Malaria transmission and insecticide resistance in *Anopheles gambiae* in Ellibou, Côte d'Ivoire

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Bédjou Prisca N'DRI

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auf Antrag von

Fakultätsverantwortlicher: Prof. Dr. Jürg Utzinger
Dissertationsleiter: PD Dr. Pie Müller
Korreferent: Prof. Dr. Alexander Mathis

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Prof. Dr. Marcel Mayor

Dekan

To my late father, N'Dri Abouo Samuel

and

my late mother, Kassi Sandé Marie…

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List of abbreviations

ACE-1	Acethylcholinesterase resistance
CDC	Centers for Disease Control and Prevention
COES	Carboxylesterase
CSRS	Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
CYP	Cytochrome P450
DDT	Dichlorodiphenyltrichloroethane
DNA	Deoxyribonucleic acid
EIR	Entomological inoculation rate
ELISA	Enzyme-linked imunosorbent assay
G119S	Glycine to serine mutation at position 119
GST	Gluthatione S-transferase
HBR	Human biting rate
HLC	Human landing catch
IRAC	Insecticide Resistance Action Committee
IRS	Indoor residual spraying
KAPB	Knowledge, attitude, practice and belief
kdr	Knockdown resistance
L1014F	Leucine to phenylalanine mutation at position 1014
L1014S	Leucine to serine mutation at position 1014
LLIN	Long-lasting insecticidal treated nets
LT	Light trap
N1575Y	Asparagine-to-tyrosine mutation at position 1575
NMCP	National Malaria Control Programme
PCR	Polymerase chain reaction
PSC	Pyrethrum spray catch
R	Resistant
RNA	Ribonucleic acid
RS	Resistance suspected
RT-PCR	Real-time reverse-transcription polymerase chain reaction
S	Susceptible
s.l.	*Sensu lato*
s.s.	*Sensu stricto*
SR	Sporozoïte rate

Swiss TPH	Swiss Tropical and Public Health Institute
VGSC	Voltage-gated sodium channel
WHO	World Health Organization

Summary

Background

Malaria is a major public health problem in Côte d'Ivoire with a countrywide prevalence exceeding 60%. The main strategies to fight the disease include early diagnosis, prompt and effective treatment as well as individual and community effective preventive measures, such as the use of long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS). The deployment of any of these preventive measures must take into account the local conditions of malaria transmission, in particular the level of endemicity, the vectors involved in the transmission and the factors which influence the level of transmission. Unfortunately, resistance of malaria vectors to pyrethroids (PYRs), the main approved class of insecticides for treatment of bednets is widespread and constitutes a problem in disease control by the National Malaria Control Programme (NMCP). Ellibou is located in the southern part of Côte d'Ivoire and apart from the LLIN distribution campaign by the NMCP in 2014, no entomological study has been conducted in this locality. However, knowledge of the local malaria vectors and their resistance status to insecticides are essential to implement vector control strategies.

Aim and objectives

The aim of this PhD study was to determine key entomological parameters of malaria transmission and to assess the insecticide susceptibility of malaria vectors in Elibou, South Côte d'Ivoire. Specifically, we (i) determined the species composition of mosquitoes in Elibou, the sporozoite rate and the seasonal variation of malaria vector density over an entire year; (ii) assessed the insecticide susceptibility of the main malaria vector to the four conventional classes of insecticides used in public health and characterise the mechanisms involved in resistance to these insecticides; and (iii) investigated the knowledge, attitudes, practices and believes (KAPB) of the population in relation to the use of insecticides and insecticide resistance in malaria vectors.

Methods

We carried out an entomological survey, including larvae and adult mosquito collections in Elibou village over a 1-year period. To catch adult mosquitoes we performed pyrethroid spray catches (PSCs) and deployed CDC light traps (LTs). Adults mosquitoes were identified morphologically and diagnosted by PCR to genus and, where possible, to species level.

The susceptibilty of female *Anopheles* moquitoes emerged from larvae to insecticides was assessed by World Health Organization (WHO) insecticide susceptibility assays, while we characterised the mechanisms involved in insecticide resistance by real-time reverse-transcription polymerase chain reaction (RT-PCR). Finally, a KAPB survey, using questionaires, focus group discussions (FGDs) and interviews, was carried out.

Results

We caught 2,383 adult mosquitoes, 884 specimens by CDC light traps and 1,499 by PSCs. We morphologically identified 10 different taxa, including three *Anopheles* taxa. *Anopheles gambiae s.l.* represented the predominant malaria vector. The other two *Anopheles* taxa were *Anopheles funestus* and *Anopheles pharoensis* representing less than 1% each. The molecular identification showed that *An. gambiae s.s.* (38.3%), *Anopheles coluzzii* (39.0%) and *Anopheles arabiensis* (19.5%) as the main malaria vectors in Elibou. To our knowledge, this is the first report of *An. arababiensis* in Côte d'Ivoire. We also found *An. gambiae/coluzzii* hybrids (4.7%). The other mosquito species included *Aedes* sp., *Culex* sp. and *Mansonia* sp. and represented 57.8% of the total of mosquitoes collected. The sporozoite rates were 5.3%. The numbers of adult *An. gambiae s.l.* mosquitoes increased considerably during the rainy seasons mainly between June and July. The main malaria vector was resistant to deltamethrin, dichloro-diphenyl-trichloroethane (DDT) and bendiocarb while susceptible to malathion. In the *An. gambiae s.l.* specimens we found the insecticide target site mutations *kdr* L1014F, L1014S and N1575Y, and the *Ace-1 G119S* at both the homozygous and heterozygous stage. While the L1014S was only found in *An. arabiensis*, nine individuals harboured simultaniousely the L1014F and L1014S alleles. The detoxification genes *CYP6P3, CYP6M2, CYP6Z1, CYP6P4, CYP6P1, CYP9K1, CYP4G16* and *GSTE2* were found to be overexpressed in all *Anopheles* tested from Ellibou. *CYP6P4* and *CYP6M2* were the most upregulated genes. The KAPB survey revealed that people have poor knowledge about insecticide resistance, while they felt that preventing measures were ineffective. The main reason given was that insecticides were diluted by the manufacturers as a marketing strategy to sell larger quantities of their products. More than a third of the farmers used agricultural pesticides for domestic purposes to kill weeds or mosquitoes.

Conclusion

The results of this study contribute to a better understanding of the main local malaria vectors and their resistance status to insecticides used in public health and agriculture as well as factors that lead to insecticide resistance and persistence of malaria affecting the NMCP activities in

the fight against malaria. Here, we report for the first time the presence *An. arabiensis* among malaria vectors in Côte d'Ivoire. This finding is an early warning sign because *An. arabiensis* shows a different behaviour and ecology than *An. coluzzii* and *An. gambiae s.s*. Thus, different methods for vector control are required.
Resistance to insecticides as observed in Ellibou is a great concern. Our results show that several resistance mechanisms are involved in insecticide resistance. In addition, the KAPB results show that the pesticides used for agriculture are also used in households for domestic purposes and might be a cause of insecticide resistance development in mosquitoes in Elibou. Given the misunderstanding of resistance by the local population, it is important to inform them about the current situation for awareness and sensitise them for a change in behaviour on the best use of insecticides and the environmental sanitation.

For future studies, we recommend to investigate on the history of the onset of *An. arabiensis* in Côte d'Ivoire, its distribution, its resistance status and its implication in malaria transmission**.** For *An. gambiae s.l.*, the use of an alternative insecticide such as pyrimiphos-methyl, an organophosphate for IRS, is recommended. However, *An. arabiensis* is primarily an outdoor biting mosquito, and hence IRS and LLINs may be less effective against this malaria vector**.** To this end, we recommend to implement an effective control using a transdisciplinary approach while involving the local population because it plays an important role in the spread of resistance. The conclusions of our study will guide decision makers to design and implement an effective vector control strategy in the Ellibou region and other localities with the same characteristics.

Key words : *Anopheles arabiensis*, *Anopheles gambiae*, Ellibou, Côte d'Ivoire, insecticide resistance, metabolic resistance, transmission, malaria.

Resumé

Introduction

Le paludisme est un problème de santé publique majeur en Côte d'Ivoire avec une prévalence nationale supérieure à 60%. Les principales stratégies de lutte contre la maladie comprennent un diagnostic précoce, un traitement rapide et efficace ainsi que des mesures préventives individuelles et communautaires efficaces, telles que l'utilisation de moustiquaires imprégnées d'insecticide à longue durée d'action (MILDA) et la pulvérisation intradomiciliaire à effet rémanent (PID). Le déploiement de l'une ou l'autre de ces mesures préventives doit tenir compte des conditions locales de transmission du paludisme, en particulier du niveau d'endémicité, des vecteurs impliqués dans la transmission et des facteurs qui influencent le niveau de transmission. Malheureusement, la résistance des vecteurs du paludisme aux pyréthroïdes (PYR), la principale classe d'insecticides approuvée pour le traitement des moustiquaires, est très répandue et constitue un problème dans la lutte contre la maladie par le Programme national de lutte contre le paludisme (PNLP). Ellibou est situé dans la partie sud de la Côte d'Ivoire. En dehors de la campagne nationale de distribution de MILDA faite par le PNLP en 2014, aucune étude entomologique n'a été menée dans cette localité. Or, la connaissance des vecteurs locaux du paludisme, son statut de résistance aux insecticides sont essentiels pour mettre en place des stratégies de lutte antivectorielle efficaces.

But et objectifs

L'objectif de cette étude de doctorat était de déterminer les paramètres entomologiques clés de la transmission du paludisme et d'évaluer la sensibilité aux insecticides des vecteurs du paludisme à Ellibou, dans le sud de la Côte d'Ivoire. Plus précisément, nous avons (i) déterminé la composition des espèces de moustiques à Ellibou, le taux de sporozoïtes et la variation saisonnière de la densité des vecteurs du paludisme sur une année entière; (ii) évalué la sensibilité aux insecticides du principal vecteur du paludisme quatre classes d'insecticides conventionnelles utilisés en santé publique et avons caractérisé les mécanismes impliqués dans la résistance à ces insecticides ; et (iii) étudié les connaissances, attitudes, pratiques et croyances (CAPC) de la population en relation avec l'utilisation des insecticides et la résistance aux insecticides chez les vecteurs du paludisme.

Méthodes

Nous avons réalisé une enquête entomologique, comprenant des collectes de larves et de moustiques adultes dans le village d'Ellibou sur une période de 12 mois (un an). Pour la collecte de moustiques adultes, nous avons effectué des captures par pulvérisation de pyréthroïdes et

déployé des pièges lumineux. Les moustiques adultes ont été identifiés morphologiquement et diagnostiqués par PCR au niveau du genre et de l'espèce. La sensibilité des moustiques anophèles femelles émergeant des larves a été évaluée par les tests de sensibilité aux insecticides de l'Organisation mondiale de la Santé (OMS), tandis que nous avons caractérisé les mécanismes impliqués dans la résistance aux insecticides par réaction en chaîne par polymerase après transcription inverse (RT-PCR). Enfin, une enquête CAPC, utilisant des questionnaires, des « focus group et discussions (FGD) » et des entretiens avec des informateurs clés, a été réalisée.

Résultats

Nous avons capturé 2 383 moustiques adultes, 884 spécimens par les pièges lumineux et 1 499 par les pulverisations. Nous avons identifié morphologiquement 10 taxons différents, dont trois taxons d'*Anopheles*. *Anopheles gambiae s.l.* représentait le vecteur prédominant du paludisme. Les deux autres taxons d'anophèles étaient *Anopheles funestus* et *Anopheles pharoensis* représentant moins de 1% chacun. L'identification moléculaire a revélé en plus d'*Anopheles coluzzii* (39,0%) et *An. gambiae s.l.* (38,3%), la présence d'un troisième vecteur: *An. arabiensis* (19,5%) à Ellibou. A notre connaissance, il s'agit du premier rapport d'*An. arabiensis* en Côte d'Ivoire. Nous avons également trouvé des hybrides *An. gambiae/coluzzii* (4,7%). Les autres espèces de moustiques comprenaient *Aedes* sp., *Culex* sp. et *Mansonia* sp., représentant 57,8% du total des moustiques collectés. Les taux de sporozoïtes étaient de 5,3%. Le nombre de moustiques adultes *An. gambiae s.l.* a considérablement augmenté pendant la saison des pluies, principalement entre juin et juillet. Le principal vecteur du paludisme était résistant à la deltaméthrine, au (dichloro-diphenyl-trichloroethane) DDT et au bendiocarbe, mais sensible au malathion. Chez les spécimens d'*An. gambiae s.l.* nous avons trouvé les mutations du site cible de l'insecticide *kdr* : L1014F, L1014S et N1575Y et *Ace-1* G119S au stade homozygote et hétérozygote. Alors que L1014S n'a été trouvé que chez *An. arabiensis*, neuf individus portaient simultanément les allèles L1014F et L1014S. Les gènes de détoxification *CYP6P3, CYP6M2, CYP6Z1, CYP6P4, CYP6P1, CYP9K1, CYP4G16* et *GSTE2* ont été surexprimés chez les anophèles testés à Ellibou. Cependant, *CYP6P4* et *CYP6M2* étaient les gènes les plus régulés. L'enquête CAPC a révélé que les participants ont une mauvaise connaissance de la résistance aux insecticides, alors qu'ils estiment que les mesures de prévention sont inefficaces. La principale raison évoquée était que les insecticides ont été dilués par les fabricants dans le cadre d'une stratégie marketing visant à vendre de plus grandes quantités de leurs produits. Plus d'un tiers des agriculteurs utilisait des pesticides agricoles à des fins domestiques pour tuer les mauvaises herbes ou les moustiques.

Conclusion

Les résultats de cette étude contribuent à une meilleure connaissance des principaux vecteurs locaux du paludisme et de leur statut de résistance aux insecticides utilisés en santé publique et en agriculture ainsi que des facteurs qui conduisent à la résistance aux insecticides et à la persistance du paludisme affectant les activités du PNLP dans la lutte contre le paludisme. Ici, nous rapportons pour la première fois la présence d'*An. arabiensis* parmi les vecteurs du paludisme en Côte d'Ivoire. Cette découverte est un signe d'alerte précoce car *An. arabiensis* a un comportement et une écologie différents de ceux d'*An. coluzzii* et d'*An. gambiae s.s.* Des techniques différentes de contrôle des vecteurs s'avèrent nécessaires pour la lutte antivectorielle.

La résistance aux insecticides, telle qu'observée à Ellibou, est une grande préoccupation. Nos résultats montrent que plusieurs mécanismes de résistance sont impliqués dans la résistance aux insecticides. Les résultats de l'étude CAPC montrent également que les pesticides utilisés pour l'agriculture sont également utilisés dans les ménages à des fins domestiques, et pourraient être une cause de résistance à l'insecticide à Ellibou. Compte tenu, de la mauvaise connaissance de la résistance par la population locale, il est important de l'informer de la situation actuelle afin de la sensibiliser à un changement de comportement sur la meilleure utilisation des insecticides et l'assainissement de l'environnement.

Nous recommandons des études futures pour investiguer sur l'histoire d'apparition d'*An. arabiensis* en Côte d'Ivoire, sa distribution, son statut de résistance et son implication dans la transmission du paludisme. Pour *An. gambiae s.l.*, l'utilisation d'un insecticide alternatif tel que le pyrimiphos-méthyl, un organophosphate pour les PID est recommandée. Etant donnée que *An. arabiensis* est principalement un moustique qui pique à l'extérieur (exophage), et donc les PID et les MILDAs sont moins efficaces contre ce vecteur. A cet effet, nous recommandons de mettre en place un contrôle efficace en utilisant une approche transdisciplinaire tout en impliquant la population locale car elle joue un rôle important dans la propagation de la résistance. Les conclusions de notre étude guideront les décideurs pour concevoir et mettre en œuvre une stratégie de lutte antivectorielle efficace dans la région d'Ellibou et dans d'autres localités présentant les mêmes caractéristiques.

Mots clés : *Anopheles arabiensis*, *Anopheles gambiae*, Ellibou, Côte d'Ivoire, resistance insecticides, resistance metabolique, transmission, paludisme.

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Chapter 1: Introduction

1.1. Malaria: global situation and situation in Africa

Malaria remains a considerable public health issue in the world, threatening more than a billion people in the poorest population group. In 2019, an estimated 229 million cases and 409,000 deaths from malaria occurred worldwide. Africa is, by far, the most affected continent with 94% of cases estimated in 2019. According to the World Malaria Report 2019, 19 countries in sub-Saharan Africa and India carried almost 95% of the global malaria burden. Six of them accounted for half of the cases, namely, Nigeria, Democratic Republic of the Congo, Mozambique, Uganda and Niger (WHO 2020a).

1.1.1. Socio-economic aspects

Malaria puts a heavy economic burden on endemic countries and contributes to poverty. The decline of malaria is heavily related to the socio-economic improvement (Hertig 2019). Indeed, in addition to causing the loss in human life, malaria reduces population activities due to absenteeism affecting the economy. It is, therefore, considered a disease of poverty. According to the United Nations Children Funds (UNICEF): "Malaria's cost to human and social well-being is enormous. It is a major cause of poverty and poverty exacerbates the malaria situation". Malaria hampers social development and children's education. The socio-economic status is closely related to the risk of malaria, namely, the morbidity and the mortality. More than half (58%) of all malaria deaths are encountered in the poorest 20% of the world's population. Indeed, the populations living in areas with difficult access to health facilities unreached by the activities carried out by the NMCP. Some studies on population KAPB revealed that the distance between household and health facility influenced malaria treatment seeking behaviour of most heads of households (Lowassa *et al*. 2012). In addition, the poorest people did not visit the health facility due to the cost of malaria treatment. Without a health insurance, the malaria treatment has to be paid up front and poor households have no insurance, and hence must pay cash. Therefore, the lack of money is driving poorer people to get inappropriate treatments for malaria, including traditional medicine or counterfeit "paracetamol" at the market to reduce fever. Several factors such as lack of money, long waiting times at the hospital, distance to the health center, socio-cultural aspects (*e.g.* perception of acceptability of insecticide treated nets), disparities in the use of public health services by the poorest, low level of education and limited sources of transport are some identified barriers that limit access to malaria treatment and the success for the disease control (Chuma, Okungu, and Molyneux 2010; Barat *et al*. 2004). Thus, training of community health workers to improve malaria treatment seeking behaviours is required.

1.1.2. Malaria situation in Côte d'Ivoire

Malaria is endemic in Côte d'Ivoire and occurs year-round. The entire population is at risk of malaria. In Côte d'Ivoire, malaria represents the primary cause of consultations of school health services and absenteeism. The prevalence due to *Plasmodium falciparum* is about 73% among schoolchildren (Houngbedji *et al*. 2015).

Côte d'Ivoire is characterised by a warm and humid climate. In Côte d'Ivoire, malaria transmission is linked to the climate conditions divided into three ecological zones:

- The southern zone is the forest area, characterised by an abundant rainfall. Malaria transmission occurs only in the clearings. In this part of the country, malaria occurs all year.
- The transition zone in the centre of the country corresponds to the pre-forest zone with so-called wet savannah malaria. The duration of malaria transmission is comparable to the forest area in the southern zone.
- The northern zone shows sudano-sahelian or sahelian savannah malaria. In this region, the transmission is permanent with an upsurge during the rainy seasons lasting for 6 to 8 months.

1.2. Malaria transmission

Malaria is a vector-borne disease caused by parasites of the genus *Plasmodium* through the bites of infective female *Anopheles* mosquitoes. Five species of *Plasmodia* are responsible for malaria in humans:

Plasmodium falciparum (Welch, 1897) is the most pathogenic malaria parasite and responsible for the most fatal cases. It is present in tropical African, Asian, South American and Oceanic regions. It is particularly dominant in Africa.

Plasmodium vivax (Grassi et Felleti, 1890) co-exists with *P. falciparum* in many parts of the world and is present in some temperate regions. This species is practically non-existent in Africa but predominates in Southeast Asia, South America and Oceania.

Plasmodium malariae (Laveran, 1881) is unevenly distributed in the world and is not fatal but can cause relapse up to 20 years after the primary infection (Werry 1995, Mouchet et *al,* 2004).

Plasmodium ovale (Stephens, 1922) is mainly found in West Africa. It does not kill, but it can cause relapses 4 to 5 years after the primary infection.

Plasmodium knowlesi (Sinton et Mulligan, 1932) is the fifth parasite of malaria that is known to infect monkeys in Southeast Asia. *Plasmodium knowlesi* also infects humans causing malaria that is transmitted from animal to human (zoonotic malaria)

1.2.1. Life cycle of *Plasmodium falciparum*

The life cycle of *Plasmodium* is characterised by two phases: a asexual or schizogonic phase within the human host and the sexual phase taking place within the mosquito vector (Figure 1.1).

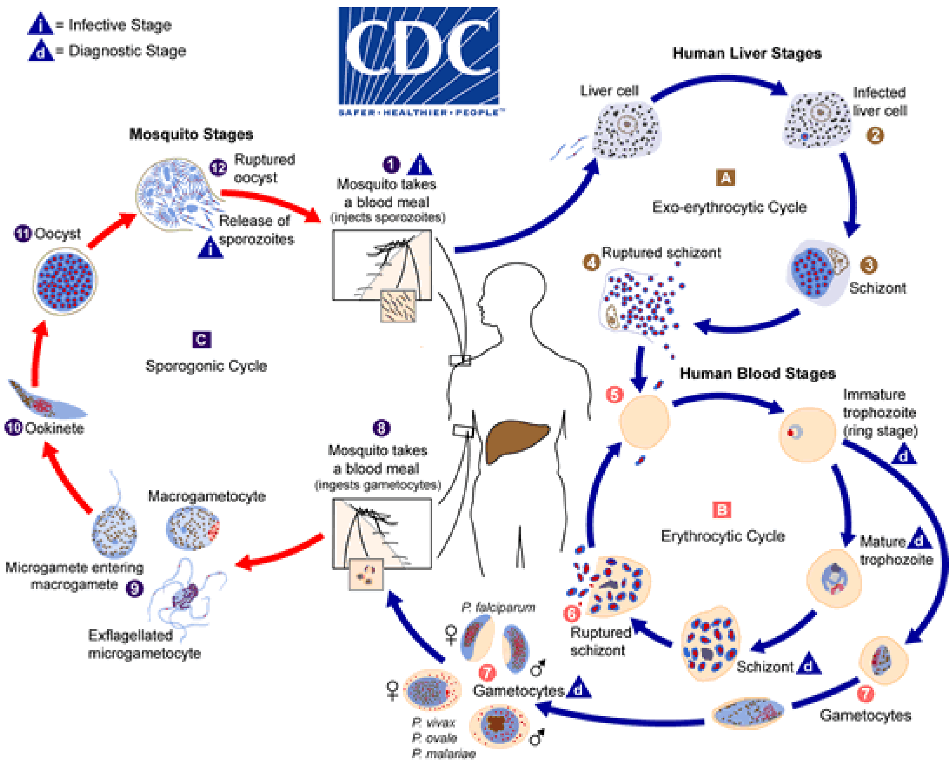

Figure 1.1. Life cycle of malaria parasites.
(https://www.cdc.gov/malaria/about/biology/index.html)

1.2.1.1. Asexual phase in humans

The evolution of the parasite in the human host has two phases: the exo-erythrocytic phase and the intra-erythrocytic phase:

Exo-erythrocytic phase: this phase takes place in the liver (A). During the blood meal of an infectious female anopheline mosquito, the human host is infected with sporozoites. The sporozoites travel into the blood and infect the liver cells (2). Here, they multiply asexually, increase in size and become schizonts (3). The liver schizonts then become mature. The mature schizonts and the infected cells burst and release the merozoites into the blood stream where they invade new cells (4). Some merozoits pass into the blood stream, enter the red blood cells and give rise to the intra-erythrocytic phase (5).

The liver stage lasts about 10 days for *P. falciparum* but other species such as *P. vivax* can remain dormant for several months in the liver (Sattabongkot et *al*. 2006).

Intra-erythrocyte phase: in the red blood cells (B) the merozoites increase in size and are transformed into schizonts. The erythrocytic schizonts result in the transformation of mature schizonts. Erythrocyte schizogony lasts 2 to 3 days varying between species. Mature schizonts and parasitic red cells burst and release merozoites that invade other red blood cells (6). After several schizogonic cycles, a part of the merozoites develops into sexual male and female gametocytes (7) that are then ingested by a mosquito and continue the parasite development cycle.

