3,000 species of Anopheles have been recorded, only about 60 transmit malaria. The African region is the most affected with 90% of malaria cases and 92% of recorded deaths. These regions present favourable ecological conditions for the development of Anopheles, the main ones being Anopheles gambiae, Anopheles funestus, Anopheles nili and Anopheles moucheti. These anopheles all belong to species complexes and can have highly variable vectorial capacities and behaviours. The Democratic Republic of Congo and Nigeria lead the way with 35% of the world's malaria prevalence [1].

Malaria remains one of the causes of mortality in the 3 vulnerable groups, namely children under five, pregnant women and the elderly. The DRC, a developing country, must face the dual challenge of economic growth and population health [2]. The rural environment, which comprises around 70% of the total population and its economy being essentially agricultural, needs healthy men to promote it. However, the latter are exposed to various vector-borne diseases, including malaria. This disease remains endemic and is the leading cause of morbidity and mortality in the DRC [3].

In 2016, 15,397,717 cases of malaria were recorded with 33,997 deaths in DRC [3].

The most common vectors are Anopheles gambiae (92%), Anopheles funestus, Anophelesnili, Anophelesmoucheti and Anophelespaludis [4]. Under these conditions, vector control seems indispensable. Vector control requires the control and identification of vector species, particularly their biology, ecology and etiology, as well as their sensitivity to insecticides [4].

This entomological component has two main prevention interventions implemented by the NMCPs, namely the massive use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), as a vector control strategy, making it possible to distinguish three malaria zones:

-Stable malaria zone: Anopheles transmission is high, with more than 100 infected bites per person per year, which means that premunition can be acquired after 5 years;

-Unstable malaria zone: transmission is seasonal and moderate, with a few infective bites per man per year, which does not allow the acquisition of premunition and the disease is epidemic.

Intermediate zone between the two previous ones.

CARNEVAL has mapped the epidemiology of malaria in sub-Saharan Africa [5]. It divided the continent into six epidemiological facies according to the biogeography of the region concerned, namely :

- Equatorial facies (forests and post-forest savannas);
- Tropical facies (humid savannas);
- Sahelian facies (dry savannas and steppes);
- Desert facies (Sahelo-Saharan steppes, deserts of the Horn of Africa);
- Southern facies (southern African plateaus); and
- Mountain facies (mountains between 1000 metres and 2000 metres (MOUCHET et al,1993) [6].

DRC has three epidemiological facies, namely equatorial and tropical, where 97% of the population live, and is the zone with stable malaria. The mountainous facies, where 3% of the population lives, is the zone with unstable malaria in Kailo, malaria transmission is very heterogeneous depending on the environment. Malaria prevalence is high in peripheral areas and low in urban areas. ASIDI and collaborators justified this situation by the disruption of the ecosystem by human activities. Unfortunately, the deployment of these two interventions coincided with the emergence of vector resistance to insecticides in some regions [7]. Despite the multitudes of ITN/AIDS distribution campaigns organised in DRC in general and Kailo in particular, malaria transmission still persists.

In 2019, the 18 Health Zones of Maniema Provincial Division reported 463272 cases of malaria with 1032 deaths [3].

These high morbidity and mortality rates can be explained by several factors:

The misuse of ITNs per household;

The inefficiency of insecticides used in the impregnation of mosquito nets; Mosquito resistance to insecticides used in net impregnation. Hence the need to carry out an entomological assessment of the behaviour of malaria vectors with regard to the insecticides commonly used in Kindu. The main synthetic insecticides belong to 5 chemical families observed:

- Organochlorines,
- organophosphates,
- carbamates,
- pyrethroids and
- the benzylureas.

The detection of insecticide resistance should therefore be a component of all national pest control efforts to ensure that the most effective vector control methods are implemented. The choice of insecticide for indoor spraying should always be a decision based on recent local data on the sensitivity

of target vectors [1].

The general objective of this study is to assess the level of sensitivity of Anopheles to common public health insecticides in order to contribute to the identification of more effective insecticides in the Basoko and RVA sites of Kindu city.

Seven species of Anopheles have been identified and recorded in Kindu, namely: An. Gambiae sl, An. funestus, An. paludis, An. hancocki, An. coustani, An. Brunnipes and An. nili.