1.2.1.2. Sexual phase in mosquitoes

Male and female gametocytes are ingested by the female mosquito (8). The gametocytes reproduce sexually by forming a motile egg, called ookynete (10) which then transforms into oocysts in the mosquito gut (11). Later, the oocysts burst and release sporozoites that migrate to the salivary glands of the mosquito (12). The cycle then continues in humans after the next bite (1). The duration of the life cycle depends strongly on the ambient temperature and the *Plasmodium* species. The life cycle of *P. falciparum* is temperature-dependent and may take 13 days at 23 ºC and 30 days at 20 °C (Garcia 2007). Malaria can also be transmitted transplacentally from mother to child by blood transfusion or contaminated slides and syringes.

1.2.2. Distribution of malaria vectors

Human malaria is transmitted by mosquitoes of the genus *Anopheles*. There are about 500 species worldwide of which 50 are able of transmitting malaria to humans (Harbach and Besansky 2014) (Figure 1. 2). *Anopheles gambiae, An. arabiensis* and *An. funestus* are the three African *Anopheles* species most responsible of malaria transmission. Globally, *An. gambiae s.s.* and *An. arabiensis* were shown to be the most dominant malaria vectors, while *An. gambiae* s.s. is the main and most widespread vector in Tropical Africa (Akpan, Adepoju, and Oladosu 2019).

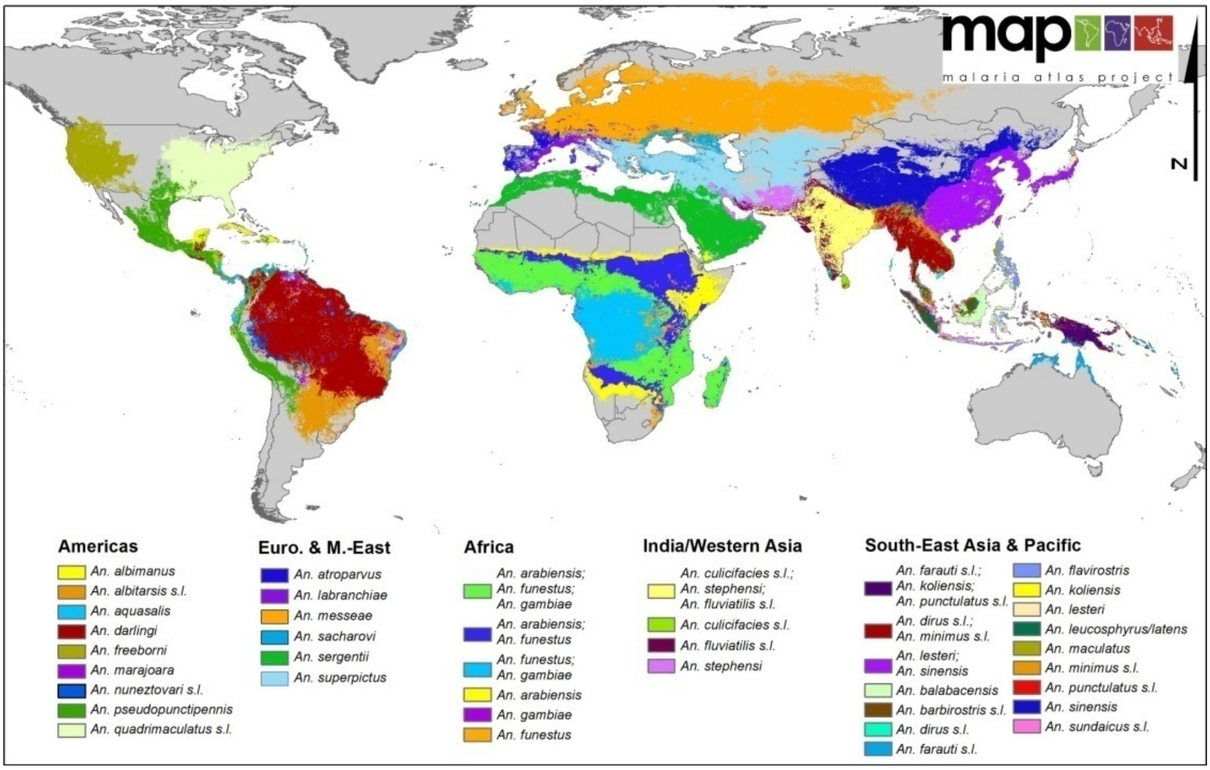

Figure 1.2. Global distribution of the dominant vector species of malaria (https://www.intechopen.com/books/anopheles-mosquitoes-new-insights-into-malaria-vectors/global-distribution-of-the-dominant-vector-species-of-malaria). Visited on 20 May 2020.

1.2.3. Malaria vectors in Côte d'Ivoire

In Côte d'Ivoire, two *Anopheles* taxa are known to transmit malaria, *An. gambiae s.l* and *An. funestus*. *Anopheles gambiae s.l.* is the main malaria vector that plays the major role in malaria transmission followed by *An. funestus. Anopheles nilii* has been described as a third vector in malaria transmission but only in the southern forest area of the country (Adja et *al.* 2011).

1.2.4. *Anopheles gambiae* complex

Among the major malaria vectors in sub-Saharan Africa, *An. gambiae* is the most widespread and most studied. *Anophele gambiae* is a species complex and covers at least eight sibling species that are morphologically very similar: *An. gambiae s.s., An. coluzzii*, *An. arabiensis*, *An. merus*, *An. melas*, *An. quadrimaculatus, An. amharicus* and *An bwambae* (Coetzee et *al.* 2013). Among them, *An. gambiae s.s.* and *An. arabiensis* are the two main African malaria vectors in the species complex (Bass et al. 2007; Kirby and Lindsay 2009). *Anopheles gambiae s.s.* and *An. coluzzii* have been reported to exist in sympatry in West Africa. *Anopheles gambiae s.s.* develops in temporary breeding sites, unlike *An. coluzzii*, that are adapted to more permanent breedings sites (*e.g.* rice fields) (Diabaté et *al.* 2005).

1.2.5. Life cycle of *Anopheles gambiae s.l.*

The life cycle of *An. gambiae s.l.* comprises of an aquatic phase with three stages (*i.e.* egg, larvae and pupa) and a terrestrial phase, the adult stage. The development of each stage depends on the species and the water temperature.

1.2.5.1. Aquatic phase

The aquatic phase lasts at least 8 days in tropical zones but it can go up to one month in temperate zones. For *An. gambiae s.l.,* the duration is on average 7 to 10 days. The duration increases when the temperature decreases and shortens when it increases.

A female mosquito mates only once in a lifetime and can lay eggs throughout its whole life. After the copulation, the eggs are fertilized and the maturation of the eggs requires a blood meal by the female *Anopheles*. The blood meal digestion normally lasts 2 to 3 days. Mature eggs are laid separately on the water surface. A female *Anopheles* can lay 100 to 150 eggs at a time measuring 0.6 to 0.8 mm. The eggs have lateral floats keeping them on the water surface. After 2-3 days, the eggs hatch into larvae.

The larvae swim on the water surface and feed by filtering organic debris and microorganisms from the water. At the end of their development, the larvae pupate. The pupa is motile and does not feed. The pupa is shaped like a comma. At this stage, the insect undergoes very deep transformations that bring it from the aquatic to the adult stage. After 2-3 days, the skin splits and the adult emerges.

Figure 1. 3. **Picture of an *Anopheles* mosquito larvae.** 10x magnification. Photo credit: Behi, 2019.

Figure 1.4. Picture of an *Anopheles* pupa. 10x magnification. Photo credit: Behi, 2019.

1.2.5.2. Terrestrial phase

The male or female adult emerges from the pupa and rest on top of the water surface. Mating occurs only once in the life of anopheles around the 3rd day after emergence. Their first meal is composed of nectar. The male does not suck because it is unable to pierce the skin of a vertebrate. However, they feed on plant juices. The development of the mosquito from the egg stage to adult lasts 11–13 days. Only, the female is hematophagous, needing blood to recover proteins essential for ovarian maturation. This defines the gonotrophic cycle that constitutes of host seeking by the female, the uptake and the digestion of the blood, the maturation of ovarioles and the search for an adequate breeding site to lay eggs (Carnevale *et al*, 1979). The duration of the gonotrophic cycle varies according to the mosquito species.

1.2.6. *Anopheles* behaviour

Due to their hematophagous diet, female *Anopheles* mosquitoes play an important role in malaria transmission and usually bite at night. *Anopheles* mosquitoes can fly over large distances from their habitat for host seeking. Mosquitoes may also be passively be transported by wind, ship, aircrafts, or motorised vehicles (WHO 2016). Usually, female *Anopheles* bite the whole night with a peak of biting activity at the middle of the night (Bradley et al. 2015; Zahouli et *al*. 2011; Dambach et *al*. 2018; Antonio-Nkondjio et *al.* 2002). Some *Anopheles* can feed on humans (anthropophilic) or animals (zoophilic) depending on the accessibility of the host species and the mosquitoes' preferences. Some species are endophagous, taking their blood meal inside the houses and some are exophagous, feeding outside the houses. Endophagous species that rest inside houses are called "endophilic" and those that leave the houses are called "exophilic" (Mouchet *et al.* 2004; Lardeux *et al*. 2007; Muriu *et al*. 2008).

1.3. Measuring malaria transmission

Malaria transmission can be measured by using parasitological indicators or entomological parameters (Shaukat, Breman and McKenzie 2010; MacDonald 1957). The entomological inoculation rate (EIR) remains the most appropriate and direct measure for quantifying the risk and intensity of malaria transmission (Shaukat, Breman and McKenzie 2010). EIR is the number of infectious bites per unit time. The human biting rate (HBR) is defined as the average number of mosquito bites per human per day. The sporozoite rate (S) is the proportion of female anopheline mosquitoes having sporozoites in their salivary glands. To estimate these parameters, mosquitoes are caught by human landing catches (HLC) that is the gold standard reference method. However, HCL has been strictly limited for ethical concerns since humens are exposed to potentially infective mosquito bites (Tangena et al. 2015; WHO 2013).

1.3.1. Factors influencing malaria transmission

The dynamic of mosquito populations is influenced by environmental and anthropogenic factors. Among them, climatic factors are essential for mosquito development. Malaria incidence changes with weather conditions. Thus, malaria transmission is linked to seasonal variations in the temperature and humidity affecting mosquito density (Craig *et al*. 2004). However, there another factors affect mosquito densities such as insecticides resistance.

1.3.1.1. Rainfall

Rainfall is one of the climatic factors recognised to drive malaria incidence as it affects mosquito densities. An increase in rainfall leads to more breeding sites; hence higher abundance of larvae. Several studies have clearly demonstrated the association between rainfall, mosquito abundance and malaria cases (Thomson *et al.* 2005; Craig *et al*. 2004). The permanent presence of water has a significant effect on mosquito abundance and their distribution because female *Anopheles* lay eggs in stagnant water where the larvae develop. In contrast, excessive rainfall may also wash out the larval breeding sites, which could even lead to a decrease in larvae number and vector density. Since *An. gambiae* larvae prefer to develop mainly in temporary breeding sites, they are very sensitive to climate change that could induce the re-emergence of vector borne diseases through spreading into new areas (Caminade, McIntyre and Jones 2019).

1.3.1.2. Temperature

Like rainfall, temperature impacts vector and parasite development (Kotepui and Kotepui 2018). Ambient temperature affects mosquito dynamics through *e.g.* mosquito survival and development rates of juvenile stages, including eggs, larvae and pupae. They may not survive certain minimum and maximum temperatures. An increase in temperature reduces the time for mosquito maturation and consequently increases the feeding frequency (Service. MW, 1980). In *An. gambiae s.l.*, larval development is optimal when the temperature rises to a peak around 28 °C. In addition, the life cycle of malaria parasites is also affected by temperature. Under optimal conditions, 10 days are required for the parasite to complete its development in the mosquito vector. However, this time can be longer, depending on the temperature. Below a temperature of 18 °C, the parasite may not develop.

1.3.1.3. Anthropogenic factors

Human pollution can have an impact on mosquito abundance. Some studies revealed that domestic water storage, rutted track, gutters, wastewater and backwaters provide favourable breeding sites for mosquitoes (Sovi *et al.* 2013). Indeed, man-made breeding sites contribute to maintain adult mosquito populations. The same effects are seen from environmental changes, urbanisation due to population growth, dam constructions and agricultural practices. For example, certain irrigated agricultural practices have an impact on malaria transmission (Sovi *et al.* 2013). Due to the growth in the human population, several dams and irrigated schemes have been created in sub-Saharan African countries for food security. Unfortunately, such projects contribute to the recrudescence of malaria transmission by increasing the density of mosquitoes (Koudou *et al.* 2007; Mwangangi *et al.* 2010). For example, in Côte d'Ivoire irrigated rice fields were recognised suitable breeding sites for mosquito development, particularly *An. gambiae s.l.* (Koudou *et al.* 2007; Keiser, Utzinger and Singer 2002). Several studies carried out across the country have found an effect of irrigation on the proliferation of vectors. However, some studies also suggest the abundance of mosquitoes may also have no influence on malaria transmission (Nzeymana *et al* 2003).

1.3.1.4. Insecticide resistance in malaria vectors

Another factor of concern is mosquitoes being resistant to insecticides. The emergence of insecticide resistance in mosquitoes may jeopardise the effectiveness of malaria vector control.

Alleles conferring resistance to insecticides may be passed to successive generations, increasing their survival while threatening insecticide based interventions such as LLIN or IRS. Unfortunately, the choice of insecticides for public health that show low toxicity to humans and are long lasting is very limited. Indeed, all current LLINs still contain pyrethroids. Unfortunately, malaria vectors resistant to pyrethroids are widespread across the African region (www.irmapper.com) and may partially explain the re-emergence of malaria (WHO 2019).

1.4. Public knowledge about malaria in Côte d'Ivoire

Knowledge, attitude and practices (KAP) among population exposed to malaria are well studied (Klein *et al*. 1995) compared to the KAP relating to insecticides used by farmers in agriculture (Abuelmaali *et al*. 2013; Tia *et al*. 2006). Population's beliefs, education levels and the socio-cultural context are factors influencing the effectiveness of vector control (Idowu *et al.* 2008). Indeed, the surveillance of the population's KAP is essential in the fight against the disease. In Côte d'Ivoire, KAP studies related to malaria revealed good population knowledge of malaria, with awareness of malaria transmission by mosquitoes and their breeding sites (Granado *et al.* 2011; Esse *et al*. 2008; Comoe *et al.* 2012). However, some studies have revealed that, according to the beliefs of ethnic groups there are several types of malaria with knowledge of proper symptoms and clinical manifestations of the disease. For example, a study conducted by Esse *et al*. (2008) in Central Côte d'Ivoire has shown that white palms, a symptoms of aneamia and yellow eyes and urine, symptoms of jaundice, are recognised as symptoms of malaria by the public affected people seek treatment from traditional medicine (Esse *et al.* 2008). Moreover, the public recognised the effectiveness of insecticide-treated nets to prevent malaria. Despite the distribution of insecticide-treated nets their usage is low. This could be because some people complain about suffocation, excess heat or their socio-cultural situation. Therefore, making people aware of environmental sanitation and educate them about the adequate use of insecticide-treated nets is required to reduce mosquito densities and ultimately malaria incidence. Moreover, implementing a malaria management programme in communities must be considered to successfully control the vectors and prevent disease.

1.5. Malaria prevention and control measures

In malaria endemic countries, populations are still at risk from malaria, while prevention remains the most important way for malaria control. Malaria control consists of measures intended to eliminate or to reduce the consequences linked to malaria, in particular, morbidity, mortality and the social and economic losses. Chemoprevention by using antimalarial drugs and protecting against mosquito bites are recommended by WHO (2019). The control of adult mosquitoes is based on indoor residual spray (IRS) and long-lasting insecticidal nets (LLINs). For the treatment or prevention of malaria with chemoprophylaxis, WHO recommends sulfadoxine pyrimethamine (SP) for pregnant women in areas with high endemicity, once they have perceived the first fetal movement, followed by intermittent presumptive treatment (IPTp) at each scheduled antenatal visit after the first trimester.

In Côte d'Ivoire, the protection measures are essentially based on IRS and LLINs that are being distributed free of charge though the NMCP, chemoprophylaxis (IPTp) in pregnant women and environmental sanitation (PNLP 2014).

However, drug resistance in the parasites compromises control efforts. Developping effective vaccine for malaria is challenging because the *Plasmodium* parasite goes through several development stages in both the human host and the mosquitoes vector during its developmental cycle. Each step ends with the release of a parasite in different forms, carrying different antigens inducing a different immune response. To this end, vector control appears to be the most effective tool to prevent malaria.

1.6. Insecticides used for vector control and their mode of action

1.6.1. Chemical insecticides

Chemical insecticides are synthetic compounds used against insects to cause their death upon contact or ingestion. Some also exert an excito-repellent effect on insects. The four classic classes of insecticides against adult mosquitoes in public health are organochlorines, pyrethroids, organophosphates and carbamates.

Organochlorines

Organochlorines are the oldest insecticide class and were widely used from the 1940s to the 1970s. Dichloro-diphenyl-trichloroethane (DDT), hexachlorocyclohexane (HCH) and dieldrin were the most used organochlorines. They act on the nervous system in both vertebrates and invertebrates. In mosquitoes, organochlorines act on the insect on the central and peripheral nervous system through contact and ingestion by targeting the voltage-gated sodium channel. DDT was the first insecticide to revolutionise vector control due to its remarkable stability and low cost. (Martinez-Torres *et al*. 1998). Unfortunately, its use had to be banned because of its persistence in the environment, the effect on human health and the appearance of resistance in vectors such as in *An. gambiae* (Mouchet 1994). However, in some countries DDT is still in used for both public health and agriculture.

Pyrethroids

Pyrethroids are synthetic insecticides derived from the natural pyrethrins. Since the 1980s, pyrethroids have become increasingly more important on the insecticide market. Their photostability and selective insecticidal toxicity while showing low toxicity for warm-blooded vertebrates have made them prime candidates for their use in the public health, particularly for the impregnation of mosquito nets (WHO 2019). Like the organochlorines, pyrethroids such as permethrin and delatamethrin are neurotoxious and act on the central and peripheral nervous system. They kill the insect by blocking the functioning of the voltage-gated sodium channels that are essential for the transmission of nerve impulses. Pyrethroids constitute the primary class of insecticides that are recommended by WHO for the impregnation of bednets (WHO 2020b). They are by far the most widely used insecticide due to their low mammalian toxicity, efficacy and acceptability (Zaim, Aitio and Nakashima 2000; Corbel *et al*. 2003).

Organophosphates

Organophosphates are highly toxic to insects but less toxic to humans and animals. They are nerve poisons. In mosquitoes, they cause an excitation followed by trembling ends and a paralysis leading to death. Organophosphates are good neurotoxic inhibitors of the enzyme acetylcholinesterase. They block the synaptic neurotransmission in the mosquito by inhibiting the enzymatic action of acetylcholinesterase (AChE). Organophosphates used in public health have a shorter residual effect than DDT. Organophosphates have been widely used in public health for the treatment of *Culex quinquefasciatus* and *Aedes aegypti* breeding sites and sometimes even for *Anopheles* breeding sites (Carnevale 1990). Among the organophosphates, malathion is the most widely used insecticide for the control of adult malaria vectors.

Carbamates

Like organophosphates, carbamates (CMs) are inhibitors of the insecticide AChE. They have the same mode of action like organophosphates and are generally applied as contact insecticides. However, they show short residuality. The molecules most used in public health are propoxur, carbonyl, bendiocarb and carbosulfan for IRS in the fight against malaria (Carnevale 1990).

1.6.2. Naturally derived insecticides

In addition to the purely synthetic insecticides used in the fight against mosquitoes, there are also naturally derived insecticides. Chlorfenapyr is a pro-insecticide derived from microbially produced pyrroles and is used in public health against mosquitoes. Pyriproxifen is an insect growth regulator, having larvicidal activity wich in combination with a second product intensifies the effectiveness of interventions; hence may reduce insecticide resistance (Maoz *et al*. 2017) of *Bacillus thurigiensis* (*Bti*) and *Bacillus sphaericus* (*BS*) products are the most frequently used in the fight against mosquito larvae. The use of *Bti*-based products has increased in Africa. These larvicidal agents significantly reduce the abundance of larvae in their natural habitats. For example, larval control of malaria vectors using *Bti* has been effective in Burkina Faso (Majori, Ali and Sabatinelli 1987). In addition, the use of medicinal plants such as *Phyllantus. amarus*, *Cissus populnea* et *Cochlospermum planchonii* in the control of larvae have shown very good efficacy against *An. gambiae* and *Cx. quinquefasciatus* larvaes (Azokou *et al.* 2013).

1.7. Insecticide resistance

Insecticide resistance is a major threat for malaria vector control. Insecticide resistance is defined by the Insecticide Resistance Action Committee (IRAC) as "the selection of a heritable characteristic in an insect population that results in the repeated failure of an insecticide product to provide the intended levels of control when used as recommended''. In contrast, WHO defines insecticide resistance as "the development of an ability in a strain of insects to tolerate doses of toxicants which would prove lethal to the majority of individuals in a normal population of the same species" (WHO 1957). According to WHO, resistance to the four common classes of insecticides, including pyrethroids, organochlorines, organophosphates and carbamates has been detected in at least one malaria vector in 23 endemic countries (WHO 2020b). In addition, resistance to at least one class of insecticides has also been detected in 73 malaria endemic countries.

The most worrying fact is that malaria vectors are becoming resistant to pyrethroid insecticides, the key class of insecticides approved by WHO for LLINs (Figure 1.4). Resistance to pyrethroids was detected for the first time in Bouaké, Central Côte d'Ivoire (Elissa *et al.* 1993).