A single vector species An. Gambiae sl is responsible for malaria transmission; it represents 93.27% of the Anopheles fauna [8].

Materials and Methods Frame and Material

The study took place in two sites in the Kailo Health Zone (Lokando and Lonioma Quarry) from 22 January to 23 March 2020 in Kailo. We selected sites on the basis of a reasoned choice taking into account the observations of ASIDI and Giovan francesco.

All water bodies likely to serve as breeding sites were selected and prospected during this study.

Surveys of Anopheles breeding sites were carried out beforehand in the 2 sites to identify the different types of breeding sites. These sites were categorised into several types according to the WHO:

Small puddles, gutters, ditches, tyre tracks where we examined the water surface;

Swamps, ponds and lakes, where larvae were usually found in the vegetation on the edges, or sometimes far from the shore if there were floating plants;

Rivers that have been examined at the shoreline, where there is vegetation and where the flow is slower;

special places, such as wells or water reservoirs, where the entire water surface has been examined [2].

The biological material for our study consisted of larval lodges and adult anopheles from the larvae and pupae collected from the Lokando and Lonioma lodges in Kailo.

The statistical units consisted of larval and anopheline breeding sites.

Methods Nature of the study

This is a descriptive study which consisted in describing the bio-ecological parameters of Anopheles sites and determining their behaviour with regard to common insecticides.

In each site, potential Anopheles larval sites were identified and characterised by travelling along different routes. The physico-chemical characteristics of the deposits were recorded by the multiparameter probe to determine the water quality of the deposits (temperature (°C), conductivity (μ s/ cm), turbidity (ppm) pH) were taken from these deposits. These parameters were measured by placing the Hanna Combo multiparameter probe 10 cm deep in each deposit to determine the water quality of the deposits.

The type of the deposits was determined to characterize them as temporary and permanent deposits.

The vegetation cover allowed us to determine the nature of the deposits (sunny and shady). Gîtes with out vegetation cover were considered sunny and those with vegetation cover were considered shady.

Larval collection was organized according to the Dupping method to estimate larval density according to the carron scale on the one hand and on the other hand for rearing in order to obtain the adults for the test. Some quantitative parameters of the deposits were described and presented in tabular form.

Mosquito mortality was calculated by adding up the number of mosquitoes that died across all tests for exposure to the different insecticides and expressing this number as a percentage of the total number of mosquitoes exposed to the insecticides :

Mortality Rate=Number Total Mosquitoes Dead/NumberTotal Mosquitoes Exposed x100

Mosquito resistance analysis was also carried out in depth using log probit analysis software, following the procedure proposed by WHO 2013 [9].

This software allowed the determination of Kdt50 and Kdt95.

Lokando

Average

28,3

8,1

694,6

1389,2

17,3

Samples of larvae and pupae were collected for adult rearing for susceptibility testing. Larvae and pupae were collected from the sites. Two teams of six people were formed. Two entomological technicians, a doctor, a biologist and two community relays, who took turns visiting the targeted sites.

In order to collect the larvae and nymphs properly, the trapper approached the site slowly to avoid any disturbance that might cause them to dive. He places himself against the shade to avoid the escape of larvae and nymphs

 Table II : Physical and physico-chemical parameters of the Lokando and Lonioma sites

Standard

deviation

2,3

0,5

427,4

851,3

30,9

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ence in the depth of the different deposits.

physico-chemical

parameters

Temperature (°C)

pН

Turbidity (ppm)

Conductivity (µs/

cm)

Depth (cm)

towards the bottom of the lodges.

The larvae and pupae were collected using a 500 ml plastic ladle with a handle whose colour makes it easy to see the larvae and pupae. This ladle was gently dipped into the water at an angle of about 45° until one edge was just below the surface.

If the larvae and pupae were disturbed/disturbed, a wait of a minute or two was observed for them to come to the surface and continue sampling. These collected larvae were placed in the numbered containers with lids that could be nested together to avoid shaking during transport, following the Williams and Pinto protocol in 2012 [10].