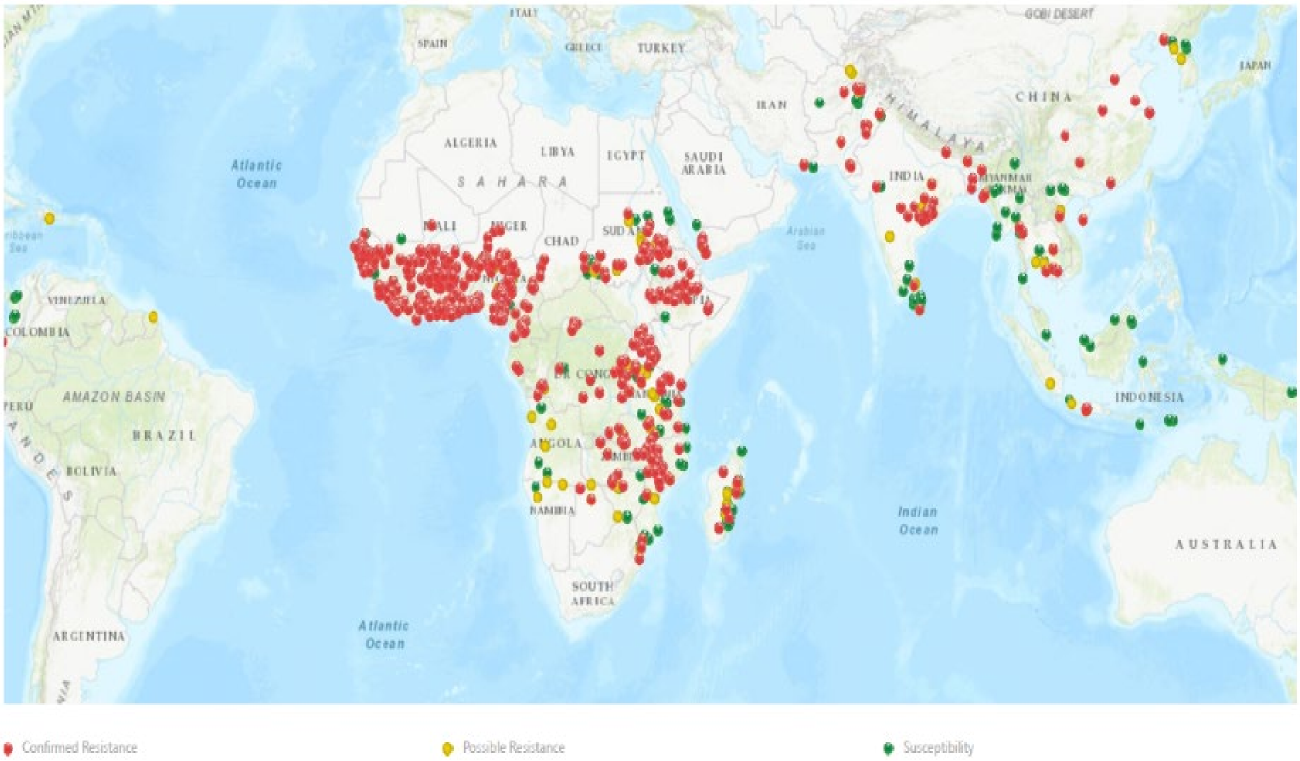

Figure 1.5. Distribution of pyrethroid resistance in *An. gambiae s.l.* between 2010 and 2020. Source: https://www.irmapper.com/, visited on 9 March 2021

1.7.1. Overview of insecticide resistance in Côte d'Ivoire

The rapid spread of resistance to pyrethroid insecticides in malaria vectors is a factor that affects malaria control efforts. In Côte d'Ivoire, pyrethroid resistance is on the rise and resistance to other insecticides classes has also been reported (Edi *et al.* 2012b) (Figure 1.6). However, *An. gambiae s.l.* remains susceptible to malathion in several part of the country (Fodjo *et al*. 2018; Camara *et al.* 2018). Studies investigating the molecular mechanisms revealed the presence of the L1014F *kdr* and *Ace-1* mutation at high frequencies. Indeed, the L1014F is closely associated with resistance to pyrethroids and DDT, while *Ace1* mutation confers resistance to carbamates in *An. gambiae* (Edi *et al*. 2012a). The L1014S *kdr* mutation has also been reported in Côte d'Ivoire (Chouaibou *et al.* 2017). Both L1014F and L1014S are involved in cross-resistance to DDT and pyrethroids (Ranson *et al*. 2000). The massive use of insecticides in vector control and agriculture may be the principal cause of resistance in malaria vectors through the increasing selection pressure. To date, five classes of insecticides are approved by WHO that are recommended to use against adult *Anopheles* mosquitoes for IRS. These are the pyrethoids, organophosphates, carbamates and neonicotinoids (WHO 2020a).

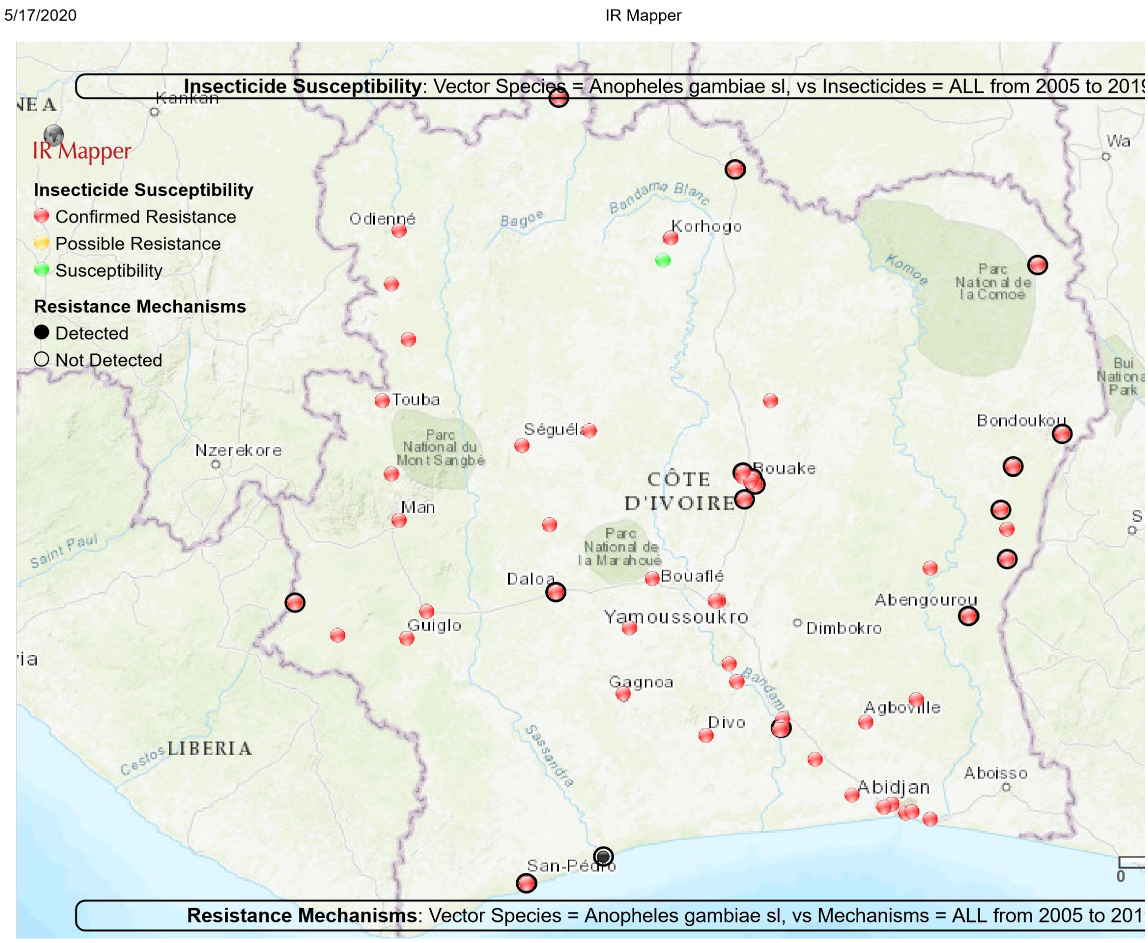

Figure 1.6 Distribution of insecticide resistance to all classes of insecticides in Côte d'Ivoire from 2005 to 2019 (Source: IR Mapper, visited on 17 May 2020).

1.7.2. Insecticide resistance mechanisms

Multiple mechanisms can contribute to insecticide resistance involving several factors. The following four insecticide resistance mechanism are key in mosquitoes:

- Metabolic resistance, which is the degradation of insecticides;
- Target-site resistance that is the modification of the insecticide target site, preventing the insecticide from binding to its site of action;
- Behavioural resistance, which is the behaviour of the insect to avoid contact with the insecticide;
- Cuticular resistance, a change in the rate of absorption or excretion of the insecticide.

The two physiological main mechanisms are metabolic and target-site resistance and have been widely studied.

1.7.2.1. Target-site resistance

Target-site resistance may be caused by a single amino acid substitution leading to alterations decrease in sodium channels or the reduction in the sensitivity of the insect nervous system. The most common of the target-site mutations are knockdown resistance (*kdr*) and acethylcholine esterase insensitive (*Ace-1^R*). According to WHO, *kdr* is widely distributed and is associated with cross-resistance to DDT and pyrethroids (WHO 2005; Weill *et al.* 2002). Molecular techniques such as the TaqMan assay is used to characterise target-site insecticide resistance mechanism.

***Kdr* mutation**

Resistance due to *kdr* alleles is characterised by a decrease in the affinity between the voltage-gated sodium channels (VGSC) and the insecticides targeting the channel, including dichlor-dipheny that confers cross-resistance to DDT and pyrethroids (Martinez-Torres *et al*. 1998). The *kdr* mutation is located at position 1014 in the domain II of the voltage-gated sodium channel with either a L1014F (leucine to phenylalanine) or L1014S (of leucine to serine) substitution. Initially, the L1014F mutation was only found in West Africa (Chandre *et al*. 1999) and the L1014S one in East Africa (Kawada *et al*. 2011). Today, both mutations are largely distributed across the African continent from West to East (Awolola *et al*. 2005; Ochomo *et al.* 2015; Djegbe *et al*. 2018). Moreover, hybrids with both the L1014S and L1014F mutations were first reported from Burkina Faso in both *An. gambiae s.s.* and *An. coluzzii* (Namountougou *et al*. 2013). In Côte d'Ivoire, L1014F is widely spreading across the country (Edi *et al*. 2012b), and the L1014S mutation has recently been detected in a single individual in the heterozygous form in Central Côte d'Ivoire (Chouaibou *et al.* 2017).

A more recently described *kd*r mutation is the N1575Y substitution within the linker between domain II-IV of the VGSC having a synergistic effect with the L1014F mutation. This mutation has emerged in West and Central Africa and is associated with pyrethroids and DDT resistance (Jones *et al.* 2012). The N1575Y mutation has never been reported in Côte d'Ivoire.

Ace 1 **mutation**

The mutation of the acetylcholinesterase is one of the most important mechanisms of resistance. This mutation is due to a single nucleotide substitution of glycine with serine at codon position 119, the target-site mutation for organophosphates and carbamates insecticides (Weill *et al.* 2004). The *Ace-1* mutation confers cross-resistance to organophosphates and carbamates. Organophosphates and carbamates act on an insect by inactivating the acetylcholinesterase enzyme site, causing the death of the insect (Weill *et al*. 2002). Several studies in West Africa including, for example, Côte d'Ivoire and Benin and Burkina Faso have reported *Ace-1* mutation in *Anopheles gambiae* species (Ahoua Alou *et al.* 2010; Aikpon *et al.* 2014) and in *An. arabiensis* in Burkina Faso and Ghana (Dabire *et al.* 2014).

1.7.2.2. Metabolic resistance

In contrast to target site mutation, metabolic resistance of insecticide is linked to overexpression of specific enzymes which detoxify the insecticide (Li, Schuler and Berenbaum 2007). Three classes of enzymes are primarily involved in the detoxification of insecticides used in vector control. These are the carboxylesterases (COE) that are involved in resistance to organophosphates, carbamates and pyrethroids; the glutathione S-tranferases (GST) that are involved in resistance to organophosphates and DDT; and the cytochrome P450 monooxygenases (CYP) that are involved in the detoxification of pyrethroids.

Cytochrome P450 monooxygenases also play a role in conferring resistance to organophosphates and carbamates. Biochemical assays may be performed to detect an increase in the activity of enzymes involved in metabolic resistance (Brogdon, McAllister and Vulule 1997). The gene expression levels of enzymes putatively involved in insecticide detoxification may also be quantified by quantitative RT-PCR. The overexpression of genes has been associated with resistance to different classes of insecticides. For example, pyrethroids may be detoxified through overexpression of CYP6P3, a member of the P450 family (Müller *et al.* 2008).

1.7.3. Factors influencing resistance development

Factors that influence the development of insecticide resistance can be grouped into three categories: biological factors, genetic factors and operational factors (Georghiou 1980):

Biological factors are population size (*i.e.* the number of offspring), life span of a generation, type of reproduction (sexual or asexual) and behavioural factors such as mobility and migration.

Indeed, population size plays an important role in the development of resistance; the larger the population is, the higher is the risk to develop insecticide resistance. Insects may also be dispersed by wind. Mosquitoes, for example, fly 50 m up to 5 km into new areas for host seeking, depending on the species (Verdonschota and Besse-Lototskayaa 2013). Therefore, individuals migrating from areas where resistance is a problem could introduce resistance alleles into susceptible populations. Short generation times may also accelerate the development of resistance, linked to the number of generations having undergone selection pressure.

Genetic factors include gene frequency, the number of resistance mechanisms and dominance of resistance alleles, the number of resistance target-sites and past selection by other active ingredients.

Operational factors: this aspect specifically refers to human intervention (*i.e.* the use of insecticides). Non-compliance to correct application dose of insecticides may increase the selection of resistant individuals. Indeed, when an insecticide is used below the recommended dosage, the insecticide will kill the sensitive homozygous but not the resistant heterozygous. Resistance appears more quickly depending on the dose of pesticides applied, which influence the expression of dominance. In addition, repeated application of the same insecticide may lead to the appearance of resistance and increase the frequency alleles in a population due to the constant selection pressure. It is therefore, important to limit the frequency of pesticide application.

1.8. Resistance management

Due to the rise of insecticide resistance to the approved classes of insecticides, developing strategies for resistance management is mostly to decrease resistance in mosquitoes and end malaria transmission. The WHO global plan for insecticide resistance management (GPIRM) developed strategies of resistance management based on the use of IRS and LLIN (WHO 2012). The goal of resistance management is to slow down or avert the spread of resistance though the target vector populations. The intention is to maintain a high percentage of sensitive, wild type genes in the pest population while keeping resistant alleles to a minimum; thus preserving the efficacy of available insecticides used for public health and agriculture. Moreover, it is crucial to know the resistance status and the mechanisms involved in resistance.

Resistance management for the use of IRS and LLIN may include:

- Rotation of insecticides with two or more insecticides in rotation between one year to another;
- Combination of interventions. A combination of two or more insecticides in a house (*e.g.* mosquito nets impregnated with pyrethroids and walls treated with an insecticide with a different mode of action;
- Mosaic spraying, consisting of applying compounds with different modes of action across the same geographic area;
- Combination of insecticides. Two or more compounds of different insecticide classes are mixed to make a single product so the mosquitoes are less likely to develop resistance.

In all cases, the success of the implementation of insecticide resistance management requires the monitoring of malarial vectors.

1.9. Aim and objectives of the thesis

Research question

The spread of resistance to insecticides appears as a threat which can deeply impact the disease control efforts. The success of any control measure requires knowledge of the main malaria vector and its sensitivity with respect to the WHO approved classes of insecticides and to characterise underlying mechanism. Ellibou is a village located in the forested zone, Southern Côte d'Ivoire and crossed by a main road connecting the South to the North Country with several traffics and has never been subject to an entomological survey. Therefore, the following research questions were addressed:

1. What is the main malaria vector and the dynamics of vector transmission of malaria over the course of a year?
2. What is the insecticide resistance profile of the local *Anopheles* population?
3. What are the key habits among the local human population that could affect resistance development in mosquitoes?
4. What are the mechanisms present in the local *Anopheles* population?

The overall goal of the study was to determine the key entomological parameters of malaria transmission and to assess the insecticide susceptibility of malaria vectors to WHO approved insecticides for public health in Ellibou, southern Côte d'Ivoire by adressing the following specific objectives:

1. To determine the species composition of culicidae fauna in Ellibou, EIR and malaria transmission over an entire year;
2. To assess the susceptibility of the main malaria vectors to different classes of insecticides used in the public health and identify potential mechanisms involved in insecticide resistance in the Ellibou *Anopheles* population;
3. To investigate the KAPB of the local populations relative to the use of insecticides both in agriculture and public health.

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Chapter 2. First detection of N1575Y mutation in pyrethroid resistant *Anopheles gambiae* in Southern Côte d'Ivoire

Authors: Ako Victorien Constant Edi [1*], Bédjou Prisca N'Dri [1,3*], Mouhamadou Chouaibou[1], Fodjo Behi Kouadio [1], Patricia Pignatelli [4] , Giovanna Raso [2,3], David Weetman [4], Bassirou Bonfoh[1,2]

[1]Research and Development Department, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, 01 BP 1303, Côte d'Ivoire

[2]Swiss Tropical and Public Health Institute, Basel, CH-4051, Switzerland

[3]University of Basel, Basel, CH-4002, Switzerland

[4]Vector Biology Department, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK

* Equal contributors

Corresponding author: Ako Victorien Constant Edi

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RESEARCH ARTICLE

First detection of N1575Y mutation in pyrethroid resistant *Anopheles gambiae* in Southern Côte d'Ivoire [version 1; referees: 2 approved]

Ako Victorien Constant Edi 1*, Bedjou Prisca N'Dri 1-3*, Mouhamadou Chouaibou[1], Fondjo Behi Kouadio[1], Patricia Pignatelli[4], Giovanna Raso[2,3], David Weetman[4], Bassirou Bonfoh[1,2]

[1]Research and Development Department, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, 01 BP 1303, Cote d'Ivoire
[2]Swiss Tropical and Public Health Institute, Basel, CH-4051, Switzerland
[3]University of Basel, Basel, CH-4002, Switzerland
[4]Vector Biology Department, Liverpool School of Tropical Medicine, Liverpool, L3 5QA, UK

* Equal contributors

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Abstract

Background. The intensification of insecticide use for both public health and agriculture in Africa has contributed to growing insecticide resistance. Today, resistance to World Health Organization (WHO)-approved insecticide classes is widespread. In an agricultural area of Southern Côte d'Ivoire, the main malaria vector *Anopheles coluzzii* shows multiple resistance across insecticides mediated by both target site mutation and metabolic mechanisms. To plan new vector control strategies and avert future resistance liabilities caused by cross-resistance mechanisms extant within populations, it is crucial to monitor the development and spread of both resistance and mechanisms.
Methods. Larvae of *Anopheles gambiae* were collected from natural breeding sites in Tiassalé and Elibou, between April and November 2016 and raised to adults. Adult female non-blood fed mosquitoes, three to five days old, were exposed to deltamethrin in WHO bioassays. Extracted DNA samples from exposed mosquitoes were used for species characterisation and genotyping.
Results. Most adult *An. gambiae* tested were resistant to deltamethrin, with mortality rates of only 25% in Tiassalé and 4.4% in Elibou. Molecular analysis of DNA from samples tested showed the presence of both *An. coluzzii* and *An. gambiae s.s* in Elibou and only *An. coluzzii* for Tiassalé. As previously, the L1014F *kdr* mutation was present at high frequency (79%) in Tiassalé and the L1014S mutation was absent. The N1575Y mutation, which amplifies resistance conferred by L1014F was detected in a single unique individual from a Tiassalé *An. coluzzii* female whereas in Elibou 1575Y was present in 10 *An. gambiae* s.s, but not in *An. coluzzii*.
Conclusion. This is the first report of the N1575Y mutation in Côte d'Ivoire, and as in other populations, it is found in both dominant West African malaria vector species. Continued monitoring of N1575Y is underway, as are studies to elucidate its contribution to the resistance of local vector populations.

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1 **Kobié H. Toe**, National Center for Research and Training on Malaria, Burkina Faso

2 **Jacob Williams**, Tropical Public Health Practice LLC, USA

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Corresponding author: Ako Victorien Constant Edi (edi.constant@hotmail.com)

Author roles: Edi AVC: Formal Analysis, Investigation, Methodology, Writing - Original Draft Preparation; **N'Dri BP**: Investigation, Methodology; **Chouaibou M**: Formal Analysis, Supervision; **Kouadio FB**: Investigation; **Pignatelli P**: Formal Analysis; **Raso G**: Supervision; **Weetman D**: Formal Analysis, Supervision; **Bonfoh B**: Supervision

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Introduction

Malaria remains an important tropical disease that requires a global effort for eradication (WHO malaria report, 2016). Scale-up of existing control measures and development of new tools for the markets (2016–2030) are key components of the global control and elimination strategies (WHO Global Malaria programme, 2016). The development of new tools is of utmost importance, since resistance in the major *Anopheles* malaria vectors of sub-Saharan Africa to WHO-approved insecticides is now widespread and increasing (Ranson & Lissenden, 2016). Increasingly, populations of mosquitoes that are resistant to more than one class of insecticides are being reported (Djouaka *et al.*, 2016; Edi *et al.*, 2014a; Nwane *et al.*, 2013).

Among insecticides, pyrethroids remain the only class approved for long-lasting insecticide treated nets, and major resistance mechanisms are metabolic detoxification, especially by P450 enzymes (David *et al.*, 2013), and mutations in the *para* voltage gated sodium channel (VGSC), the target site of pyrethroids and DDT. VGSCs are transmembrane proteins that transfer sodium ions inside the cell in order to achieve the depolarizing phase of action potentials, an essential phase of nervous impulses (Catterall *et al.*, 2005). Mutations in the VGSC cause a phenotype known as knock down resistance (*kdr*). The most common *kdr* mutations in *Anopheles* are substitutions at the 1014 leucine codon to either phenylalanine (Martinez Torres *et al.*, 1998) or serine (Ranson *et al.*, 2000). Both are now widely distributed across Africa, and sometimes co-occur (Fryxell *et al.*, 2012; Nwane *et al.*, 2011; Pinto *et al.*, 2006; Reimer *et al.*, 2008; Tripet *et al.*, 2007). Moreover, their frequency could differ from a mosquito population tested with insecticide to another (Antonio-Nkondjio *et al.*, 2015). An additional asparagine-to-tyrosine mutation at codon 1575 within the linker between domains III-IV of the VGSC has also been documented in *An. gambiae* and/or *An. coluzzii* from Burkina Faso, Ghana, Benin and Cameroon (Jones *et al.*, 2012; Fossog Tene *et al.*, 2013). The N1575Y mutation provides a synergistic effect on pyrethroid and DDT resistance by elevating the insensitivity of the sodium channel gates produced by the 1014F and 1014S mutations (Wang, 2013), although to date it has only been found on the 1014F haplotype (Jones *et al.*, 2012).

Vector control requires improved management of insecticides and identification of mechanisms of resistance, which can be readily screened to forewarn increases in resistance or represent possible cross-resistance liabilities to new insecticides when they become available. Previous studies in Southern Cote d'Ivoire have documented resistance to multiple insecticides, mediated by multiple mechanisms (Edi *et al.*, 2012; Edi *et al.*, 2014a); however, all samples screened were wild type at the 1575 codon (Edi *et al.*, 2012). Here we report the first detection of N1575Y mutation in deltamethrin resistant populations of *An. gambiae* and *An. coluzzii* from Southern Côte d'Ivoire.

Methods

Study site

All collections were carried out in Tiassalé (latitude 5.89839, longitude -4.82293) and Elibou (latitude 5.69000, longitude - 4.50000), Southern Côte d'Ivoire (Figure 1). The study sites are located in the evergreen forest zone. The primary agricultural activity is cash

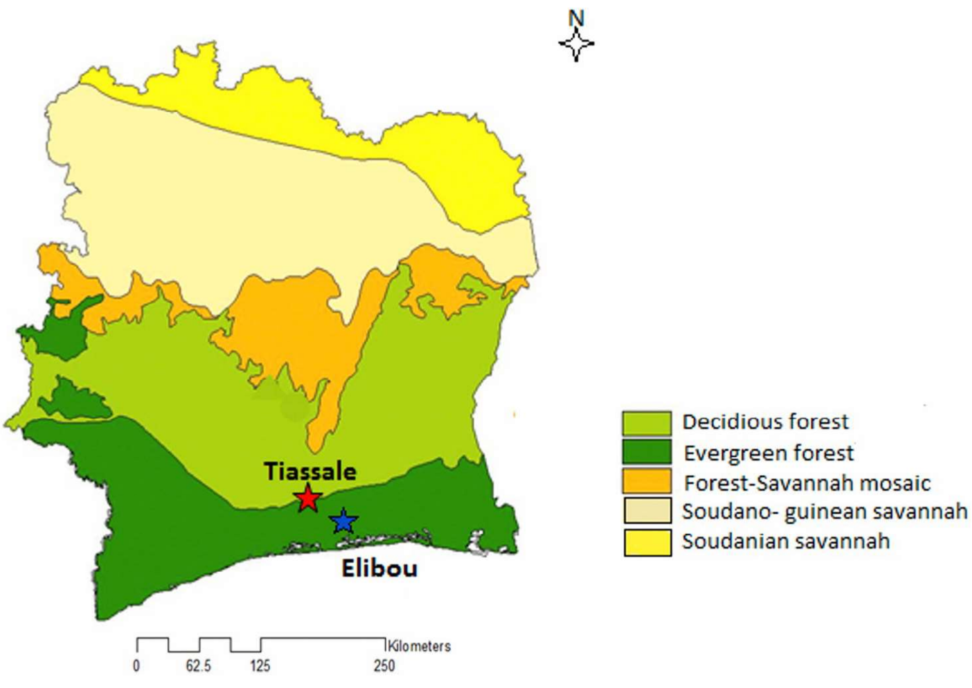

Figure 1. Vegetation map of Côte d'Ivoire, showing the study areas Tiassalé and Elibou.

crop farming of vegetables in Elibou and irrigated rice fields in Tiassalé. High malaria transmission occurs during the rainy seasons, between May and November.

Larval collections

Larval collections of *An. gambiae s.l.* were obtained by dipping from breeding sites in each study location during the peak malaria transmission periods between April and November 2016**.** All larvae were provided a diet of Tetra Mikromin fish food (Tetra; Melle, Germany) until adult emergence. All mosquito rearing was performed under controlled ambient environmental conditions (27°C±2°C, 80% ±4% relative humidity). Adult mosquitoes were given access to 10% sucrose solution.

WHO diagnostic bioassays

Adult female non-blood fed mosquitoes, two to five days old, were exposed to 0.05% deltamethrin for one hour, using WHO tubes and criteria (WHO, 2013). Mosquitoes surviving insecticide exposure were given access to 10% sucrose solution in WHO holding tubes. Mortality was assessed after 24 hours. For each replicate, fifty mosquitoes were exposed to non-treated filter papers as a control in two separate tubes. Exposed mosquitoes were then stored with silica gel until DNA extraction. Levels of mortality between study locations were compared using Chi-square test run on the open source software package R, version 3.4.0 (R Core Team, 2008). For statistical testing, the level of significance was set at $\alpha = 0.05$.

DNA extraction

Genomic DNA was extracted according to the LIVAK method (Livak, 1984) from individual *An. gambiae s.l.* mosquitoes from Tiassalé (n=92) and Elibou. In Tiassalé, extraction was made on individuals surviving exposure to deltamethrin. In Elibou, additional 110 untested females (control samples) obtained from larval collection were considered. Mosquitoes were individually ground in 100µl of preheated grind buffer made with 1.6 ml 5M Nacl, 5.48 g sucrose, 1.57g Tris, 10.16 ml 0.5M EDTA and 2.5 ml 20% SDS (Thermo Fisher Scientific). Mixed buffer-mosquito solution was incubated at 65°C for 30 minutes and then 14 µl 8M K-acetate was incorporated and gently mixed. The resulting thicker mixture was incubated for 30 min on ice and then centrifuged at 13,000 rpm for 20 min (4°C). 200 µl 100% EtOH was then added to the supernatant (later transferred in new Eppendorf tube 1.5 ml) and the mixture was centrifuge again at 13,000 rpm for 15 min (4°C). The supernatant was discarded and the pellet was rinsed with 100 µl ice cold 70% EtOH. Dried pellets were re-suspended in 100 µl TAE buffer. The DNA was used for species ID and target site genotyping.

Species and molecular form identification

The SINE-PCR method was used to identify *An. gambiae s.l.* to species (Santolamazza *et al.*, 2008). A volume of 24.75 µl of master mix was considered per reaction. Overall the master mix (Applied Biosystems) contained 18.83 µl DNase free water, 2.5 µl buffer 10X, 0.75 µl MgCl2 (25mM), 1 µl dNTP (10mM) and respective 1 µl of Sine 6.1a (10 µM) 5'-CGCTTCAAGAATTCGAG ATAC-3' and Sine 6.1b (10 µM) 5'-TCGCCTTAGA CCTT-GCGTTA-3'and 0.17 µl Kappa Taq. Each PCR product containing 23 µl of mix and 2 µl of genomic DNA was amplified for 3 min at 94°C, followed by 35 cycles of 94°C, 62°C, and 72°C for 30 s respectively. The last cycle of was 5 min at 72°C. Products were run on 1.5% agarose gels. In Elibou, untested female samples were used for species and molecular form identification. In Tiassalé, only individual surviving exposure to deltamethrin were considered.

Genotyping assays

TaqMan assays with two labelled Fluorochromes probes FAM and HEX were used to screen for the L1014F and L1014S *kdr* mutations (Bass *et al.*, 2007) and the N1575Y mutation (Jones *et al.*, 2012). A total volume of 9 µl per reaction was used for the mix, containing DNase free water (3.875 µl), Bioline sensimix (5 µl) and specific primer/probe (0.125 µl) (*kdr*-Forward 5'-CATTTTTCTTGGCCACTGTAGTGAT-3', *kdr*-Reverse 5'-CGATCTTGGTCCATG TTAATTTGCA-3') for *kdr* 1014 and (3′NFQ-ATTTTTTTCATTGCATTATAGTAC-5' for N1575 and 3′NFQ-TTTTTCATTGCATAATAGTAC-5'for 1575Y, respectively. The mix was centrifuged at 2000 rpm for approximately 10 seconds. 9 µl of the mix with 1 µl of each gDNA were added to each TaqMan PCR (Applied Biosystems), and centrifuged at 2000 rpm for 15 seconds. Reactions were performed on the Agilent MX3005P qPCR system (Agilent Technologies). The genotype was determined from the fluorescence profiles and bi-directional scatter plots generated in the MX3005P software. The PCR condition was 95°C for 10 minutes (1 cycle) follow-ing by 40 cycles of 95°C at 10 seconds and 60°C at 45 seconds, respectively for Kdr genotyping. For N1575 Y mutation, PCR conditions of 10 min at 95°C followed by 40 cycles of 15 s at 92°C and 1 min at 60°C were considered (Jones *et al.*, 2012).

Results

A total of 291 mosquitoes were tested with deltamethrin in Tiassalé (n=200) and Elibou (n=91) during the rainy season, using standard WHO susceptibility assays. Mortality rates were 25% in Tiassalé and 4.4% in Elibou. The prevalence of *An. gambiae* resistance was different between study locations (Chisq=13, p=0.0003).

All mosquitoes were collected at larval stage. In Elibou, the first emerged (n= 91) were used for bioassay, as previously described, and a sample of 110 from the remaining untested ones were used for molecular form and species identification. All these mosquitoes were from the same batch. Thus, from a subset of DNA samples analysed in Elibou (n= 110) and Tiassalé (n=92), no *An. arabiensis* were detected. All individuals were found to be *An. coluzzii* (100%) in Tiassalé. In Elibou, both *An. coluzzii* (53.6%, n= 59) and *An. gambiae* ss (35.5%, n= 39) and additional hybrid individuals (10.9%, n= 12) were present.

Of the two potential substitutions screened at codon 1014, 1014S was absent, whereas the 1014F *kdr* allele was observed in Tiassalé at high frequency (79.3%). In Elibou, the frequencies of L1014 mutation were not investigated. From the 92 surviving *An. coluzzii* mosquitoes exposed to deltamethrin, a single individual was found to carry the N1575Y mutation (in heterozygous form) in Tiassalé (Table 1). In Elibou, both heterozygous (20.4%) and a single homozygous resistant individual (2.2%) were detected in *An. gambiae* s.s (Figure 2). The overall 1575Y allele frequency in Elibou was 25% (Table 1).

First detection of N1575Y mutation in pyrethroid resistant *Anopheles gambiae* in Southern Côte d'Ivoire

3 Data Files

https://dx.doi.org/10.6084/m9.figshare.5319250.v1

Dataset 1: Raw data for mortality rates from WHO bioassays in Tiassalé.

Dataset 2: Raw data for mortality rates from WHO bioassays in Elibou.

Dataset 3: Raw data for N1575Y genotypes.

Discussion

The aim of this study was to investigate the level of resistance to the pyrethroid deltamethrin in Southern Cote d'Ivoire. It was found that the prevalence of *An. gambiae* resistance was different between study locations. In Tiassalé, the mortality rate to deltamethrin was 25%, while this was six-folds lower in Elibou. Resistance to deltamethrin in the Southern region could be explained by the insecticide pressure due to development of agriculture and use of insecticide in the region Reid & McKenzie, 2016. Indeed, deltamethrin insecticides are largely used by farmers for both pest control and increase of yield. A recent finding also showed that among the main pyrethroid insecticides used in Tiassalé, deltamethrin was the most common (46.9%) followed by lambdacyhalothrin (35.2%) and cypermethrin (10.5%) (Chouaïbou *et al.*, 2016). To our knowledge, there is no cultivation in the Elibou village, but in the surroundings, there is intense rubber (hevea) farming, where insecticides are also applied. Thus, potential contamination of existing breeding sites could occur given the

Table 1. Prevalence of N1575Y allele in Tiassalé and Elibou, Côte d'Ivoire, 2016.

Strains	Species	Phenotype	No. tested	No. per genotype			Frequency (%)
				NN	NY	YY	
Tiassalé	*An. coluzzii*	Alive	92	91	1	0	0.5
Elibou	*An. coluzzii*	Alive	22	22	0	0	0
	An. gambiae s.s	Alive	22	12	9	1	25

Y and N represent mutant resistant (tyrosine) and wild types alleles (asparagine), respectively.

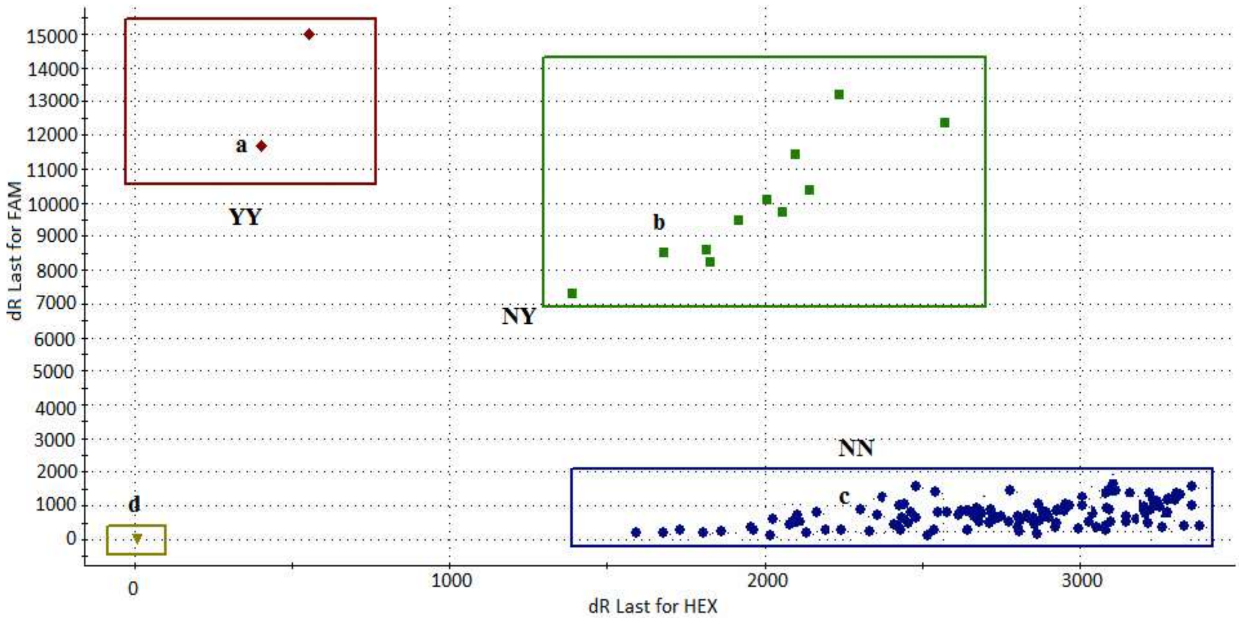

Figure 2. Distribution of N1575Y alleles in Southern Côte d'Ivoire. The letters a, b, c and d represent the positive controls for the homozygous mutant allele (Y), heterozygous mutant (NY) and the homozygous susceptible allele (N) and blank, respectively. Nine individual mosquitoes displayed the heterozygous mutant/susceptible genotype (NY), only one individual carried the homozygous resistant genotype (YY). All the other samples carried the susceptible genotype (NN).

ecology of the region (see description of study sites), and further investigations are required to better understand the emergence of resistance to deltamethrin in this area.

In Tiassalé, the presence of the 1575Y resistance allele was detected for the first time. This is surprising because previous consecutive data from 2011 (0%, n= 184) and 2012 (0%, n=92) did not report the N1575Y mutation (Edi *et al.*, 2012; Edi *et al.*, 2014b). In Elibou, our finding revealed the presence of both heterozygous and homozygous resistant tyrosine alleles in *An. gambiae s.s.* Elibou is located within a 100 km radius from Tiassalé and characterized by the presence of both *An. coluzzii* and *An. gambiae s.s.* The detection of N1575Y mutation in *An. coluzzii* in Tiassalé and *An. gambiae s.s* in Elibou revealed the potential for both species to carry the asparagine-to-tyrosine resistance mechanisms, as previously documented in Burkina Faso (Jones *et al.*, 2012).

The detection of N1575Y mutation now in southern Côte d'Ivoire requires more investigation to better characterize its expected synergistic relationship with 1014F *kdr*. This resistance mechanism could spread very rapidly, and threaten the fragile gains that have been made in reducing the malaria burden in this region through vector control interventions.

Conclusion
The present study showed the presence of the N1575Y mutation in Côte d'Ivoire. The discovery of an additional mechanism that could further reduce insecticide efficacy in the already pyrethroid resistant mosquitoes in this region is concerning. Continued monitoring of N1575Y is underway to elucidate its contribution to the resistance of local vector populations.

Data availability
Figshare

First detection of N1575Y mutation in pyrethroid resistant *Anopheles gambiae* in Southern Côte d'Ivoire

Dataset 1: Raw data for mortality rates from WHO bioassays in Tiassalé.

Dataset 2: Raw data for mortality rates from WHO bioassays in Elibou.

Dataset 3: Raw data for N1575Y genotypes.

https://doi.org/10.6084/m9.figshare.5319250.v1 (Edi *et al.*, 2017)

Competing interests
No competing interests were disclosed.

Grant information
This work was supported by the Wellcome Trust [110430] to AVCE and [103995] to CM; Swiss Government Excellence Scholarships for Foreign Scholars and Artists (EKAS) to BPN'D; DELTAS Africa (Wellcome Trust, AAS, NEPAD, UKAID) [107753] to BB.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgements
We thank the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire; Mr Jackson Kouamé, Mr Kassoum Bamogo, and Mr Yves Kadio for their technical assistance at Tiassalé. Finally, we would like to acknowledge the proof reading from Jasmina Saric at CSRS and thank the populations and the authorities of Tiassalé and Elibou for facilitating this study.

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Open Peer Review

Current Referee Status: ✔ ✔

Version 1

Referee Report 29 September 2017

doi:10.21956/wellcomeopenres.13258.r25868

Jacob Williams
Tropical Public Health Practice LLC, Washington, DC, USA

The primary purpose of reporting on the presence of N1575Y mutation in pyrethroid resistant *Anopheles gambiae* – is well demonstrated. This study provides crucial additional information to the body of knowledge on the evolving scenario of insecticide resistance in the West Africa sub-region. There are few issues requiring the attention of the authors:

1. Consider a slight modification of the title of the paper to indicate evaluation of *Anopheles gambiae* **sensu lato**, since both *An. coluzzii* and *An. gambiae s.s.*, were evaluated:

   "First detection of N1575Y mutation in pyrethroid resistant *Anopheles gambiae (s.l.)*, in Southern Côte d'Ivoire"

2. While the mosquitoes tested were 2-5 days old and varies from the WHO recommendation of 3-5 days old test samples, in this case, the discrepancy does not present a significant problem since the objective of the study to detect the presence of the resistance gene mutation. However, comparison of phenotypic expression from this study (i.e. mortality rates from WHO bio-assay), with results obtained from other datasets elsewhere, may be constrained. This is because mortality rates have been shown to vary with the age of mosquito and the addition of 2-day old samples, will be a confounding factor.

3. It is unclear if the mosquito samples evaluated in Elibou (*n*=44, Table 1) were those surviving the WHO deltamethrin bio-assay, or a subsample from the additional 110 samples the authors indicated were "untested" but also evaluated. I am inclined to believe that the 44 mosquitoes evaluated for N1575Y, were survivals from the 91 samples tested via WHO bio-assay. Please clarify.

Authors indicate ongoing monitoring. They are encouraged to ensure robust concurrent evaluations of the progress/frequency of mutation and the resistance rate (phenotypic) among the various subspecies of *An. gambiae s.l.* in the area, to capture the very likely differences among the species.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Referee Report 18 September 2017

doi:10.21956/wellcomeopenres.13258.r25343

Kobié H. Toe
National Center for Research and Training on Malaria, Ouagadougou, Burkina Faso

The paper looks interesting, clear and well written. The report of the N1575Y mutation, which now contribute to pyrethroid resistance although only reported in some countries. This paper is the proof that it could be spread also in other countries. However I have noticed that some useful details which could lead to better understanding and avoid confusion of the work were missing. Please see comments to author.

Abstract: In the method paragraph it said that three to five days old mosquitoes were used for the bioassays. Which contradict what is mentioned in **the Methods-WHO diagnostic bioassays** part where it is mentioned that two to five day old mosquitoes were used. It is confusing. Give the right age of mosquitoes used for the bioassays. If it was two to five days mosquitoes used, explained why, as the WHO procedure you have cited recommends to use three to five days old mosquitoes. I do not know which is right.

Methods:
In Elibou untested mosquito were used for molecular analysis, and in Tiassalé surviving mosquito to deltamethrim exposure were used.
In Elibou the frequency of 1014F has not been investigated, there is a specific reason for that? It is known that the 1014F is linked to 1575Y. The data of the 1014F should be interesting. Providing this data should be important.
Table1: in this table presenting the results the frequencies of the 1575Y mutation in Tiassalé (n= 92) and Elibou (n= 44), I am a bit confused. 110 untested mosquitoes from Elibou were used for molecular. Why in the table the total number of mosquitoes from Elibou was only 44? These mosquitoes are from those surviving deltamethrim exposure or are subset of the 110 untested mosquitoes used for molecular form and species identification.

Of the most recent paper (http://www.ncbi.nlm.nih.gov/pubmed/25766412), reporting the presence of the 1575Y mutation in Tengrela area in the Burkina Faso, of the closest area from Côte d'Ivoire where this mutation is reported with a frequency around 0.25- is not cited anywhere in the paper. It could be interesting to report to this paper, as it is one of the most recent talking also about this mutation.

I have hope that the authors will continue the monitoring of the mutation as mentioned in the conclusion.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Medical entomologist (field and lab works)

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Chapter 3. Species composition and insecticide resistance in malaria vectors in Ellibou, southern Côte d'Ivoire and first finding of *Anopheles arabiensis* in Côte d'Ivoire

Authors: Bédjou P. N'Dri[1,2,3*], Nadja C. Wipf[2,3], Jasmina Saric[2,3], Behi K. Fodjo[1], Giovanna Raso[2,3], Jürg Utzinger[2,3], Pie Müller[2,3], Chouaïbou S. Mouhamadou[1,4]

Affiliation: [1]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303, Abidjan 01, Côte d'Ivoire. [2]Swiss Tropical and Public Health Institute, Kreuzstrasse 2, CH-4123 Allschwil, Switzerland. [3]University of Basel, P.O. Box, CH-4001 Basel, Switzerland.[4]North Carolina State University, Raleigh, NC, 27695-7508, USA.

*Corresponding author: N'DRI Bedjou Prisca

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Malaria Journal

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Species composition and insecticide resistance in malaria vectors in Ellibou, southern Côte d'Ivoire and first finding of *Anopheles arabiensis* in Côte d'Ivoire

Bédjou P. N'Dri[1,2,3*], Nadja C. Wipf[2,3], Jasmina Saric[2,3], Behi K. Fodjo[1], Giovanna Raso[2,3], Jürg Utzinger[2,3], Pie Müller[2,3] and Chouaïbou S. Mouhamadou[1,4]

Abstract

Background Knowing the species composition and insecticide resistance status of the target vector population is important to guide malaria vector control. The aim of this study was to characterize the malaria vector population in terms of species composition, insecticide susceptibility status and potential underlying resistance mechanisms in Ellibou, southern Côte d'Ivoire.

Methods A 1-year longitudinal entomological survey was conducted using light traps and pyrethroid spray catches to sample adult mosquitoes in combination with larval sampling. The susceptibility status of *Anopheles gambiae sensu lato* (s.l.) to bendiocarb, deltamethrin, DDT and malathion was assessed using the World Health Organization insecticide susceptibility test. Additionally, *An. gambiae* specimens were screened for knockdown (*kdr*) and acetylcholineesterase (*ace1*) target site resistance alleles, and the expression levels of eight metabolic resistance genes, including seven cytochrome P450 monooxygenases (P450s) and one glutathione *S*-transferase (GST), measured with reverse transcription quantitative real-time polymerase chain reaction (qPCR).

Results Overall, 2383 adult mosquitoes from 12 different taxa were collected with *Culex quinquefasciatus* and *An. gambiae* being the predominant taxa. Molecular identification of *An. gambiae* s.l. revealed the presence of *Anopheles arabiensis*, *Anopheles coluzzii*, *An. gambiae sensu stricto* (s.s.) and *Anopheles coluzzii/An. gambiae* s.s. hybrids. *Anopheles gambiae* mosquitoes were resistant to all insecticides except malathion. PCR diagnostics revealed the presence of *ace1*-G280S and the *kdr* L995F, L995S and N1570Y target-site mutations. Additionally, several genes were upregulated, including five P450s (i.e., *CYP6P3*, *CYP6M2*, *CYP9K1*, *CYP6Z1*, *CYP6P1*) and *GSTE2*.

Conclusion This is the first documented presence of *An. arabiensis* in Côte d'Ivoire. Its detection – together with a recent finding further north of the country – confirms its existence in the country, which is an early warning sign, as *An. arabiensis* shows a different biology than the currently documented malaria vectors. Because the local *An. gambiae* population was still susceptible to malathion, upregulation of P450s, conferring insecticide resistance to pyrethroids, together with the presence of *ace1*, suggest negative cross-resistance. Therefore, organophosphates could be an alternative insecticide class for indoor residual spraying in the Ellibou area, while additional tools against the outdoor biting *An. arabiensis* will have to be considered.

*Correspondence:
Bédjou P. N'Dri
prisca.b.ndri@gmail.com
Full list of author information is available at the end of the article

Keywords *Anopheles arabiensis*, *Anopheles gambiae*, Côte d'Ivoire, Insecticide resistance, Metabolic resistance, Malaria

Background

Malaria is a major public health problem in sub-Saharan Africa. In Côte d'Ivoire, malaria is the leading cause of mortality and hospitalization in children under the ageof 5 years [1]. Across sub-Saharan Africa, the key malaria vectors are *Anopheles gambiae sensu stricto* (s.s.), *Anopheles coluzzii*, *Anopheles arabiensis* and *Anopheles funestus* [2]. In Côte d'Ivoire, *An. gambiae* s.s., *An. coluzii*, *Anopheles nili* and *An. funestus* are known vectors driving malaria transmission [3] with *An. gambiae* s.s. and *An. coluzzii* being the key vectors [4]. Recently, the presence of *An. arabiensis*, another important malaria vector, has been reported from Bouaké in the central part of Côte d'Ivoire, an observation that might be linked to the urbanization process [5].

The control of malaria vectors primarily relies on indoor residual spraying (IRS) and long-lasting insecti- cidal nets (LLINs) [6] with pyrethroids being the main class of insecticides for bed nets. However, susceptibility to pyrethroids has generally declined in *Anopheles* mosquitoes [4], including in Côte d'Ivoire, where insecticide resistance is widespread [4], representing a threat to the success of existing malaria control and elimination programmes as well as for the control of other vector-borne diseases.

Several molecular mechanisms are involved in conferring insecticide resistance, while the key mechanisms are metabolic resistance and target-site insensitivity. The most studied target-site insensitivity loci in *An. gambiae sensu lato* (s.l.) are point mutations in the voltage-gated sodium channel, conferring cross-resistance to pyrethroids and dichlorodiphenyltrichloroethane (DDT), including the L995F (formerly known as L1014F or *kdr* 'west') and L995S (formerly known as L1014S or *kdr* 'east') at the same codon position [7, 8]. Both *kdr* point mutations are present in *An. gambiae* across most of Africa and may co-occur in the same individual [9–13]. While L995F has been first detected in Côte d'Ivoire [14] and is now widely present, the L995S *kdr* has only recently been detected in Côte d'Ivoire in a single hybrid mosquito carrying both *kdr* mutations [15]. An additional *kdr* mutation—N1570Y (formerly known as N1575Y), the so called "super *kdr*"—occurs with and intensifies the effect of the L995F-mediated pyrethroid resistance and has recently also been detected in southern Côte d'Ivoire [16, 17]. Another key point mutation is the acetylcholinesterase *ace1*-G280S (formerly known as G119S) mutation and is associated with resistance to carbamates and organophosphates [18, 19]. Both *kdr* and *ace1*-G280S mutation were present in central and northern Côte d'Ivoire [20–22]. In contrast to target-site insensitivity, metabolic resistance is linked to overexpression of specific enzymes that break down, export or sequester insecticides. The three major enzyme families associated with metabolic insecticide resistance are carboxylesterases (COE), glutathione *S*-transferases (GSTs) and cytochrome P450 monooxygenases (P450s) [23].