Presentation of Results

Lonima

Average

31.9

7,8

292,2

589,9

16,8

Minimum

Maximum

Min: 25,2

Max: 32,3

Min: 7,1

Max: 9,2

Min: 176

Max: 2000

Min: 353

Max: 3999

Min: 2

Max: 150

Table II shows that the waters of all larval sites had comparable pH values that were slightly basic. Temperature was higher at the RVA (31.95 ± 3.57). In contrast, the Basoko site was characterised by high turbidity (694.6 ± 427.4 ppm) and conductivity (1389.2 ± 851.3 µs/cm). However, there was no difference of the state of th

These results concern a total of 81 larval deposits, characterized in 5 types of water collection.

Table I : Types of g	gites by site		
TYPES OF GITE	SITES		(n=81)
	Lokando	Lonioma	Total
Gutter	1 (1,2%)	0 (0%)	1 (1,2%)
Market garden water collection	28 (34,6%)	0 (0%)	28 (34,6%)
Stagnant rain- water	9 (11,1%)	31 (38,3%)	40 (49,4%)
Presence of marshes	7 (8,6%)	1 (1,2%)	8 (9,9%)
Well	1 (1,2%)	3 (3,7%)	4 (4,9%)
TOTAL	46 (56,8%)	35 (43,2%)	81 (100%)

Table 1: shows that the distribution of gites varies according to the site. Anopheles colonised breeding sites at Lokando and Lonioma were 56.8% and 43.2% respectively. At Lonioma, Anopheles breeding sites were created by stagnant water (31 sites or 38.3%). However at Lokando, the market gardening collections constituted 28 gites or 34.6% identified and characterised.

Standard

deviation

3,5

0,6

324,6

643,9

15,5

Minimum

Maximum

Min: 26.2

Max: 39,2

Min: 6,57

Max: 10,1

Min: 97

Max: 1870 Min: 189

Max: 3697

Min: 2

Max: 70

Sites	Insecticides	n	Kdt50 (min)	Kdt95 (min)	Mortality24h(%)	Status*
	Deltaméthri n 0,05 %	100	35.7 (33.7-37.7)	74.1 (66.4-86.2)	52	R
	Deltaméthri n 0,05 % + PBO 5%	100	30.1 (28.3-31.8)	46 (42.3-52.1)	98	S
Lokando	Perméthri n 0,75 %	100	55.2 (52.7-58.6)	97.6 (85.9-118.2)	31	R
	Perméthri n 0,75 % + PBO 5 %	100	40.6 (39.3-41.9)	58.3 (55.1-62.7)	100	S
	Bendiocarb 0,1 %	100	23.45 (22.1-24.8)	35.4 (32.6-39.7)	100	S
	Malathion 5 %	100	26.7 (25.3-28.2)	39.6 (36.7-44.1)	100	S
	DDT 4%	100	158.7(100.7-827.9) Pas d'effet	440.8(193.9- 9169.5) Pas d'effet	16	R
Lonioma	Deltaméthrin 0,05 %	100	31.0 (29.3-32.7)	49.1 (46.2-53.0)	99	S
	Perméthrin 0,75 %	100	47.1 (45.6-48.7)	66.0 (62.9-70.1)	79	R
	Perméthrin 0,75 % + PBO 5 %	100	42.1 (40.4-43.7)	61.9 (58.7-66.3)	98	S
	Bendiocarb 0,1 %	100	21.0 (19.8-22.2)	30.7 (28.8-33.4)	100	S
	DDT 4 %	100	69.3 (64.4-95.6) Pas d'effet	79.6(70.6-130.2) Pas d'effet	10	R
	Malathion 5 %	100	31.7 (29.8-33.6)	45.9 (36.7-44.1)	100	S

*Status: R=resistant (≤ 90) RP=probable resistance S=sensitive (98-100%)

Kdt50 and Kdt95 were highly variable depending on the type of insecticide and the test site.

Bendiocarb and Malathion were the effective insecticides at both sites (Lokando and Lonioma). Anopheles gambiae sl tested with these insecticides were all stunned (100%). Kdt50 and Kdt95 were too early in Lonioma, respectively at 21.0 minutes with 95% CI (19.8-22.2) and 30.7 (28.8-33.4) minutes. However in Basoko, Kdt50 and Kdt95 were slightly late, respectively at 23.45 (22.1-24.8) minutes and 30.7 (28.8-33.4) minutes.

Similarly for Malathion, Kdt50 and Kdt95 were reached too early in Lonioma (31.7 (29.8-33.6) minutes and 45.9 (36.7-44.1) minutes) compared to Lokando (26.7 (25.3-28.2) minutes). No effect of Kdt50 and Kdt95 observed at the Anopheles tested with DDT in the sites. In Lonioma, Deltamethrin was effective. These anopheles were stunned with Kdt50 and Kdt95 at 31.0 (29.3-32.7) minutes and 49.1 (46.2-53.0) minutes respectively.

In Lokando, on the other hand, Kdt95 was not reached. Anopheles tested against Bendiocarb and Malathion were sensitive with 100% mortality in both sites. These anopheles showed very high resistance to permethrin and DDT in both sites with low mortality. The mortality of anopheles exposed to Deltamethrin was more marked at Lonioma, i.e. 99%.

Discussion

The main objective of this work was to determine the level of sensitivity of Anopheles to insecticides used in public health in order to contribute to the improvement of resistance management of Anopheles to these insecticides in the Lokando and Lonioma sites in the Kailo Health Zone.

Characteristics of Larval Sites

According to Mouchet et al, the different species of Anopheles exploit a wide variety of water collections as lodgings, including residual pools of sunny stagnant surfaces, pools with upright vegetation, brackish water, etc.

This study had shown that the majority of the larval deposits observed in the two sites of Lokando and Lonioma, consisted of stagnant rainwater (49.4%) and market garden water collection (34.6%). The majority of these sites were sunny (85.2%).

This difference could be justified by the anthropic activities that are carried out in each site and also the nature of the soil which is sandy in Lokando and silty-clayey in Lonima.

This same observation was observed in Kinshasa by ASIDI and collaborators and METELO and collaborators in Bandundu-ville [11].

In the rainy season, in most rural areas the numerous ruts, small water collections created by depressions in the land, human activities and poor urbanisation provide excellent breeding sites [11].

Physical and Physico-Chemical Parameters

The Anopheles. Gambiae sl preferentially uses residual pools from sunny stagnant surfaces in several parts of Africa (BOBANGA et al, 2007). mean temperature values at Basoko were 28.3±2.3°C (min: 25.2°C; max: 32.3°C) and at Lonioma 31.9±3.5°C (min: 26.2°C; max: 39.2°C) [11].

This difference could be due to the fact that 60% of Lonioma's lodgings were created by collections of market garden water in which the water is

renewed daily by watering the flowerbeds. Whereas at Lokando the stagnant water from the rains (88.5%) was very exposed to the sun. These observations differ from those recorded in Morocco by OUALILALAMI and collaborators where the average temperature was 23.7°C (min: 20.4°C; max: 31.4°C), a difference that can be justified by the climate [12].

All larval sites prospected had a slightly basic pH.

These results corroborate those found by METELO and collaborators in Bandundu-ville [11].

The low conductivity and turbidity of the deposits had an influence on larval density and entomological parameters of malaria transmission. In this study, it was observed that turbidity and conductivity were high in both surveyed sites with an average of 494.9 \pm 376 ppm and 989.6 \pm 747.6 µs/ cm respectively [11]. Despite the cloudy quality of these larval sites, larval production was maximal.

These results are similar to those found by Metelo1 et al. in Bandundu-ville [11]. This is justified by the vectorial capacity of Anopheles gambiae to adapt in these water collections.

It was observed that the mean turbidity and conductivity values at Lonioma were double those of Lokando. These observations can be justified by the poor waste management that is believed to be at the root of the suspended matter and ions.

Conclusion

The studies carried out at the Kailo health zone in the Lokando and Lonioma sites have led to the following observations:

5 types of gites were characterised (market gardening water collections, stagnant rainwater, swamps, wells and gutter). In Lokando, the Anopheles larval deposits were created by the stagnant waters, while in Lonioma by the market gardening collections.

The majority of the larval sites prospected were sunny and temporary. The larval sites had comparable pH values which were slightly basic. The breeding sites were characterised by high turbidity and conductivity values.

Anopheles gambiaea showed cross-resistance to permethrin and DDT as well as sensitivity to Bendiocarb and Malathion. This demonstrates the total involvement of oxidases in resistance [13-59].

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