Knowing the species composition and insecticide resistance status of the target vector population is important to guide malaria vector control. While several studies were conducted on malaria transmission, vector population and insecticide resistance in Côte d'Ivoire, highlighting the impact of irrigated rice farming on malaria transmission [22, 24–27], the picture across the country is still very patchy. This motivated the study reported here, carried out in the southern part of Côte d'Ivoire in the village of Ellibou, situated in a forest and ground swamp area suitable for malaria vectors. The village belongs to the region of Agneby-Tiassa and is located along the main motorway connecting Abidjan with the capital Yamoussoukro. Along this route, mosquitoes may be displaced through motorized vehicles. Thus far, Ellibou has not been the subject of entomological surveys. Given the behavioural and ecological differences among malaria vectors and the spread of resistance to public health insecticides across the country, a deeper understanding of local species composition and an accurate and updated insecticide resistance profile are central to the success of vector control. Hence, the overarching aim of this study was to characterize the local malaria vector population in Ellibou in terms of species composition, sporozoite rates, insecticide susceptibility status and potential underlying resistance mechanisms.

Methods

Study site

The study was carried out in Ellibou (geographical coordinates: 5°40′59.4″ N latitude, 4°30′31.1″ W longitude; Fig. 1) from January to December 2015. Ellibou has a population of approximately 12,000 inhabitants according to "Recensement Géneral de la Population et de l'Habitat 2021" and belongs administratively to the Agneby-Tiassa region. The village is located in a forested area, some 60 km north-west of Abidjan with direct access to a national motorway. Agriculture and trade are the main economic activities in Ellibou. The village is surrounded by uneven ground leading to many small

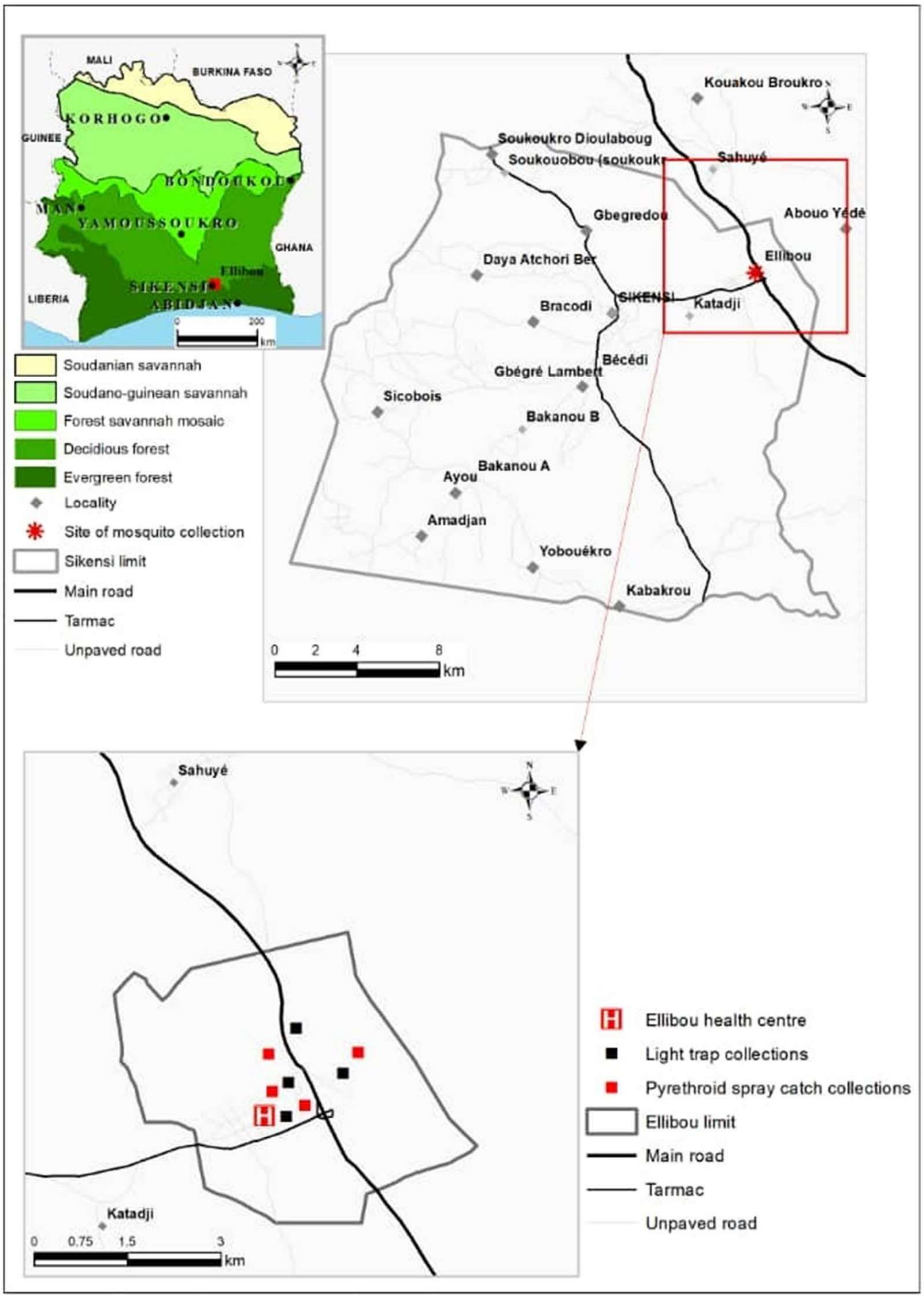

Fig. 1 Map of Côte d'Ivoire showing the study site,Ellibou, where the mosquitoes were collected. The map was created using ArcGISDesktop 10.7.1 (ESRI Inc., Redlands, CA, USA). Source of backgroundmap: CNTIG data (Comité National de Télédétection et d'InformationGéographique), 2020

temporary ponds that serve as breeding sites for mosquitoes during the rainy season. At the time of the study, insecticide-treated nets (ITNs) were the only vector control intervention implemented in Ellibou.

Meteorological data

Meteorological data, including monthly average temperature and precipitation, were obtained from the local weather station of the Meteorological Department of the Society for Airport, Aeronautical and Meteorological Operations and Development in Côte d'Ivoire. The climate of Ellibou is characterized by four seasons: (i) a long rainy season from April to July; (ii) a short dry season in August and September; (iii) a short rainy season in October and November; and (iv) a long dry season from December to March.

Mosquito sampling and morphological identification

Mosquitoes were sampled as either adults or larvae. Adult mosquitoes were collected from houses using Centers for Disease Control and Prevention (CDC) light traps and pyrethroid spray catches (PSC) twice per week from January to December 2015. Both collection techniques were performed on the same days. In addition, the number of people that slept in the rooms the previous night was recorded.

The CDC light traps (John W. Hock Company; Gainesville, FL, USA) were placed in four arbitrarily chosen houses across four sectors given by the main road and the bridge, dividing Ellibou into four sectors (Fig. 1). In each sector, two houses were selected, one house for PSC and another one for CDC light traps with a distance of approximately 100 m between the houses. In each house, a CDC light trap was placed inside one bed room. Inside the bed room, the trap was mounted at 1.5 m above the floor and close to the bed of the study participants that slept under a long-lasting insecticidal net (LLIN). While all other lights in the room were switched off, mosquitoes that entered the room were attracted by the incandescent light of the trap and then aspirated into a collection bag by the trap's fan. The traps ran for two consecutive days in each week from 20:00 to 06:00 h. Each morning, the mosquitoes caught in the traps were removed and transferred to the laboratory at Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS) in Abidjan for morphological identification. The mosquito species and sex, as well as the gonotrophic stage of the females (i.e. unfed, fed, semi-gravid or gravid) were recorded.

PSC were performed on two consecutive days per week in the mornings from 05:30 to 07:30 h in four additional, arbitrarily chosen houses by spraying a commercially available product "ORO" (Químicas ORO SA; San Antonio de Benagéber, Spain) containing pyrethrin and the synergist piperonyl butoxyde. Before spraying, a white sheet was placed on the floor, then the room was sprayed and any mosquitoes knocked down within 10–15 min after spraying were collected with forceps, put in Petri dishes and transferred to the entomology lab at CSRS for morphological identification.

For the insecticide susceptibility assays, the mosquitoes were collected as larvae during the long rainy season in June and July 2015. The larvae were sampled using a standardized dipper from breeding sites such as puddles on unpaved roads and around household courtyards within the village. Larvae that were morphologically identified as *Anopheles* sp. were transferred to the insectary at the CSRS and reared to adult stage. The larvae were fed ground Tetra MikroMin fish food (Tetra; Melle, Germany), while the emerging adult mosquitoes were provided with 10% honey solution *ad libitum*. All mosquito rearing was performed under controlled ambi- ent environmental conditions with a temperature of 27 ± 2 °C, a relative humidity of 80 ± 4% and a 12:12 h light: dark photoperiod.

Sporozoite rate

In addition to species identification and insecticide resistance testing, the female *Anopheles* caught inside the houses with PSCs were assessed for their gonotrophic status and for the presence of *Plasmodium falciparum* sporozoites. For the presence of sporozoites, the mosquitoes were screened using the circumsporozoite protein-based enzyme-linked immunosorbent assay (ELISA) from heads and thoraces from all PSC-sampled *Anopheles* mosquitoes following the protocol by Wirtz et al. [28].

Insecticide susceptibility tests

The World Health Organization (WHO) insecticide susceptibility assays [29] were performed to assess the susceptibility of 3- to 5-day-old adult *An. gambiae* females reared from larval collections to diagnostic concentrations of deltamethrin (0.05%), DDT (4%), malathion (5%) and bendiocarb (0.1%). These insecticides were chosen because they represent the conventional insecticide classes for adult mosquitoes. The test papers were purchased from the official WHO provider, University Sains Malaysia.

In the experiments, batches of 20–25 mosquitoes per tube with four replicates (i.e. 80–100 mosquitoes) were exposed to each insecticide for 60 min and the number of specimens knocked down was recorded. Subsequently, the mosquitoes were transferred into holding tubes and supplied with 10% honey solution. After 24 h, the number of dead mosquitoes was recorded and insecticide resistance classification was performed based on WHO criteria [29]. The insecticide susceptible *An. gambiae* s.s. Kisumu strain was included in all assays as a control for the assay and for assessing the quality of the insecticide-impregnated test papers.

Nucleic acids extraction

Non-blood fed adult *An. gambiae* females, emerging from the larval collections that had not been exposed to insecticides, were stored in RNA*later* (Ambion, Inc.; Austin, TX, USA). Nucleic acids were extracted from a subset of 141 specimens using MagaZorb® DNA Mini-Prep kits (Promega Corporation; Madison, WI, USA) with slight modifications of the manufacturer's protocol. The MagaZorb® kit extracts both DNA and RNA simultaneously. In addition, nucleic acids were extracted from 2- to 3-day-old females of Kisumu ($n = 50$) and Ngousso strains ($n = 50$) and served as insecticide susceptible references.

Mosquitoes were ground on ice in 200µl TE buffer using a plastic pestle in a 1.5 ml Eppendorf tube. Subsequently, 200 µl of lysis buffer was added and vortexed for 15 s. During a 10 min incubation period at room temperature (25 °C), the sample was repeatedly vortexed for 15 s every 2 min. To pellet the mosquito debris, the lysed sample was centrifuged for 2 min at 16,000 rcf and the clear supernatant was transferred into a 1.5 ml Eppendorf tube. Twenty microlitres of magnetic beads (MagaZorb® Reagent) and 500 µl of binding buffer were added to the supernatant and vortexed for 15 s. The sample was again incubated for 10 min at room temperature and repeat-edly vortexed. After letting the magnetic beads sediment settle by placing the tube on a magnetic rack for 2 min, the supernatant was discarded. Subsequently, the mag- netic beads were washed two times as follows: 200 µl of wash buffer was added, then the mixture was vortexed and incubated at room temperature for 1 min. After sedimentation of the beads on a magnetic rack for 2 min, the supernatant was discarded. Finally, 180 µl elution buffer was added, the mixture was vortexed and incubated for 10 min at 50 °C while repeatedly vortexing. The sample was vortexed again, spun down and placed on a magnetic rack for 2 min. On ice, a new 1.5 ml tube was prepared in which the supernatant containing the purified DNA and RNA was collected. The nucleic acid extracts were stored at − 80 °C until subjecting them to a series of quantitative PCR (qPCR) assays.

Molecular species identification

All qPCR reactions, including the species identification, target-site and metabolic resistance assays, were performed on a Bio-Rad CFX 96 real-time PCR machine (Bio-Rad; Hercules, CA, USA). In each reaction, 1 µl of the nucleic acids extract from individual mosquitoes was added to the one-step reverse transcription qPCR (RT-qPCR) master mix supplied by Fast Track Diagnos- tics (Esch-sur-Alzette, Luxembourg) together with the primers and probes at the concentrations described by Wipf et al. [30] to reach a total reaction volume of 10 µl. The following thermal cycle conditions were used for all assays: 50 °C for 15 min, 95 °C for 3 min, followed by 40 amplification cycles of 95 °C for 3 s and 60 °C for 30 s. The purified nucleic acids were subjected to two complementary TaqMan qPCR assays to identify the 141 specimens to species level within the *An. gambiae* complex with previously described adaptations to the original protocols put forth by Wipf et al. [30].

The first assay differentiated *An. coluzzii*/*An. gambiae* s.s. (Ag+) as a group from *An. arabiensis* (Aa+) and *Anopheles bwambae*/*Anopheles melas*/*Anopheles merus*/*Anopheles quadirannulatus* (Aq+) as a group [31]. The second assay distinguishes between *An. gambiae* s.s. (former molecular S-form) and *An. coluzzii* (former molecular M-form) in Ag + samples based on a short interspersed nuclear element (SINE) using common primers published by Santolamazza et al. [32] and probes by Wipf et al. [30].

For confirmation and in view of no prior records of *An. arabiensis* from Côte d'Ivoire at the time of this study in 2015, amplicons from a subset of 10 qPCR assays that identified *An. arabiensis* were repeated and their amplicons sequenced. The amplicons were sent to Microsynth AG (Balgach, Switzerland) for Sanger sequencing and searched using BLAST [33].

Insecticide target-site mutation assays

Previously published qPCR protocols were followed to identify the presence of insecticide resistance alleles, including the *kdr* mutations N1570Y [17], L995F and L995S [34, 35] and the *ace1* mutation G280S [35].

Gene expression levels of metabolic resistance loci

The expression levels of eight detox genes previously associated with metabolic resistance in *An. gambiae* were measured in nucleic acids extracted from RNA*later*-preserved, individual specimens that were raised from field-collected larvae. These genes included seven P450s (i.e. *CYP4G16*, *CYP9K1*, *CYP6M2*, *CYP6P1*, *CYP6P3*, *CYP6P4* and *CYP6Z1*) and one GST (*GSTE2*). The expression levels were measured by RT-qPCR Taqman triplex assays, developed by Mavridis et al. [36], in which the transcripts of the ribosomal protein S7 (*RPS7*) serves as an internal reference to adjust for the overall expression level. Gene expression levels of field-collected specimens from Ellibou were calculated relative to two insecticide-susceptible laboratory strains of the same species. *Anopheles gambiae* s.s. from the field were compared to *An. gambiae* s.s. Kisumu, originating from Kisumu, Kenya, and *An. coluzzii* from the field were compared to *An. coluzzii* Ngousso, originating from Yaoundé, Cameroon. No gene expression data for *An. coluzzii*/*An. gambiae* s.s. hybrids and *An. arabiensis* were included in this study, because of the low number of field samples from Ellibou and because there was no susceptible laboratory reference stain of the same species available. Gene expression assays were ran for 50 individuals per species both from the field and laboratory reference strain, but only qPCR results that pass pre-set quality thresholds were included, e.g. individuals whose *RPS7* quantification cycle (Cq) values were above 27.10 have been excluded from the analysis.

Statistical analysis

The monthly frequencies of mosquitoes collected from both CDC light traps and PSC collections were determined. Data were double entered into an Excel spreadsheet (Windows 10, Microsoft Corporation; Redmond, WA, USA).

The level of significance was set at $\alpha = 0.05$. Pearson product-moment correlation tests were performed in order to assess linear associations between the entomological parameters and meteorological data. The sporozoite rate was determined by calculating the proportion of mosquito found to be positive to the circumsporozoite protein. The 95% confidence interval (CI) for frequency was calculated by using the formula: $\hat{p} + / - z^{*}(\hat{p}(1-\hat{p})/n)^{0.5}$, where z is 1.96. Data from the WHO insecticide susceptibility assays were interpreted according to WHO criteria [29] in the respective mosquito population. For all qPCR assays, the Cq values were determined from the measured amplification curves using the Bio-Rad CFX Maestro 1.0 software (Bio-Rad Laboratories; Hercules, CA, USA). For the target-site qPCR assays, the allele frequencies of each *kdr* and the *ace1* mutation were calculated by using the Hardy-Weinberg formula: $f(R) = (2n.RR + n.RS)/2N$ where n represents the number of mosquitoes of a given genotype and N the total number of mosquitoes analyzed. For the gene expression analysis, Cq values of the metabolic resistance genes were normalized against the Cq values of the 'housekeeping' gene *RPS7* to account for total gene expression in each individual. Then, using the method of Pfaffl et al. [37] implemented the REST 2009 software version 2.0.13, the expression levels of the resistant *An. gambiae* s.s. and *An. coluzzii* field population were calculated relative to the susceptible Kisumu and Ngousso laboratory colonies, respectively.

Results

Mosquito species composition

During the 1-year study, 2383 adult female mosquitoes from 10 taxa were collected by CDC light traps and PSC. Almost two-thirds of all mosquitoes were caught in the PSCs (Table 1). About half of the mosquitoes were morphologically identified as *Culex quinquefasciatus*, constituting the most abundant mosquito species in Ellibou, followed by the malaria vector species of the Gambiae complex. In addition to *An. gambiae* s.l., the malaria vectors *An. funestus* s.l. and *Anopheles pharoensis* were present. Among *Anopheles* sp., members of the Gambiae complex were by far the most predominant taxon with higher proportions in the PSCs. *Aedes, Culex* and *Mansonia* were the other culicine genera collected in Ellibou, representing 57.8% and 72.0% of the total mosquito population using CDC light traps and PSCs, respectively.

The numbers of adult *An. gambiae* mosquitoes collected inside the houses increased considerably when precipitation was above 200 mm per month with about a 1-month lag. The monthly average temperature in Ellibou was 26 °C, ranging between 20 and 30 °C and was not correlated with mosquito abundance (Fig. 2).

A total of 141 *An. gambiae* s.l. female adults reared From larvae collected in Ellibou were subjected to molecular species identification assays. For 13 individuals the qPCR reaction did not yield a signal; hence,

Table 1 Species and numbers of adult Culicidae sampled indoors from Ellibou, Côte d'Ivoire, in 2015

Subfamily	Species	Sampling method		
		Both methods	CDC light trap	PSC
Anophelinae	*Anopheles gambiae* s.l.	865	238	627
	Anopheles pharoensis	8	5	3
	Anopheles funestus s.l.	7	4	3
Culicinae	*Culex quinquefasciatus*	1265	471	794
	Culex nebulosus	106	79	27
	Culex decens	45	37	8
	Mansonia africana	41	19	22
	Culex cinereus	26	17	9
	Mansonia uniformis	11	9	2
	Aedes aegypti	9	5	4
	Total	2383	884	1499

n: number of specimens caught by both sampling methods

PSC pyrethroid spray catch

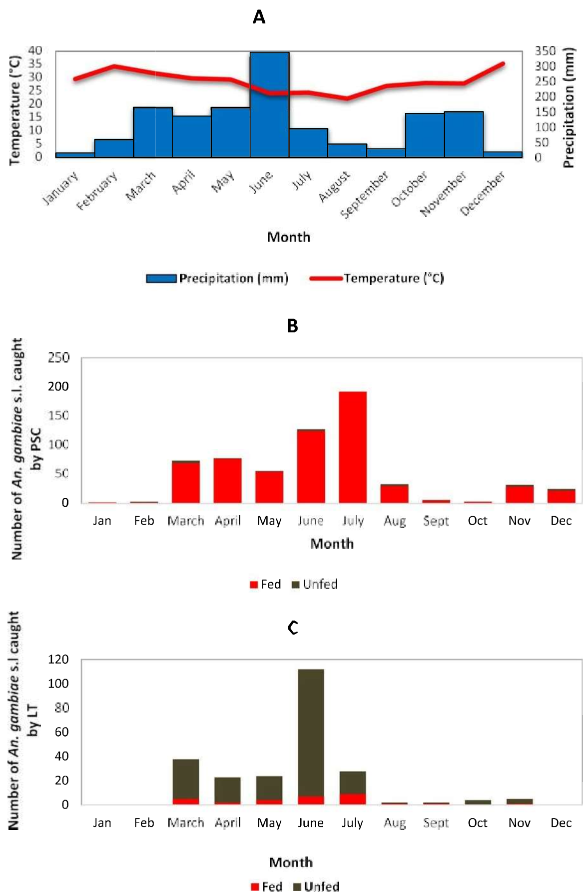

Fig. 2 Seasonal weather conditions and changes inmosquito densities, Ellibou, Côte d'Ivoire, in 2015. Curves showing monthly variationin *An. gambiae* s.l. caught by PSC andCDC light trap: **A.** Monthlytemperature and precipitation; **B.** Monthlycaught fed and unfed *An. gambiae*s.l. by PSC; **C**. Monthlycaught fed and unfed *An. gambiae*s.l. by CDC light trap

these samples were excluded from further analysis. The molecular diagnostics revealed the presence of *An. arabiensis*, *An. coluzzii*, *An. gambiae* s.s. and hybrids between *An. coluzzii* and *An. gambiae* s.s. *Anopheles gambiae* s.s. (38.3%) and *An. coluzzii* (39.0%) were almost equally represented and 4.7% were *An. coluzzii*/*An. gambiae* s.s. hybrids. As the *An. arabiensis* finding is the first in Côte d'Ivoire at the time of the study, the amplicons of 10 specimens identified as *An. arabiensis* were sequenced for confirmation. All 10 sequences were identical with the *An. arabiensis* isolate NMNH2 ribosomal RNA intergenic spacer (GenBank: EU091308.1).

Blood feeding and sporozoite rates

The number of blood-fed mosquitoes was linearly correlated with rainfall ($r = 0.6$, $p < 0.05$). Overall, 5.3% (95% CI 3.8–7.3%; $n = 607$) of tested blood-fed female *An. gambiae* were positive for sporozoites, while infective mosquitoes were detected throughout most of the year, except during the long dry season in January, February and December (Table 2).

Table 2 Seasonal variation in the number of blood-fed and proportion of sporozoite-positive *An. gambiae* s.l. in Ellibou, Côte d'Ivoire, in 2015

Season	Month	Blood-fed mosquitoes (n)	Sporozoite positive (%)[a]
Long dry	January	2	0.0 [0.0–65.8]
	February	2	0.0 [0.0–65.8]
	March	69	1.5 [0.1–7.8]
Long rainy	April	76	7.9 [3.7–16.2]
	May	54	9.3 [4.0–19.9]
	June	124	6.5 [3.3–12.2]
	July	191	0.5 [0.0–2.9]
Short dry	August	30	10.0 [3.5–25.6]
	September	5	100 [56.6–100]
Short rainy	October	3	66.7 [20.8–98.3]
	November	29	3.5 [0.2–17.2]
Long dry	December	22	0.0 [0.0–14.9]
Entire year		607	5.3 [3.8–7.3]

n: number of blood-fed *An. gambiae s.l.* mosquitoes caught using pyrethroid spray catches (PSC)

[a] The number in brackets indicate the lower and upper limits of the 95% confidence intervals

Insecticide susceptibility tests

To assess the insecticide resistance status of *An. gambiae* in Ellibou, adult females were raised from larvae collected from across Ellibou and then exposed to discriminating concentrations of the four conventional classes of insecticides (i.e. organochlorines, pyrethroids, organophosphates and carbamates).

A sample of 87 – 100 mosquitoes was exposed to an insecticide representing each insecticide class. In the positive controls, all *An. gambiae* Kisumu tested exhibited 98–100% mortality to all insecticides, indicating a good quality of the insecticide-impregnated papers, while exposure to control papers without insecticide resulted in mortality rates below 5%. The local *An. gambiae* population in Ellibou showed resistance to all insecticides tested, except for malathion (Table 3).

Molecular resistance mechanisms

All four target-site resistance markers for which mosquito samples were screened were present, including the *kdr* alleles L995S, L995F and N1570Y, and the *ace1*-G280S mutation. The L995F *kdr* mutation was

Table 3 Insecticide resistance status of *An. gambiae* s.l. from Ellibou, Côte d'Ivoire, in 2015

Colony	Insecticide	*n*	Knockdown (%)	Mortality (%)	Status
Ellibou	DDT 4%	94	0	0	Resistant
	Deltamethrin 0.05%	91	18	4	Resistant
	Bendiocarb 0.1%	87	69	52	Resistant
	Malathion 5%	87	60	98	Susceptible
Kisumu	DDT 4%	100	77	98	Susceptible
	Deltamethrin 0.05%	97	92	100	Susceptible
	Bendiocarb 0.1%	99	85	98	Susceptible
	Malathion 5%	97	85	100	Susceptible

n: total number of An. gambiae s.l. exposed in the WHO insecticide susceptibility test

predominant across *An. arabiensis*, *An. gambiae* s.s., *An. coluzzii* and *An. coluzzii*/*An. gambiae* s.s. hybrids with allelic frequencies ranging from 23 to 100% (Table 4). The highest rate was found in *An. gambiae* s.s. (100%). In contrast to L995F *kdr* mutation, the L995S mutant allele was only detected in *An. arabiensis*–at both homozygous and heterozygous state–with an allelic frequency of 65%. In addition, 7 out of 23 *An. arabiensis* individuals were found carrying the double 995 F and 995 S mutant alleles simultaneously. In contrast, the N1570Y *kdr* mutation was only found in *An. gambiae* s.s. albeit only at low frequency (8%). The *ace1*-G280S mutation was found in *An. arabiensis*, *An. coluzzii* and *An. gambiae* s.s. as well the *An. coluzzii*/*An. gambiae* s.s. hybrids with frequencies between 6% and 50% (Table 4 and Additional file 1: Table S4).

Expression levels of the measured metabolic resistance genes are shown in Fig. 3. All genes included in the expression analysis were significantly different expressed in *An. coluzzii* from Ellibou when compared to the susceptible Ngousso laboratory strain. The most upregulated genes were *CYP6P4* and CYP6M2, while *CYP4G16* was downregulated in the *An. coluzzii* field population. In *An. gambiae* s.s. from Ellibou less genes were expressed. The other genes *CYP6P4, CYP4G16* and *GSTE2* were statistically not significantly different expressed in the *An. gambiae* s.s. comparison (Additional file 2).

Discussion

Anopheles gambiae was the principal malaria vectors identified in Ellibou, southern Côte d'Ivoire, with *P. falciparum*-infected individuals present throughout the year except for the long dry season. Species identification of larval collections showed that *An. coluzzii* and *An. gambiae* s.s. constituted the major malaria vectors at almost

Table 4 *kdr* and *ace1* allele frequencies *in An. gambiae* s.l. from Ellibou, Côte d'Ivoire in 2015

Allele	Species	*n*	Genotype			Allele frequency (%)
			SS	RS	RR	
kdr-L995F	*An. arabiensis*	23	13	9*	1	23.9 [13.9–37.9]
	An. coluzzii	47	7	17	23	67.0 [57.0–75.7]
	An. gambiae s.s.	48	0	0	48	100 [92.6–100]
	An. coluzzii/*An. gambiae* s.s.	6	1	2	3	66.7 [22.2–95.7]
kdr-L995S	*An. arabiensis*	23	3	10*	10	65.2 [42.7–83.6]
	An. coluzzii	47	47	0	0	0 [0.0–7.5]
	An. gambiae s.s.	48	48	0	0	0 [0.0–7.4]
	An. coluzzii/*An. gambiae* s.s.	6	6	0	0	0 [0.0–45.9]
kdr-N1570Y	*An. arabiensis*	23	23	0	0	0 [0.0–14.8]
	An. coluzzii	47	47	0	0	0 [0.0–7.5]
	An. gambiae s.s.	48	41	6	1	8.3 [2.3–19.9]
	An. coluzzii/*An. gambiae* s.s.	6	6	0	0	0 [0.0–45.92]
ace1-G280S	*An. arabiensis*	23	20	3	0	6.5 [2.2–17.5]
	An. coluzzii	47	14	31	2	37.2 [28.1–47.3]
	An. gambiae s.s.	48	23	21	4	30.2 [21.9–40.0]
	An. coluzzii/*An. gambiae* s.s.	6	0	6	0	50 [11.8– 88.1]

n: total number of individuals screened

RR homozygous mutant; RS: heterozygous, *SS* homozygote wild type

* Seven individuals harboured the double 995 F and 995 S mutant alleles simultaneously

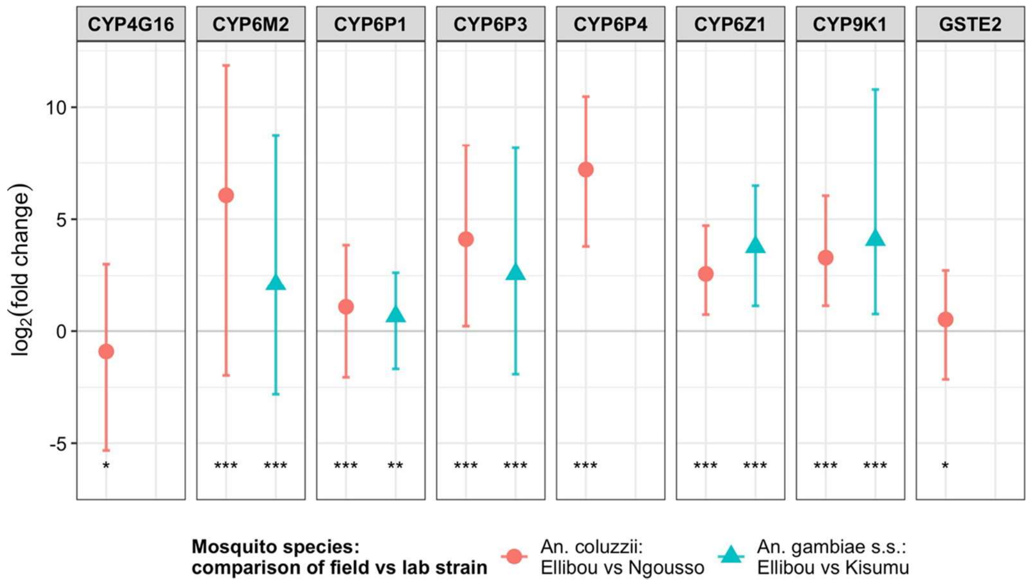

Fig. 3 Expression levels of eight metabolic resistance genesin *An. coluzzii* (blue) and *An. gambiae* s.s. (red) fromEllibou compared to insecticide susceptible laboratory strains of the samespecies: *CYP4G16*, *CYP6M2*, *CYP6P1*, *CYP6P3, CYP6P4, CYP6Z1, CYP9K1* and *GSTE2*. Genes with a $\log_2$ fold change abovethe thick, gray horizontal line ($\log_2FC > 0$) were significantlyhigher expressed in the field population than in the reference lab populations,while genes below the horizontal line ($\log_2FC < 0$) were significantlylower expressed in the field than in the lab population; Levels of significance:*p-value ≤ 0.05; **p-value ≤ 0.01; and ***p-value ≤ 0.001, not plotted whenp-value > 0.05

equal proportions, while 4.7% of the *An. gambiae* s.l. population were *An. coluzzii*/*An. gambiae* s.s. hybrids.

Surprisingly, 18% of the *An. gambiae* population were *An. arabiensis*, this being the first documented finding of *An. arabiensis* in Côte d'Ivoire at the time this study was conducted in 2015.

Recently, a study from Bouaké in the central part of Côte d'Ivoire also reported the presence of *An. arabiensis* [5] albeit the finding was 5 years following the one made here. Moreover, *An. arabiensis* is found in Côte d'Ivoire's neighbouring countries, including Burkina Faso, Mali and Ghana [38–40] and across the West Africa region in countries, such as, Benin [41], Cabo Verde [42], Gambia [43], Guinea Bissau [44], Mauritania [45], Nigeria [46], Senegal [47] and Togo [48]. *Anopheles arabiensis* generally breeds and predominates in semi-arid and savannah areas [49, 50]. However, *An. arabiensis* may also breed in similar breeding sites to those of *An. gambiae* such as small temporary, sunlit and clear freshwater pools [50] and is found in more polluted breeding sites which might be linked to increased expression of detoxifying enzymes and target-site resistant loci [51].

In 2002, Ellibou experienced a transformation of its environment, explained by a sudden increase in population due to the socio-political crisis in Côte d'Ivoire, which could have indirectly led to more suitable habitats for *An. arabiensis*. Moreover, the motorway connecting Abidjan with the country's capital, Yamoussoukro, crosses Ellibou village that constitutes a checkpoint for the control of all transport vehicles and their passengers. Therefore, it is conceivable that *An. arabiensis* was introduced to Ellibou by road traffic from neighbouring countries while the species was already pre-adapted to the altered local environment.

The presence of *An. arabiensis* in Côte d'Ivoire, now confirmed by two independent studies, is worrying and suggests that this major malaria vector is spreading further into new areas in West Africa. Indeed, in several West African cities, *An. arabiensis* has already become a dominant malaria vector [51–53]. In view of *An. arabiensis* primarily feeding outdoors [54], the effectiveness of IRS and LLINs might diminish should *An. arabiensis* become more dominant, requiring alternative intervention strategies. Worryingly, several point mutations conferring insecticide resistance were also identified in the *An. arabiensis* population of Ellibou, further challenging its control using insecticides.

The co-occurrence of *An. coluzzii* and *An. gambiae* s.s. is on par with other observations made across Côte d'Ivoire [55–57]. The presence of both sibling species at almost equal proportions, together with the presence of hybrids, suggests that Ellibou is an area were hybridization of the two sibling species takes place. In this respect it is conspicuous that both sibling species show a rather similar insecticide resistance profile which could be a result of hybridization. This is on par with the hypothesis that hybridization between the two species might have implications for the introgression of insecticide-resistance loci and wider consequences for malaria transmission [58].

A weakness of the current study is that, in contrast to the specimens reared from larval collections, the adult *An. gambiae* specimens were not further identified to sibling species level. Moreover, the collection methods used for the adult mosquitoes, including the CDC light traps and PSC, are biased towards mosquitoes biting and resting indoors. Therefore, the extent to which each sibling species and their hybrids contribute to malaria transmission in the Ellibou region—and Côte d'Ivoire as a whole—requires further investigations.

The *An. gambiae* population of Ellibou showed phenotypic resistance to deltamethrin, DDT and bendiocarb, yet was still susceptible to malathion. Parallel to phenotypic insecticide resistance, several target-site resistance alleles were identified, including the *kdr* L995F, L995S and N1570Y as well as the *ace1*-G280S mutation, although the *kdr* allele L995S was only present in *An. arabiensis*, where it was present in both homozygous and heterozygous state. In addition to the target-site resistance alleles, overexpression of P450s genes (mainly *CYP6M2, CYP6P3* and *CYP9K1*) confirm the observed pyrethroids resistance in the local malaria vectors. The phenotypic insecticide resistance data in the present study are similar to those reported from other settings in Côte d'Ivoire [4, 16, 30, 59] as is the high allelic frequency of the L995F *kdr* mutation in *An. gambiae* s.s. [59, 60]. The presence of the *kdr* N1570Y allele, in combination with the *kdr* L995F in *An. gambiae* s.s., may explain part of the resistance observed to deltamethrin in the Ellibou *An. gambiae* s.l. population [17]. With regards to *An. arabiensis* in Ellibou, similar results have been reported from Benin [61], Burkina Faso [62], Mauritania [45] and Senegal [63]. Another finding is that 7 *An. arabiensis* specimens possessed both the L995F and L995S *kdr* simultaneously. This genotype has already been reported in *An. gambiae* s.s. from Uganda [64] and Côte d'Ivoire [59], but the present study seems to be the first record of an *An. arabiensis* L995F/L995S hybrid from Côte d'Ivoire. In addition to the *kdr* mutations, the overexpression of the five elevated P450 metabolic genes might also add to the pyrethroid and DDT cross-resistance in *An. gambiae* s.l. from Ellibou [65].

Intriguingly, despite the susceptibility of *An. gambiae* s.l. to malathion, *ace1*-G280S mutations conferring cross-resistance to carbamates and organophosphates were found across all three *An. gambiae* species from Ellibou. A possible explanation for this phenomenon is negative-cross resistance conferred by overexpression of P450s [30]. Indeed, several metabolic P450 enzymes, including *CYP6M2*, *CYP6P1, CYP6P3*, *CYP6Z1* and *CYP9K1*, were found to be upregulated and might have increased the toxicity of malathion [66, 67].

Conclusion

The detection of *An. arabiensis* in southern Côte d'Ivoire, predating another finding further north of the country, confirms the presence of this malaria vector across the country. The presence of *An. arabiensis* is an early warning sign, as it shows a different behaviour and ecology than *An. coluzzii* and *An. gambiae* s.s., requiring different types of vector control interventions. It will be important to further investigate its spatial distribution, insecticide resistance status and contribution to malaria transmission for effective malaria control. Upregulation of P450s conferring insecticide resistance to pyrethroids together with the presence of *ace1* suggests negative cross-resistance since the local *An. gambiae* s.l. population was susceptible to malathion. Therefore, organophosphates could be an alternative insecticide class for IRS in the Ellibou area of southern Côte d'Ivoire.

Abbreviations

ace1	acetylcholinesterase
CDC	Centers for Disease Control and Prevention
CI	Confidence interval
COE	Carboxylesterases
CSRS	Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
DDT	Dichlorodiphenyltrichloro ethane
ELISA	Enzyme-linked immunosorbent assay
GST	Glutathione *S*-transferase
IRS	Indoor residual spraying
kdr	Knockdown resistance
LLIN	Long-lasting insecticidal net
PSC	Pyrethroid spray catch
RDT	Rapid diagnostic test
RT-qPCR	Reverse transcription quantitative polymerase chain reaction
SINE	Short interspersed nuclear element
SR	Sporozoite rate
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12936-023-04456-y.

Additional file 1. Genotype of each individual mosquito of all analyzed insecticide resistance loci.

Additional file 2: Table. Genes expression analysis results between field strains and laboratory strains): *Anopheles gambiae* s.s. from the field were compared to *An. gambiae* s.s. Kisumu, originating fromKisumu, Kenya, and *An. coluzzii* from the field were compared to *An. coluzzii* Ngousso, originating fromYaoundé.

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Author contributions

BPN, GR and CSM designed the study. BPN collected the data, pursued data analysis and wrote the first draft of the manuscript. BKF contributed to mosquito collection. BPN, NCW and PM performed the molecular analysis. GR, JU and CSM supervised the study. NCW, JS, GR, JU and PM provided scientific inputs to the manuscript. All authors read, revised and approved the final manuscript prior to submission. All authors read and approved the final manuscript.

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Availability data and materials

Data and materials of this study are included in this article and its additional files.

Declarations

Ethics approval and consent to participate

This study received approval from the "Comité National d'Éthique des Sciences de la Vie et de la Santé de Côte d'Ivoire" (reference no. 054/MSHP/CNER-kp). Participants provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declared that they have no competing interests.

Author details

[1]Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire. [2]Swiss Tropical and Public Health Institute, Kreuzstrasse 2, 4123 Allschwil, Switzerland. [3]University of Basel, 4001 Basel, Switzerland. [4]North Carolina State University, Raleigh, NC 27695-7508, USA.

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Chapter 4. Use of insecticides in agriculture and the prevention of vector-borne diseases: population knowledge, attitudes, practices and beliefs in Elibou, south Côte d'Ivoire.

Authors: Bédjou Prisca N'Dri[1,2,3]*, Kathrin Heitz-Tokpa[2,3], Mouhamadou Chouaïbou[3,4], Giovanna Raso[1,2], Amoin Jeanne d'Arc Koffi[3], Jean T. Coulibaly[3], Richard B. Yapi[3,5], Pie Müller[1,2] and Jürg Utzinger[1,2]

Affiliation. [1] Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland, [2] University of Basel; P.O. Box, CH-4003 Basel, Switzerland; [3] Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303, Abidjan 01, C ôte d'Ivoire; [4] University of North Carolina State North, Raleigh, NC, 27695-7508, USA; [5] Université Alassane Ouattara, Bouaké, BP V 18, Bouaké 01, Côte d'Ivoire

*Corresponding author: Bédjou Prisca N'Dri

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Article

Use of Insecticides in Agriculture and the Prevention of Vector-Borne Diseases: Population Knowledge, Attitudes, Practices and Beliefs in Elibou, South Côte d'Ivoire

Bédjou P. N'Dri [1,2,3,*], Kathrin Heitz-Tokpa [2,3], Mouhamadou Chouaïbou [3,4], Giovanna Raso [1,2], Amoin J. Koffi [3], Jean T. Coulibaly [1,2,3,5], Richard B. Yapi [3,6], Pie Müller [1,2] and Jürg Utzinger [1,2]

1. Swiss Tropical and Public Health Institute, P.O. Box, CH-4002 Basel, Switzerland; giovanna.raso@promotionsante.ch (G.R.); couljeanvae@yahoo.fr (J.T.C.); pie.mueller@swisstph.ch (P.M.); juerg.utzinger@swisstph.ch (J.U.)
2. Department of Epidemiology and Public Health, University of Basel, P.O. Box, CH-4003 Basel, Switzerland; kathrin.heitz-tokpa@csrs.ci
3. Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire; mouhamadou.chouaibou@csrs.ci (M.C.); koffiamoinjeanned39arc@yahoo.fr (A.J.K.); richard.yapi@csrs.ci (R.B.Y.)
4. Department of Entomology and Plant Pathology, North Carolina State University, Raleigh, NC 27695-7508, USA
5. Unité de Formation et de Recherche Biosciences, Université Félix Houphouët-Boigny, 22 BP 770 Abidjan 22, Côte d'Ivoire
6. Centre d'Entomologie Médicale et Vétérinaire, Université Alassane Ouattara, Bouaké, BP V 18 Bouaké 01, Côte d'Ivoire

* Correspondence: prisca.ndri@swisstph.ch; Tel.: +41-77-987-3278 or +225-5227-6117

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Abstract: People's knowledge, attitudes, practices and beliefs (KAPB) pertaining to malaria are generally well described. However, little is known about population knowledge and awareness of insecticide resistance in malaria vectors. The aim of this study was to investigate KAPB related to insecticide resistance in malaria vectors due to the use of insecticides in agriculture and the prevention against mosquitoes. In mid-2017, we carried out a cross-sectional survey in Elibou, South Côte d'Ivoire, employing a mixed methods approach. Quantitative data were obtained with a questionnaire addressed to household heads. Interviews were conducted with key opinion leaders, including village chiefs, traditional healers, heads of health centres and pesticide sellers. Focus group discussions were conducted with youth and elders. A total of 203 individuals participated in the questionnaire survey (132 males, 65%). We found that people had good knowledge about malaria and mosquitoes transmitting the disease, while they felt that preventing measures were ineffective. Pesticides were intensively used by farmers, mainly during the rainy season. Among the pesticides used, insecticides and herbicides were most commonly used. While there was poor knowledge about resistance, the interviewees stated that insecticides were not killing the mosquitoes anymore. The main reason given was that insecticides were diluted by the manufacturers as a marketing strategy to sell larger quantities. More than a third of the farmers used agricultural pesticides for domestic purposes to kill weeds or mosquitoes. We observed a misuse of pesticides among farmers, explained by the lack of specific training. In the community, long-lasting insecticidal nets were the most common preventive measure against malaria, followed by mosquito coils and insecticide sprays. The interviewees felt that the most effective way of dealing with insecticide resistance was to combine at least two preventive measures. In conclusion, population attitudes and practices related to insecticides used in agriculture and the prevention against mosquitoes could lead to resistance in malaria vectors, while people's knowledge about insecticide resistance was limited. There is a need to raise awareness in communities about the presence of resistance in malaria vectors and to involve them in resistance management.

Keywords: attitude; belief; Côte d'Ivoire; insecticide resistance; knowledge; malaria; practice; resilience

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1. Introduction

Malaria remains a major public health problem with an estimated 228 million clinical cases and 405,000 deaths in 2018 [1]. Nevertheless, in the past 20 years, considerable progress has been made in malaria control by using long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS) [2–4]. Unfortunately, this progress is threatened by the development and spread of insecticide resistance in mosquitoes. Indeed, insecticide resistance is increasing throughout Africa, where most of the malaria burden is concentrated [5–9]. Due to practices linked to intensification of agriculture, resistance to insecticides has emerged and spread [10]. Several studies suggest that intensive use of insecticides in agriculture selects for resistant genes in malaria vectors [11,12]. Although research has been conducted previously on farmers' knowledge, attitudes, practices and beliefs (KAPB) on insecticides used in agriculture [10,12], there is a paucity of studies on people's knowledge about resistance to currently utilized insecticides and the causes leading to resistance, including how people cope with the growing resistance.

According to the World Health Organization, malaria is endemic in Côte d'Ivoire and the entire population is at risk of malaria transmission [1]. The main preventive measure is sleeping under LLINs [13]. Unfortunately, resistance to pyrethroids, which is the single class of insecticides currently approved for net impregnation, is widespread in Africa [14,15]. Additionally, pyrethroid insecticides are widely used in agriculture, i.e., for rice and vegetable farming [10]. Recently, an entomological study reported a high level of resistance to insecticides in *Anopheles gambiae* s.l. collected in Elibou in the southern part of Côte d'Ivoire [16]. Elibou is characterized by the absence of irrigated rice farming; other crops are cultivated at a distance of 2.5 km away from the village. The following research question was asked: what are the key practices of the population that might govern insecticide resistance?

To identify the potential root causes driving insecticide resistance in malaria vectors in Elibou, we carried out a KAPB survey using a mixed methods approach. We employed a household questionnaire to determine socio-demographic factors, population KAPB regarding the use of insecticides in agriculture and public health and resilience to resistance. In addition, we interviewed key informants, had focus group discussions (FGDs) and made direct observations.

2. Materials and Methods

2.1. Ethics Statement

The current study was approved by the ethics committees of North-Western and Central Switzerland (reference, 2017-00421 EKNZ) and the Comité National d'Éthique des Sciences de la Vie et de la Santé de Côte d'Ivoire (reference, 054/MSHP/CNER-kp). The head of Sikensi health district and village authorities gave permission to conduct the survey. The study procedures have been explained in lay terms to all participants, including objectives, potential risks and benefits related to the study.

2.2. Study Area

The study was designed as a cross-sectional survey. It was carried out in May and June 2017 in Elibou, a forested area located in the southern part of Côte d´Ivoire (geographical coordinates:

5°40'57.99" N latitude and 4°0'30.29" W longitude). Elibou is characterized by a long rainy season (April–July) and a long dry season (December–March), interspaced by a short rainy season (October–November) and a short dry season (August–September) (see: https://fr.climate-data.org; accessed on 6 January 2020). There is a swamp area with an environment suitable for mosquito breeding and larval development. Subsistence agriculture and trading are the main economic activities of the population. Cocoa, rubber tree (hevea), palm oil, tomato, chilly and cassava are the main crops.

2.3. Sample Size Calculation and Selection of Participants

We wanted to be 95% certain that the relative estimation error would not exceed 20% of the true proportion p, provided that p equals 1/3. This led to Equation (1):

$$0.2 \times \frac{1}{3} \geq 1.96\sqrt{(1/3 \times 2/3)/n} \quad (1)$$

providing $n > 192$. Hence, we aimed to draw a random sample of at least 200 households.

If the head of a selected household was absent during the survey, he/she was replaced by someone from the household aged 20 years and above. If no such household member could be found, the neighboring household was chosen instead.

2.4. Questionnaire and FGDs

A KAPB survey was conducted with an emphasis on people's use of insecticides in agriculture and for prevention of vector-borne diseases in the context of resistance in malaria vectors. The questionnaire was addressed to 203 heads of household and was structured around (i) socio-economic and demographic aspects of households; (ii) knowledge related to malaria and mosquito resistance; (iii) attitude, practices and beliefs related to the use of insecticide in agriculture and for disease prevention; and (iv) resilience to resistance of mosquitoes. Households were visited in the morning. A catch-up visit was carried out for those who were absent during our initial visits.

Four FGDs were carried out with elders and youth, separate by gender, each including eight individuals. Key informant interviews were conducted with five individuals (i.e., village chief, a pesticide seller, an elder person, a traditional healer and the head of the health centre). Two interview guides were employed; one for FGDs and one for key informant interviews.

Pesticides were defined as a substance used to control pests considered being harmful for humans and agricultural production, including weeds. There are three groups of agricultural pesticides: (i) insecticides used to fight insects; (ii) herbicides against weeds that impact the growth of plant; and (iii) fungicides against parasitic fungi that cause various diseases in plants.

2.5. Statistical Analysis

Data from the questionnaire survey were double entered into EpiInfo version 3.5.3 (Centers for Disease Control and Prevention; Atlanta, GA, USA) and cross-checked. The cleaned dataset was transferred into STATA IC 14 (Stata Corporation; College Station, TX, USA). Results are reported as counts and percentages. Chi-square (χ^2) test was used to compare proportions between groups. Key informant interviews and FGDs were tape-recorded and transcribed in Word and converted into MAXQDA version 10 (VERBI Software Consult; Berlin, Germany) for qualitative data analysis and interpretation.

3. Results

3.1. Characteristics of Study Population

The socio-economic and demographic profile of the 203 respondents is summarized in Table 1. Most of the respondents were aged between 25 and 49 years (60.1%). With regards to education, slightly more than a quarter never attended school, while more than four out of 10 respondents only had obtained primary schooling (42.4%). Almost half of the respondents were famers (48.3%).

Table 1. Characteristics and socio-economic status of the study populations in Elibou, South Côte d'Ivoire in 2017 (n = 203).

Characteristic	No. of People Interviewed	Percentage
Sex		
Male	132	65.0
Female	71	35.0
Age group (years)		
20–24	12	5.9
25–49	122	60.1
≥ 50	69	34.0
Educational attainment		
None	53	26.1
Primary school	86	42.4
Secondary school	48	23.6
High school	16	7.9
Occupation		
Farmer	98	48.3
Housewife	50	24.6
Trader	16	7.9
Civil servant	14	6.9
Unemployed	25	12.3
Population group		
Autochtones (Abidji)	146	71.9
Allochtones	43	21.2
Allogènes	14	6.9
Wealth tertile		
Poorest	71	35.0
Less poor	65	32.0
Least poor	67	33.0

In Côte d'Ivoire's multi-ethnic agrarian societies, the population is generally categorized into three groups of origin. In French, these are known as (i) *autochtone*s (indigenous; first settler population); (ii) *allochtones* (allochthonous; internal or national migrants, people stemming from another region of Côte d'Ivoire); and (iii) *allogènes* (allogenic; non-national migrants and foreigners). The *autochtones* of Elibou belong to the "Abidji", which is the predominant ethno-linguistic group (71.9%).

3.2. Awareness Related to Malaria and Insecticide Resistance

Most of the respondents (93.1%) complained about mosquitoes. Key informant interviews and FGDs suggested that the abundance of mosquitoes had increased over time. For instance, the village chief said: "*In the past, we did not feel the presence of mosquitoes or if they were there, they did not do so much damage*". By the "past", respondents referred to the 1980s and 1990s. During that time, the village chief and other elderly interviewees said that aerial sprayings were performed every couple of months to fight against onchocerciasis and human African trypanosomiasis. According to the village authorities, the spraying had the effect that the population did not feel the presence of mosquitoes and other disturbing insects. However, around the new millennium, the situation changed. Aerial sprayings were stopped and people began complaining about the large abundance of mosquitoes.

Regarding seasonal patterns, 61.1% of respondents stated that mosquitoes were most abundant during the rainy season. Only 9.9% stated that the highest abundance of mosquitoes occurs in the dry season. The remaining 29.1% gave no peak time of mosquito abundance. Insalubrity and backwater were the most frequently reported causes of mosquito abundance in the village, as illustrated by a quote from an elderly man: "*In former times, the village was maintained, and we did not have polluted water like this.*

There was a corner where we put waste. We did not have open wells, so there were fewer mosquitoes". The fact that the village is no longer well maintained was explained by the demographic expansion and the arrival of migrants during the political crisis of 2002–2011. Some Christians interviewed referred the negative changes to prophecies in the Bible. According to a young man: *"The abundance and resistance in mosquito is a punishment of God. These are warning signs of the end of the world"*. Finally, people linked the mango and maize season—which correspond to the long rainy season—to the presence of large numbers of mosquitoes.

Most respondents (98%) answered having good knowledge about malaria with no statistically significant difference according to educational attainment ($\chi^2 = 1.8$; $p = 0.6$). In the local language (i.e., Abidji), malaria is termed "*djèkouadjo*". There are different types of *djèkouadjo*. For instance, the traditional healer distinguished between two types of malaria (i) "*djèkouadjo lébé*", which is the yellow malaria and (ii) "*djèkouadjo lofou*", which means white malaria. The latter is more dangerous and can cause madness.

Respondents mentioned that mosquitoes are responsible for diseases, such as malaria, typhoid fever and anaemia. Indeed, 94.1% of the respondents knew that malaria is caused by mosquito bites. The results revealed that malaria was not so common in earlier times, and people found it easier to treat with traditional remedies. To date, residents still rely on traditional medicine. They only go to the hospital when the disease has become severe. According to the head of the local health centre: "*[People] go to the traditional healer and, when they see that it's complicated, they send the child here. You see the child in a comatose state*".

Table 2 summarises people's knowledge about insecticide resistance in mosquitoes. Regarding knowledge of insecticide resistance in the malaria vector, a similar percentage of respondents felt that insecticides are effective (42.9%) or ineffective (44.3%), while the remaining 12.8% had no clear opinion. Hence, if one exclusively takes this response into consideration, it is not possible to determine whether insecticides are, or have become, ineffective. Nevertheless, among those who perceived a decline in the effectiveness of insecticides, 57.8% stated that the root cause was the dilution of insecticides by the vendors.

Table 2. Responses given by study participants in relation to the knowledge of insecticide resistance in malaria vectors from a cross-sectional survey conducted in Elibou, South Côte d'Ivoire in 2017.

Question	n (%)
Are insecticides effective against mosquitoes? (n = 203)	
No	90 (44.3)
Yes	87 (42.9)
I do not know	26 (12.8)
What are the causes of ineffectiveness of insecticides? (n = 90) *	
Insecticides have been diluted	52 (57.8)
Mosquitoes have become numerous and dangerous	12 (13.3)
Due to vector control	4 (4.4)
I do not know	22 (24.4)
Since when have you observed that insecticides have been ineffective? (n = 90) *	
After regional vector control was stopped	28 (31.1)
Always	9 (10.0)
For more than 10 years	1 (1.1)
I do not know	52 (57.8)

* n = 90: number of participants who responded that insecticides were not effective.

For some participants it is not conceivable why the same insecticides that effectively killed mosquitoes in the past are no longer effective today. Other participants see a link between insecticide resistance and the business strategy of the insecticide manufacturers and vendors. An elderly man explained during a FGD: "*Manufacturers do not dose insecticides well. They know that if they dose it well and (the product) kills mosquitoes, we won't buy their products any longer. So they have reduced the dose to continue selling their products*". Yet for others, the fact that it is the same treatment used in the past and today proves that mosquitoes have become resistant to the products used. This observation was articulated as follows: *"The way our organism adapts, this is how mosquitoes have changed, too. So I think if the treatments don't kill them anymore, it is because their organism have become used to the same treatment"*.

3.3. Attitudes, Practices and Beliefs Related to Insecticide Use in Agriculture and Disease Prevention

Table 3 illustrates attitudes and practices of participants in relation to insecticides used while pursuing agricultural activities. Three quarter of the respondents have a farm (n = 152, 74.9%). Among them, 75% use pesticides. Farmers mentioned that the choice of pesticides they use depends on their crops. A seller of pesticides explained during the interview: "*There are several types of products; there is one for pepper, okra, eggplant, rubber and cocoa. Some people also use them to kill insects that attack plants, others on the contrary use them to weed their fields*". The respondents' believe that using the products will increase their production, and hence their income. The rainy season is the period when respondents are most likely to buy and apply insecticides and herbicides. As quoted by a pesticide seller: "*It is in the rainy season that things move. During this period, the people use a lot of herbicides*".

Table 3. Attitudes and practices of respondents from Elibou, South Côte d'Ivoire regarding the use of insecticides, as revealed by a cross-sectional survey in 2017.

Agricultural Practices	n (%)
Do you have a farm? (n = 203)	
Yes	152 (74.9)
No	51 (25.1)
Do you use pesticide on your farm? (n = 152)	
Yes	115 (75.7)
No	37 (24.3)
Period of pesticides usage? (n = 115)	
All seasons	63 (54.8)
Rainy season	39 (33.9)
Dry season	13 (11.3)
Do you use agricultural insecticides in your home? (n = 115)	
Yes	45 (39.1)
No	70 (60.9)

As shown in Table 3, most farmers use agricultural pesticides in their homes against weeds and for mosquito control (39.1%). It is important to note that the same pesticides mentioned are used at home. A famer in a FGD said: *"When we spray the products, it kills the weeds and all the insects that are inside. There are other products that repel and kill snakes, so it is unlikely that the mosquitoes will stay alive. For us, the effect that these products can have on mosquitoes is that it kills them"*. Moreover, the use of herbicides for weeding allows saving time and energy, as illustrated by a man in his 40s: *"We prefer to take a bottle of herbicides to spray the grass around the house, it is fast and then it is less tiring than taking a machete or hoe"*.

Farmers interviewed have the habit of using agricultural pesticides for mosquito control and weeding at home without considering the risks that this might have on their health. One of them, a household head, confirmed to usually spray *"decis"* insecticide in his room due to the fact that mosquitoes are resistant to other preventive measures. Regarding farmers' educational attainment, 29% who used pesticides were illiterate, 40% only attended primary school, 26.3% secondary school and 5.3% had high school education.

Insecticides, herbicides, fungicides and growth regulators were mentioned during the cross-sectional survey. Insecticides were frequently used by farmers (45.7%), followed by herbicides (40.0%). Fungicides and growth regulator accounted for 8.6% and 5.7%, respectively. Among insecticides used, pyrethroids (e.g., deltamethrin, cypermethrin, D-althrin, bifenthrin and lambdacyalothrin) accounted for 57.1%. Neonicotinoids (e.g., acetamiprid and thiamethoxin; 28.6%) and organophosphates (e.g., etonophos and profenofos; 14.3%) were additional important classes of insecticides.

Regarding different preventive measures used by those who perceived a change in the effectiveness of insecticides, LLINs were most often stated (25.6%). Yet, the actual use of LLINs was low. Reasons given for not using LLINs are that sleeping under a LLIN causes discomfort by suffocation and excess heat (18.2%) or that people did not receive LLINs (18.0%). The use of fumigating coils emerged as another important tool to protect against mosquito bites, as stated by 15.3% of the respondents. In practice, most of the participants in the FGDs and those interviewed, used fumigant coils with the commercial name *"moustico"*. This device is considered as a cheaper option compared to LLINs. Its use is not only price-related but also by habits. A farmer in a FGD explained: *"We don't have money, so it is "moustico" that we use. Since we were children, it is what our parents used to repel mosquitoes."*

Slightly more than half of the participants (53.2%) used a single tool to prevent mosquitoes (Table 4). With regard to using multiple preventive measures, the combination of LLINs and IRS was most often given (19.7%). A man in his 40s said: *"When we use one insecticide and mosquitoes are still there, we start using both insecticide spray and fumigant coils at the same time, so that, the dose of insecticides becomes strong and we get to sleep a little."*

Table 4. Tools used for protection against mosquitoes mentioned by respondent from Elibou, South Côte d'Ivoire who perceived insecticides as inefficient.

Preventive Measure	n (%)
None	11 (5.4)
Single tool	
LLIN	52 (25.6)
Fumigant coil	31 (15.3)
Insecticides spray	13 (6.4)
Electric fan	1 (0.5)
Multiple tools	
LLINs + insecticide spray	40 (19.7)
LLINs + insecticide spray + fumigant coil	19 (9.4)
LLINs + fumigant coil	18 (8.9)
Insecticide spray + fumigant coil	15 (7.4)
LLINs + fan	2 (0.9)
Fumigant coil + fan	1 (0.5)

Even though people use insecticide sprays, the reported quantity is small. During our FGDs, participants reported not to spray in all the rooms and only to spray a small amount, because the product was too expensive. Respondents believe that it is the smell of the product that repels mosquitoes. This practice is a strategy to make savings, given that the spray is quite expensive.

Taken together, there is a good management of insecticide sprays used. An elderly man explained: "*I use insecticide spray, so in the evening, we spray at least 30 minutes before sleeping. Moreover, we spray a little bit because it costs FCFA 1,000, we cannot buy it each week. The smell is enough; this is why we spray just a little bit*." This attitude from respondents could also be linked to the fact that participants cannot afford to spray frequently at large quantities. This is illustrated by a woman in her 50s: "*Me, I used to use insecticide spray. But when I saw that mosquitoes were coming a lot, that's when I stopped, because I do not have money*".

3.4. Population Resilience Regarding Resistance to Insecticides

According to the results from the FGDs and interviews, the main tool to fight against mosquito bites in the past was by burning the bark of a tree, locally known as "*gnanman*". The traditional healer of the village said: "*At the time, there was a bark of a tree which served to repel mosquitoes and this tree is called gnaman. Now it does no longer exist due to the production of coal and the destruction of the forest*".

For the majority of respondents, alternative strategies consist of using a bed sheet to cover themselves or a piece of cloth to repel mosquitoes. Although they developed strategies to avoid mosquito bites, 55.2% of respondents ranked LLINs as the most effective tool. Participants in FGDs said that they used LLINs when they noticed the limitations of the above strategies. This is illustrated by the following quote from an elderly man: *"After we have tried everything and it did not work finally, we used mosquito nets. But again, it did not work, because even being under the net, we still had mosquito bites".* Beside the use of LLINs, respondents have adopted additional strategies such as environmental sanitation as the most important one, as stated by 72.4% of the respondents.

Results from the FGDs and interviews revealed that participants keep their surrounding clean. Key activities are focused on weeding and cleaning the compound. However, these activities were limited to the immediate surrounding of participants' homes and not the public space of the village. A woman in her 60s said in a FGD: *"Well, to avoid mosquitoes, I clean the house, I sweep the room. But without effect, mosquitoes are still there".*

Regarding the options given for enhanced control, respondents have identified people who should be involved in vector control activities in Elibou. Respondents felt that the following actors should deal with vector control: the general population (47.8%), municipality (22.7%) and traditional authorities (e.g., village chief and other local authorities; 12.8%). However, about one of seven participants did not know who should deal with vector control. FGDs and interviews revealed that everyone has to promote environmental sanitation in his or her neighbourhood*: "I propose that we group young men and women by neighbourhood. Specifically, clean, sweep [and] empty garbage. We can collect garbage, because it is a source of mosquito production".*

4. Discussion

In the current study, 39% of respondents confirmed to have a habit of using agricultural pesticides in their houses against weeds. Among them, one household head affirmed to spray "*decis*" in the rooms due to the ineffectiveness of disease prevention tools commonly used against mosquitoes. While this insecticide belongs to the class of the pyrethroids, it is intended for treatments of various plant cultures. The use of insecticides in agriculture and for disease prevention is an important cause for the development and spread of resistance in mosquitoes. Indeed, the spread of insecticide resistance is a public health threat, which can jeopardize vector-borne disease control efforts. In Côte d'Ivoire, resistance to insecticides is widespread, presumably as a result of the heavy use of pyrethroids in agriculture [10,17,18]. In 1993, resistance to pyrethroids was reported for the first time from Bouaké in the central part of Côte d'Ivoire [18]. More recently, a high level of resistance to insecticides in malaria vectors has been reported from Elibou where the current study was conducted [16]. Awareness of resistance in mosquitoes was less known by respondents. A deeper understanding of the population's KAPBs in a given community regarding resistance in malaria vectors might help improve control strategies [19].

Our results revealed that the same insecticides used in agriculture are also employed athome against weeds and for mosquito control. About 75% of field owners in the current study used pesticides in agriculture. Thus, the concomitant use of insecticides in agriculture and for disease prevention are likely causes for the observed resistance to insecticides in malaria vectors inElibou [16]. Moreover, the aerial spraying in the last decades before the new millennium, using DDT, organophosphates, carbamates and pyrethroids against black flies and tsetse flies, as reported during our FGDs, might also explain the selection for resistance in mosquitoes. Considering that all LLINs contain pyrethroids, our observations are worrying with regards to vector-borne disease control and the potential re-emergence and persistence of malaria. Several studies emphasized the potential role of agricultural insecticides in accelerating the selection of insecticide resistance due to their excessive use [10,17,20,21].

Pesticides are primarily applied during the rainy season and might then be washed out by the rains and contaminate the village of Elibou. As mosquito breeding sites are abundant during the rainy season, the pesticides may also affect larvae breeding sites. The pesticide residues seep into the soil, likely exerting a selection pressure on the larvae [8]. Among the pesticides identified, insecticides and herbicides were used most commonly. Pyrethroids, neonicotinoids and organophosphates were the main classes of insecticide applied by farmers. Among them, pyrethroid insecticides were the principal class. The increased use of pyrethoids could also explain the high resistance to this class of insecticides observed in malaria vectors in Elibou. Moreover, most farmers have not received specialists' advice on the best choice of a product and adequate use of pesticides, such as the exact dosage and frequency of use. Given that 29% of pesticides users are illiterate [10], they may also ignore label instructions. Finally, there is a lack of control of the quality of products that are used by the farmers for crop production leading to sales and use of pesticides not registered in Côte d'Ivoire.

In contrast to the general unawareness of how to properly use pesticides, most participants had good knowledge about malaria. Half of the participants perceived a decline in the effectiveness of malaria vector prevention tools over time. Hence, we conjecture that villagers have observed some kind of mosquito resistance development. Interestingly, 93% of participants noticed an increase in mosquito densities and half of them attributed the increase to the ineffectiveness of insecticides used to prevent mosquitoes. According to participants' responses, the products sold on the Elibou market have been adulterated by manufacturers, which might explain the reduced efficacy. Participants attributed it to the business of manufacturers to increase the return on investment with fraudulent means. In fact, they cannot imagine that the same insecticides that effectively killed and repelled mosquitoes in the past do not work any longer. Hence, there is a pressing need to inform users on the current situation to raise awareness. As protective measure, LLINs remain the most effective tool for prevention of mosquito bites, and thus the transmission of malaria [4]. However, its usage is low due to the fact that people do not like the extra heat while sleeping under a LLIN or unavailability of LLINs, among other reasons. These factors constitute a weakness that impedes malaria control activities. Similar observations were made in previous studies conducted in Ghana, Nigeria and other parts of the world [22,23].

A follow-up of nets distributed, and their proper use is required. The majority of respondents have low socio-economic status that compromised their attitude and practices on the use of insecticides. Indeed, the use of prevention measures depend on the cost of the device. Education and specific training on indoor spray usage is required because participants have the habit to spray only a little amount of insecticides in only a part of their rooms. Even though they might have a large house, they preferred to spray in the main room only. Actually, the amount sprayed was not enough to repel and kill mosquitoes throughout the house. This practice is also governed by the cost of the products. Fumigant coils are being used as an alternative to LLINs as they are thought to be cheaper. Moreover, participants used *"moustico"* not only because of its perceived low cost, but also out of habit. According to them, "*moustico*" has been used for a long time by their parents and therefore, they continue to use it despite its ineffectiveness against mosquitoes.

Owing to the ineffectiveness of the use of a single tool to protect against mosquito bites, respondents developed a more effective strategy, such as combining two or more preventive measures. Quite often, LLINs and insecticide sprays were used in conjunction, as it is believed that relying on both products simultaneously is more effective than a single tool. Several studies reported the significant effect to combine both LLINs and IRS usage on malaria, particularly in areas where pyrethroid resistance is high [2,24]. However, the rate of multiple tools observed in our study was still low, perhaps, because the spray is too expensive. Thus, the practice of covering oneself in a bed sheet to avoid mosquito bites at night appears as an additional solution to prevent from mosquito bites.

Vector control requires community engagement. Interestingly, participants have made some suggestions in order to reduce and eliminate resistance. First, the involvement of the population to promote cleanliness in neighbourhoods, which should be enforced by local authorities. We suggest regular monitoring and training of LLINs distributed by the national malaria control programme to their correct use, education of the community for the proper use of insecticides applied for agriculture and disease prevention and implementation of a rigorous control of pesticides sold to avoid the hazards of toxic products non authorized by the government of Côte d'Ivoire.

Only a few studies have been carried out on KAPB of populations on their habits related to the use of insecticides in agriculture and disease prevention, which could impact on insecticide resistance [10,17]. The current study is the first report on data of population knowledge about resistance in malaria vectors. Unlike malaria, we recorded that knowledge of the resistance phenomenon is not well understood by the community. Hence, there is a need to inform people about this issue. This is important, since the main users of pesticides for cultivation are illiterate or have only attained primary schooling. The use of insecticides in agriculture depends on the culture and farmers' financial resources [17]. Similar observations were made in our study. Herbicides and insecticides were the pesticides most often used by the respondents. Our results corroborate with those found by Chouaïbou et al. [10] for vegetable cultivation. In Elibou, there is no irrigated rice farming in the main village. The nearest fields with other crops are located 2.5 km away. Our study also revealed that pesticides are more heavily used during the rainy season. As the rainy season is the period where mosquitoes are most abundant, it is essential that people use prevention tools to avoid mosquito bites. To sum up, the more frequently insecticides are being used, the higher the risk of selection and spread of resistance.

Our study has several limitations that are offered for consideration. First, the study focussed on a single village, due to constrained financial resources. Hence, generalizability to other parts of Côte d'Ivoire is currently not possible. Second, the cross-sectional study design impedes causal inference. A strength of our study was that the estimated sample size of 200 was attained, thanks to the enthusiastic participation of villagers. Indeed, we were able to enrol slightly more people who generously provided answers to all questions.

5. Conclusions

The simultaneous use of insecticides in agriculture and disease prevention is a driver for resistance in malaria vectors. Further studies are required to deepen the understanding of this issue, including investigations on insecticide residuals in water and soil in the village and surrounding farms. Additionally, in order to reduce the effect of pesticides used on the environment and people's health, knowledge of proper handling and use are required. This is the first study investigating KAPB about insecticide resistance in Côte d'Ivoire. For the national malaria control programme, experiences and lessons should be utilized while developing and implementing a strategy for resistance management.

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Chapter 5. General discussion

In this thesis, I determined the main malaria vectors, key entomological parameters of malaria transmission and assesed the insecticide susceptibility of the malaria vectors to the four conventional classes of insecticide in Ellibou, southern Côte d'Ivoire. The thesis is structured into five chapters. Chapter 1 includes the general introduction; Chapter 2 contains a manuscript, which has been published in the Wellcome Open Access Journal; Chapter 3 has been published in the Malaria Journal; and Chapter 4 has been published in Tropical Medicine and Infectious Disease journal; Chapter 5 includes the general discussion. In this section, I provide a general discussion and my conclusions. While in Chapter 5, the findings of my thesis are recapitulated, the limitations will be discussed and I will propose how the work could be extended.

5.1. Limitations of the study

First, the study was carried out in a single site due to constrained financial ressources. Second, larvae collection and the assessment of resistance to insecticides in *An. gambiae s.l.* should have ideally been conducted repeatedly at the same periods, covering both rainy and dry seasons in the first and the third year of the thesis. My initial goal was to follow the evolution of resistance over consecutive years. Third, the CDC light traps and PSC collection methods used for the adult mosquitoes were biased in direction of mosquitoes biting and resting indoors. The usual measure of malaria transmission, specifically the EIR and the HBR were not determined. While, the HLC is a more realistic method for adult mosquito collection that allow to measure malaria transmission. Therefore, in furthers studies, the HLC must be considered in the collection methods to measure malaria transmission in Ellibou.

5.2. Species composition and malaria transmission

The entomological survey revealed *An. gambiae s.s.*, *An. coluzzii* and *An. arabiensis* as the main malaria vectors in Ellibou. Our study report for the first time, *An. arabiensis* among the *Anopheles* species complex in Côte d'Ivoire. This finding was five years ago and at the time no *An. arabiensis* had been detected in Côte d'Ivoire. Indeed, *An. arabiensis* are considered as species that cover arid savannah and semi-arid area (Coetzee, Craig and le Sueur 2000) contrary to Ellibou, located in the forest area. Recently, *An. arabiensis* was also reported in Central Côte d'Ivoire by Fournet *et al.* (2022). Both studies suggest that *An. arabiensis* is spreading across Côte d'Ivoire. The presence of *An. arabiensis* in Ellibou may be due to migration from the neighbouring countries, primarily from Burkina Faso, where *An. arabiensis* is widespread, by the main road connecting the southern to the northern country. The finding is worrying because *An. arabiensis* shows a different behaviour and ecology than *An. coluzzii* and *An. gambiae s.s.*; hence, different vector control methods are required. Furthermore, investigate of the onset and spread to better understand driving factors of *An. arabiensis* species in Côte d'Ivoire are required to implement control measures to decrease malaria incidence. In addition, *An. gambiae s.s.* and *An*. coluzzii almost equal in the Southern forested Côte d'Ivoire. To our knowledge, *An. gambiae s.s.* is dominant in Savannah, while *An. coluzzii* has been reported to be the most dominant species in the forested area of Côte d'Ivoire (Tia *et al*. 2017). The co-occurrence of the sibling species *An. gambiae s.s.* and *An. coluzzii* in Ellibou as well as hybrids suggesting Ellibou an area of hybridisation in South Côte d'Ivoire. The observed results corroborate with those of Caputo *et al.* (2022) (identifying Côte d'Ivoire as a new zone of hybridisation among *An. gambiae s.s* and *An. coluzzii.*

In addition, the density of *Anopheles* mosquitoes varied according to the seasons. The highest number in *An. gambiae s.l.* was observed during the long season. Rainfall constitutes an important climate factor that drives mosquito development (Adja *et al*. 2011). In this study, we also assessed the correlation between entomological parameters and rainfall and the malaria transmission in the Ellibou setting. The sporozoite rate is most important in Ellibou since in Africa mosquitoes preferredly feed on humans and they are adapted for long term survival (Beier 1998). Our results are consistent with those of Konan *et al*. (2009), confirming that rainfall is one of the factors that contribute largely to mosquito abundance, which then leads to increased malaria transmission.

5.3. Insecticide resistance status and characterisation of mechanisms involved in resistance to insecticides in *Anopheles gambiae s.l.*

We also investigated the insecticide resistance status of the malaria vectors to WHO approved insecticides using the WHO insecticide susceptible bioassay (WHO 2013) on adults mosquitoes that had emerged from larvae collected within Ellibou. The insecticide resistance status is important for monitoring resistance in a population of vectors. Our study results revealed that *An. gambiae s.l.* are resistant to DDT, deltamethrin and bendiocarb, yet not to malathion. Given the low mortality observed with delatmethrin, we extended our study to deepen our understanding of this result. Therefore, in Chapter 2, we investigated the N1575Y mutation, from a subset of DNA samples analysed since the N1575Y allele has not been detected before in Côte d'Ivoire.

The results also show the presence of both *An. coluzzii* and *An. gambiae s.s.* species and additional hybrid individuals. Surprisingly, N1575Y mutation was detected in *An. gambiae s.s.* both in heterozygous and homozygous forms in Ellibou. This mutation provides a synergistic effect by enhancing resistance due to L1014F (Jones *et al.* 2012). The detection of N1575Y mutation is associated with resistance to deltamethrin as observed in Ellibou malaria vectors. The results contribute to the understanding of the strong resistance to pyrethroids insecticides observed in *An. gambiae s.l.* mosquitoes in Ellibou. This means that used LLINs impregnated with only pyrethroids for malaria prevention in Ellibou may fail. As an alternative tool that showed good results could be IRS based on chlorfenapyr combined with LLINs. Chlorfenapyr is a pyrole pro-insecticide that shows neither cross-resistance to DDT, nor to pyrethroids (Ngufor *et al.* 2011).

In Chapter 3, we characterised the mechanisms conferring resistance in *Anopheles* mosquitoes in Ellibou. We identified several loci putatively involved in conferring resistance to these insecticides. Our results revealed the presence of L1014F, L1014S, N1575Y *kdr* and the 119S *Ace -1* mutation in *An. gambiae* s.s. and *An. coluzzii*, *An. arabiensis* and *An. gambiae* s.s./*An. coluzzii* hybrids both at homozygous and heterozygous state. For the first time, we also detected nine *An. arabiensis* specimens carrying L1014F–L1014S simultaneously in Côte d'Ivoire. The study also reports for the first time the L1014S mutation in *An. arabiensis* only, while the mutation was found in a different study in *An. coluzzii* in Côte d'Ivoire (Chouaibou *et al.* 2017). Results from the current study confirm the spread of L1014S in Ellibou. The co-occurrence of L1014F-L1014S *kdr* mutations are likely to be responsible for cross-resistance to DDT and pyrethroids.

In addition to target-site resistance, metabolic mechanisms were shown to be implicated in resistance of malaria vectors (Hemingway *et al.* 2004; Hemingway and Ranson 2000).

The detoxification genes *CYP6P3, CYP6M2, CYP6Z1, CYP6P1, CYP9K1* and *GSTE2* were shown to be overexpressed in wild *Anopheles* populations from Ellibou compared to the reference susceptible strains Kisumu and Ngousso. It was found that *CYP6P3* and *CYP6M2* were upregulated and then confirm the strong deltamethrin pyrethroids resistance in Ellibou mosquito populations.

In this study, the *ace1* G119S was the second frequent mutation after the L1014F and was identified in the three main malaria mosquito species. While *Ace 1* mutation confers cross-resistance to carbamates and organophosphates. *Anopheles gambiae s.l.* seems to be susceptible to malathion 5%; the mortality rate was still high around 98%. In contrast, mortality to bendiocarb (0.1%) was low (52%). Given the result, target-site *ace-1* G119S is confering resistance to bendiocarb. A possible explanation for this phenomenon could be negative cross-resistance conferred by overexpression of P450s and IRS based on organophosphates such as pyrimithos-methyl could potentially be a valuable alternative.

It is important to monitor the spread of resitance in Ellibou and other settings in southern Côte d'Ivoire due to the fact that pyrethroid insecticides are highly used for vector control. Thus, the efficacy of LLINs may be compromised because of resistance to pyrethroids.

In addition, the involvement of the population and health agents is also required to succeed with the fight against malaria.

5.4. Key habits within population which could impact the resistance to insecticides

In Chapter 4, we investigated the population KAPB related to the use of insecticides in agriculture and disease prevention to identify the main causes driving insecticides resistance in malaria vectors in Ellibou. To conduct the study, we employed a mixed method (*i.e.* quantitative and qualitative). Our results show that the population has noticed a change in mosquito densities and behaviour in the face of prevention tools used to fight against malaria. They have poor knowledge about insecticide resistance in mosquitoes. Unlike malaria, we noticed that knowledge of the resistance phenomenon is misunderstood by the community. According to respondents, the preventive tools were not effective and cannot kill mosquitoes anymore. According to the responses from questionnaires, interviewees and focus group discussions (FGDs), respondents thought that there is no resistance; however, it is felt that manufacturers have diluted the insecticides as a marketing strategy to sell more of their products. Results of the study revealed that pesticides used for agriculture are also used for domestic purposes to kill weeds or mosquitoes. Pesticide users are mostly illiterate or have only reached primary school level. This aspect may be considered due to the risk linked to the misuse of compounds by respondents. In terms of preventive measures, a fumigant coil called "*moustico*", perceived as a cheap product was frequently used by households. The socio-economic aspect such as the income level of household heads, the education level and awareness about resistance are important factors to control vectors. In the community, the most useful preventive measure against mosquitoes is LLINs followed by insecticide spraying and finally fumigant coils.

Moreover, respondents felt that the most effective tool is to combine at least two preventive measures. Several studies confirmed the effectiveness of combining two preventive measures to fight against resistant mosquitoes (Awolola *et al.* 2014; Ngufor *et al.* 2016).

To our knowledge, this study is the first report with data of population knowledge about resistance in malaria vector. Hence, there is a need to inform people about this issue. This is important since lack of population knowledge can lead to the increase of resistance and the failure of vector control.

Results from the KAPB study improve our understanding on the main cause of the growing threat of resistance in Ellibou malaria vectors.

This research is very important, because the success of malaria control requires the involvement of all stakeholders, especially the population that also play an active role in insecticide resistance. Their implication and awareness of malaria as well as the current situation regarding resistance to insecticides, mainly the ineffectiveness of preventive tools due to resistant mosquitoes is crucial.

Regardless of the increase of mosquito densities and the resistance to insecticides, respondents have made some suggestions that are based on population involvement in the environment sanitation.

5.5. Extension and future research

Due to high pyrethroid resistance it is important to implement a new strategy mainly the use of IRS such as chlorfenapyr combined with a LLINs could be a valuable option. In the future, we would like to monitor and evaluate resistance mechanisms in vectors from Ellibou at the same period, in order to identify changes trends in insecticide resistance over time. A regular follow up of resistance in Ellibou area could prevent and roll back malaria in the area.

In future research, we would like to identify sibling species of adult *Anopheles* mosquitoes collected by the different collection method.

We also identified *An. arabiensis* by RT-PCR, N1575Y while this is the first report of this species and genes mutation, and additional studies have to confirm their wider spread in Côte d'Ivoire.

In addition, to conduct a KAPB survey with interviews and FGDs in order to assess the effectiveness of preventive tools and compare the current results to the previous. Finally, collect water and a soil sample compounds for analysis by high-performance liquid chromatographic (HPLC) to identify further insecticide residues. Indeed, pesticides carried by wind may be washed out by the rain and seep into the ground. The residues of theses pesticides could contaminate the larvae breeding sites and then leading to a selection of resistance in mosquitoes.

Most importantly, Ellibou is a site that has never been the subject of any entomological study. However, the reports of new findings in our study area constitute the strength of our study. This means that Ellibou is a new area to explore results of studies and could help decisions makers for the implementation of strategies for resistance management. I am primarily interested in nice publications.

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5.7. Curriculum vitae

Personal information

Name: N'DRI Bédjou Prisca
Date of birth: 17 November 1978, Dabou
Nationality: Ivoirian
Marital status: Married
Address: 01 BP 1303 Abidjan 01, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
E.mail: prisca.b.ndri@gmail.com / prisca.ndri@csrs.ci

Research interests

My general interest is tropical diseases towards the development and implementation of control strategies. My principal interest is research focusing on Malaria transmission and vector control. With a keen interest in the assessment of malaria prevention tools My specific interest is however focusing on insecticide resistance in the development of new insecticides for malaria prevention and the monitoring of resistance in malaria vectors.

Education

Ph.D in Epidemiology, Department of Epidemiology and Public Health (EPH), Ecosystem Health Unit, Swiss Tropical and Public Health Institute (SwissTPH), University of Basel, Switzerland", Sept 2015-2021. Thesis title: Malaria transmission and insecticide resistance in *Anophles gambiae* in Ellibou, Côte d'Ivoire.

Master 2 degree in Biodiversity and Sustainable Management of Ecosystems, University Nangui Abrogoua, Côte d'Ivoire. 2012-2013. Master 2 thesis: Evaluation of the physical status and bio-efficacy of Permanet®2.0 long-lasting impregnated mosquito nets after 18 months of use in a forest camp: case of Pokola, Congo.

Master 1 degree of Natural Sciences, University of Nangui Abrogoua, Abidjan, Côte d'Ivoire. 2008-2009. Master 1 thesis: Inventory of the fauna and evaluation of the culicidal nuisance in a post-conflict urban area: Case of the city of Bouaké, Côte d'Ivoire.

Bachelor Degree of Natural Sciences, University of Abobo-Adjamé, Abidjan, Côte d'Ivoire. 2006-2007.

Work experiences

06/2017 -2020. Project lead, PhD. Malaria transmission and resistance to insecticide in *Anopheles gambiae s.l.*, south Côte d'Ivoire

2012-08/2015. Assistant of the head of research group Malaria and Neglected Tropical diseases, at Centre Suisse de Recherches Scientifiques en Côte d´Ivoire (CSRS).

11/2011- 02/2012. **Mission Team Leader** (National survey Project Co-infection: Effects of co-distribution of *Plasmodium* and intestinal helminths on clinical outcomes and self-rated quality of lif *Plasmodium-helminthiasis*) facilitated by the Swiss National Science Foundation (SNSF), (CHF 331,000)

01/09/2010 – 30/10/2010. **Assistant of the Director of Department Environment and Health** at Centre Suisse de Recherches Scientifiques en Côte d'Ivoire

01/11/2010 – 09/2011. **Laboratory Assistant at Vestergaard-Frandsen, Côte d'Ivoire**

Reseach & competence areas

- Epidemiology of tropical mosquito-borne diseases
- Medical Entomology : transmission of vector borne diseases, vector ecology and behaviour, insecticide resistance, monitoring
- Testing vector control tools (Bioassays), (field works – collection and identification of mosquitoes) and community intervention, evaluation of preventive tools
- Insectary management (Breeding of susceptible Kisumu and wild strain mosquitoes)
- Molecular technics: Enzyme-Linked Immunosorbent Assay (Test ELISA), Polymerase Chain Reaction (PCR) and WHO bioassay test
- Parasitology: Kato-katz, saf, urine test
- Drafting research projects and article
- Submission of project to ethic committee
- Design, Management, implementation, planning and monitoring of project activities
- Team support, surveys
- Reporting, Monitoring, evaluation plans for projects and program
- Water-borne disease: schistosomiasis
- Biodiversity and ecosystems conservation

Training

- **2022**. I was trained on Good Clinical Practices (GCP)
- **07-08/2020.** I was trained in Monitoring and Evaluation (M & E) of Projects and Programs
- **13-14/11/2019.** Workshop in drafting projects and preparing for responses; organized by the CSRS**,**
- **17-21/06/2019.** I was trained on simulations of vector control strategies at CSRS
- **21-22/02/2017.** I was trained on Introduction to the statistical software R, SwissTPH.
- **20/01-01/02/2017**. I was trained on Introduction on "Qualitative Health Research: Advanced Module" Basel, Switzerland; organized by "Swiss School of Public Health (SSPH+)".
- **09-12/2015**. I was trained in Epidemiology/ Biostatistic, Swiss Tropical and Public Health Institute
- **08-10/05/2012**, I was trained on Research project elaboration, multi-disciplinary and interdisciplinary, ethics and scientific English, safety in laboratories by Afrique One CSRS, Côte d'Ivoire
- **11/2010-2011.** I was trained on Entomological technics, Insectary management at Vestergaard-Frandsen laboratory in Côte d'Ivoire

Language and informatic skills

- French (fluent); English (Fluent)
- Word, Excel, PowerPoint, EpiInfo, MAXQDA, STATA, R, MS, ODK & KoBoCollect.

Research awards

- **09/2021**. Laureate Best Poster Presentation: International Colloc on "La Recherche Transformationnelle en Afrique subsaharienne". Abidjan, Côte d'Ivoire.
- **22-23/03/ 2021**. Fellowship to attend the: First Woman in Malaria Conference
- **14 /09/ 2020.** Laureate **Travel Award 69th** Annual meeting of American Society of Tropical Medicine and Hygiene (ASTMH) 2020**.**
- **1/09/2015-31/08/2018**. Doctoral Fellow "Eidgenössische Stipendienkommission für ausländische Studierende **ESKAS (**Swiss)**"**. (CHF 1920/Year)
- **26/07/2017.** Laureate "9th Awards CSRS – Eremitage Fund for Scientific Research in Partnership, Switzerland – Côte d'Ivoire (Team: **Ms.Prisca Bédjou N'Dri**, Dr.Richard Brou Yapi, Dr Eveline Hurlimann, and Dr Clarisse Abikpo Houngbedji) (CHF15000)
- **16-24 August 2016.** Fellow to attend the "**International Graduate School** (IGS) North-South Summer School Basel and Delemont, Swiss**).**

Conferences

- **30/10 – 03/11/2022.** 71st Annual Meeting of American Society of Tropical Medicine and Hygiene (ASTMH), Seattle, Washington, USA**.**
- **09-11/09/2021.** International conference: "De la nécessité de repenser le monde pour la durabilité: le rôle de la Recherche transformationnelle en Afrique subsaharienne". Abidjan, Côte d'Ivoire.
- **22-24/03/2021.** First virtual International conference: Women in Malaria (WiM)" British Society for Parasitology.
- **15-19/11/2020.** 69^{th} Virtual annual meeting: American Society of Tropical Medicine and Hygiene (ASTMH).
- **15-19 /11/2020.** Poster Presentation to the virtual annual meeting conference "American Society of Tropical Medicine and Hygiene " (ASTMH)
- **18- 22/11/2019.** Oral Presentation. 23^{rd} Conference of African Association of Insect Scientists (AAIS) at the national institute of public hygiene, Abidjan, Côte d'Ivoire, Abidjan, Côte d'Ivoire**.**
- **12/2018.** First Entomological Conference of Côte d'Ivoire, at University Nangui Abrogoua, Abidjan.
- **08-09/12/2016.** Malaria Symposium "Building on success-Malaria control and Elimination"at Congress Center, Basel, Switzerland.
- **23/04/2015.** Poster Presentation "World malaria day" University Félix Houphouët Boigny, Abidjan, Côte d'Ivoire.
- **3-5/12/2014**. "First Congress of the Ivorian Society of Parasitology and Mycology University Félix Houphouët Boigny, (Côte d'Ivoire) "
- **1^{er}-03/10/2013**, Oral presentation "First Regional Conference in Africa of Association Internationale for EcoHealth at N'sa hôtel, Grand –Bassam, Côte d'Ivoire.
- **22-23/04/2013.** First Edition of Scientific Colloc, World malaria day, at CRRAE-UEMOA, Plateaux, Abidjan**.**

Publications

1. **Bédjou P. N'Dri*,** Nadja C. Wipf, Jasmina Saric, Behi K. Fodjo, Giovanna Raso, Jürg Utzinger, Pie Müller, Chouaïbou S. Mouhamadou .Species composition and insecticide resistance in malaria vectors in Ellibou, southern Côte d'Ivoire and first finding of *Anopheles arabiensis* in Côte d'Ivoire. ***Malaria Journal.*** **2023; doi:10.1186/s12936-023-04456-y.**
2. **Bédjou P. N'Dri*,** Kathrin Heitz-Tokpa, Mouhamadou Chouaïbou, Giovanna Raso, Amoin J. Koffi, Jean T. Coulibaly, Richard B. Yapi, Pie Müller and Jürg Utzinger**.** Use of Insecticides in Agriculture and the Prevention of Vector-Borne Diseases: Population Knowledge, Attitudes, Practices and Beliefs in Ellibou,South Côte d'Ivoire. ***Trop. Med. Infect. Dis*****. 2020, 5, 36; doi:10.3390/tropicalmed5010036.**

3. Behi K. Fodjo, Benjamin G. Koudou, Emmanuel Tia, Jasmina Saric, **Prisca B. N'dri,** Marius G. Zoh, Christabelle S. Gba, Alida Kropf, Nestor B. Kesse, and Mouhamadou S. Chouaibou.Insecticides Resistance Status of *An. gambiae* in Areas of Varying Agrochemical Use in Côte D'Ivoire. ***BioMed Research International.*** **2018. Article ID 2874160, 9**

4. Ako Victorien Constant Edi*, **Bédjou Prisca N'Dri***, Mouhamadou S Chouaibou, Fodjo Kouadio Behi, Patricia Pignatelli, Giovanna Raso, David Weetman, Bassirou Bonfoh. First detection of N1575Y mutation in pyrethroid resistant *Anopheles gambiae* in Southern Côte d'Ivoire. ***Wellcome Open Research***. **2017** Aug.2:71.

5. Yapi RB, Frédérique Chammartin, Eveline Hürlimann, Clarisse A Houngbedji**, Prisca B N'Dri**, Kigbafori D Silué, Jürg Utzinger, Eliézer K N'Goran, Penelope Vounatsou, and Giovanna Raso. Bayesian risk profiling of soil-transmitted helminth infections and estimates ofpreventive chemotherapy for school-aged children in Côte d'Ivoire. ***Parasites & Vectors***, **2016**; 9:162. doi:10.1186/s13071-016-1446-0.

6. Clarisse A. Houngbedji, Frédérique Chammartin, Richard B. Yapi, Eveline Hürlimann, **Prisca B. N'Dri**, Kigbafori D. Silué, Gotianwa Soro, Benjamin G. Koudou, Serge-Brice Assi, Eliézer K. N'Goran, Agathe Fantodji, Jürg Utzinger, Penelope Vounatsou and Giovanna Raso. Spatial mapping and prediction of Plasmodium falciparum infection risk among school-aged children in Côte d'Ivoire**. Parasites & Vectors**. **2016**. 9:494.

7. Clarisse A Houngbedji, **Prisca B N'Dri**, Eveline Hürlimann, Richard B Yapi, Kigbafori D Silué, Gotianwa Soro, Benjamin G Koudou, Cinthia A Acka, Serge-Brice Assi, Penelope Vounatsou, Eliézer K N'Goran, Agathe Fantodji, Jürg Utzinger and Giovanna Raso. Disparities of Plasmodium falciparum infection, malaria-related morbidity and access to malaria prevention and treatment among school-aged children: a national cross-sectional survey in Côte d'Ivoire. ***Malaria Journal.*****2015.** 14*:7.*

8. Hürlimann E, Houngbedji CA, Yapi RB, **N'Dri PB**, Silué KD, Soro G, Kouamé FN, Fürst T, Utzinger J, N'Goran EK, Raso G. Health-related quality of life among school children with parasitic infections: findings from a national cross-sectional survey in Côte d'Ivoire. ***PLoS Negl Trop Dis***, 2014. 8(12):e3287. doi: 10.1371/journal.pntd.0003287.

9. Hürlimann E, Houngbedji CA, **N'Dri PB**, Bänninger D, Coulibaly JT, Silué KD, N'Goran EK, Raso G, Utzinger J. Effect of deworming on school-aged children's physical fitness, cognition and clinical parameters in a malaria-helminth co-endemic area of Côte d'Ivoire. ***BMC Infectious Diseases***. **2014**.14, 411.

10. Yapi RB, Hürlimann E, Houngbedji CA, **N'Dri PB**, Silué KD, Soro G, Kouamé FN, Vounatsou P, Fürst T,N'Goran EK, Utzinger J, Raso G. Infection and Co-Infection with Helminths and *Plasmodium* among School Children in Côte d'Ivoire: Results from a National Cross-Sectional Survey. ***PLoSNeglected Tropical Diseases.*****2014**. 8, e2913.

Referees

1. Prof. Dr. Jürg Utzinger: Director of Swiss Tropical Public Health Institute (SwissTPH). Department of Epidemiology and Public Health
   Address: Kreuzstrasse 2, CH-4123 Allschwil, Switzerland
   Tel: +41 79 648 24 14 /+41 61 284 81 29/ E-mail: juerg.utzinger@swisstph.ch

2. Pie Müller: Head of Vector Biology Unit at SwissTPH/ Department of Epidemiology and Public Health
Address: Kreuzstrasse 2, CH-4123 Allschwil, Switzerland
Tel: +41 79 315 58 93 / E-mail: pie.mueller@swisstph.ch

3. Dr Chouaibou S. Mouhamadou. Vector control, Department of Environment and Health, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire
Address: 01BP1303, Km 17 Adiopodoumé
Tel: +225 0777031006/ +225 0103488886/ E-Mail: mouhamadou.chouaibou@csrs.ci